MOLECULAR CHARACTERISATION OF PREDOMINANT LACTIC ACID BACTERIA IN FERMENTED BREADFRUIT (*Artocarpus communis*) AND PIGEON PEA (*Cajanus cajan*) AND QUALITY ATTRIBUTES OF THEIR PRODUCTS

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ABSTRACT

Breadfruit and pigeon-pea are high yielding crops. However, breadfruit is highly susceptible to deterioration while pigeon-pea is hard-to-cook. Fermentation improves crop preservation, nutritional value and utilisation. Literature on fermentation of Breadfruit (BF) and Pigeon-pea (PP) is sparse. The study was designed to characterise fermenting organisms and determine physicochemical and sensory properties of fermented breadfruit and pigeon-pea products.

Breadfruit (BF) and pigeon-pea (PP) were fermented individually using liquid state fermentation at 28±2 ^{o}C and 37±1 ^{o}C for 24, 48, 72, 96 and 120 h. Biochemical, DNA extraction, Phylogenetic tree, Alignment and 16S rRNA sequencing of fermenting organisms were characterised by molecular methods. The fermented crops oven-dried and milled into flours. Chemical (proximate, pH, Total Titratable Acidity (TTA), anti-nutrients), functional [Water and Oil Absorption Capacities (WAC and OAC), Bulk Density (BD), Foaming Capacity (FC) and Stability (FS), Gelation Capacity (GC)] and pasting properties of fermented samples were determined using standard methods. Based on preliminary trials, flours were blended at ratios 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (BF: PP) and analysed for proximate composition using AOAC method. Breakfast meals and cookies were prepared from the flours using standard procedures. Sensory attributes of the products were determined by panelists. Data were analysed using ANOVA at $\alpha_{0.05.}$

Sugar fermentation and gram staining of the selected isolates showed diverse sugars and improved acidity as fermentation proceeded. Sequences of purified DNA products were significantly similar to GeneBank samples. Phylogenetic tree indicated high homology among the identified lactic acid bacteria with change in fermentation duration up to 120 h, reflecting taxonomical relationships among identified species. Alignment established similarity level through the nucleotide numbers across the region. High sequence homology of *Lactobacillus plantarum* and *fermentum* with sequence codes of CP011536.1 and CP015308.1, respectively as the dominant lactic acid bacteria were identified. Fermented BF flour contained 4.2-3.6% protein, 8.1-9.3% moisture (dry basis), 2.7-3.0% ash, 3.5-3.0% fibre. The protein, moisture, ash and fibre contents of PP were 24.8-4.5, 8.8-9.2, 3.7-4.0 and 1.4-1.8%, respectively. The pH of BF flour decreased with increased TTA and the same trend was observed in PP samples. The phytate, tannin, cyanide and alkaloid contents of BF and PP were 0.5-0.2 mg/g, 6.2-4.7 mg/g, 1.0-0.1 mg/100g and 1.2-0.2%, and 0.5-0.1 mg/g, 0.9- 0.1 mg/g, 1.2- 0.1 mg/g and 0.9-0.5%, respectively. Breadfruit WAC (346.1-224.8%) decreased while OAC (256.7-286.4%) increased as fermentation progressed. Loose bulk and packed densities were 0.4-0.5 and 0.4-0.6 g/mL,

respectively. Decrease in FS and increase in GC values were observed as fermentation progressed at 28±2 and 37±1 ^{o}C, respectively. The WAC, OAC, BD, GC of PP increased with decrease in FC and FS. Fermentation improved pasting properties of BF. Meals and cookies prepared with 10-20% PP had significantly higher acceptability levels of 7.7 to 6.4 and 5.8 to 5.0, respectively.

Molecular characterisation established genetic variations in *Lactobacillus plantarum* and *fermentum*. Fermentation improved the sensory attributes of breadfruit and pigeon-pea flours. Production of breakfast meal and cookies from fermented breadfruit and pigeon-pea flours are recommended.

Keywords: Fermented breadfruit, Pigeon-pea, DNA extraction, Composite flour

Word count: 490

DEDICATION

I dedicate this work to Jehovah Shammah, the giver of my strength and wisdom for being there.

And to my late father, James Iyiola Atanda Popoola who instilled in me the love for learning and the idea that as a lady, I needed a career.

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CERTIFICATION

This is to confirm that this work was carried out by AJANI, Alice Olapade of Food Technology Department, University of Ibadan.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Breadfruit (*Artocarpus communis*) originated from Malaysia,South Pacificand Caribbean. It is a vital crop in Pacific islands, whichblow-out to the Africa and Caribbean (Taylor and Tuia, 2007).It is an essential food in Caribbean,besidesendangeredthroughinternational agreementforplant genetic (Ragone, 1997).Breadfruits can be found in Nigeria,Cameroun,Sierra Leone, Liberia,Senegal and Ghanain Africa (Appiah*et al*., 2011). It can be found in 3 zones (south-west, south-south, south-east) including part of northcentral.The production level has been projected to be almost 10 million tonnes dry weight within a year,with abilities of higher than 100 million tonnes every yearin South Western Nigeria (Adewusi *et al.,* 1995). Breadfruit tree yields fruit two timeswithin year, this occurs around March to June,then July to September and so it bears fruitall through year.It is highlynutritive, inexpensive and freelyaccessible in irresistiblelarge quantity, particularly at the topmost of the two ripeningperiods in May and August. The fruits are butunder abused in Nigeria as a result of its littlesocietalapproval (Omobuwajo, 2007).Breadfruit can be consumedat different phases of ripeness and usually at mature green and ripe stage. Not-fully ripedbreadfruits are preferred in some areas. It might be consumed at all phases of growth as a starchy staple like banana and plantain,to replace potato, or prepared as a fruit (Ragone, 2011)

Breadfruit reported asoutstanding basis of carbohydrate,vitamins, minerals but low fat (Rincon, 2007). It is well-thought-outto be good basis of potassium,calcium, magnesium,copper,iron, thiamin, niacin with appreciable anti-oxidants and carotenoid (Ragone, 1997; Deivanai and Subhash, 2010).However, the noticeably low level of protein in breadfruit makes it nutritionally deficient and predisposes the consuming population to protein malnutrition (Adebayo-Oyetoro *et al*., 2012). Also, the fruits areunderutilised due toquick physiological deterioration which results in short shelf life;as farmer powerlessly look at their reaped breadfruits decayingas a result of insufficient methods of processing to use theharvested breadfruits.Breadfruit is extremely perishable in fresh form (Amusa *et al.,* 2002) and shipment for lengthenedstoring period in commercial form is not practicable with current technical development (Medlicott, 2002).

Breadfruit produced (60-80%) in South-West Nigeria is lost because of deterioration and lack of use (Steve *et al.,* 1995).

Although, breadfruitshad been developed into numerous forms for utilisation;fruits can becooked, crushed and eaten like pounded yam (Adepeju *et al*., 2011).Mukesh *et al.,* (2014)discovered that maturefruits can beroasted,bakedand replaceable fornumerous potato formulas, the unripe fruits can cure, marinated or simmered to give flavour like artichoke hearts. Breadfruit in sliced form can use to produce chips or French fries (Morton, 1987). Breadfruits are eaten as snacks in Ghana by numerous rural dwellers andis use for food security (Appiah *et al*., 2011a). Breadfruit waspreparedto make starches, flours, complementary foods reported (Olatunji and Akerele, 1978; Ajani *etal*., 2012, Adepeju *et al*., 2014.

Strong determinations are presently made in pursuit of inexpensive protein bases with nutritious and useful properties to mitigate unruly malnutrition broadlyblow-out in developing Countries (Siddhuraju *et al*.1996). Breadfruitenrichment which have potential of lessening protein-energy malnourishment has not received considerable attention. In this regard, pigeon-pea is an important legume with excellent nutrients and inexpensive source of plant protein consumed in Africa can be used for enrichment.

Pigeon-peas (*Cajanus cajan*) are lesser knownnearbyobtainablebut inexpensive legume in the tropics andsub-tropics. Pigeon-peas protein content ranged from 23-26%(Onweluzo and Nwabugwu, 2009) and richin lysine.Protein content is equivalentwithlegumes such as cowpea, groundnut and it is high infibre contentas well as mineral quality (Fasoyiro *etal.*, 2009a). Pigeon-pea was underutilized owing to its hard texture that results to extensive cooking periodas well as incidence of some anti-nutrients (Francis *et al*., 2001;Odeny 2007, Fasoyiro*etal*., 2009). Pigeon-pea remainsdearthacceptingpulseembraced through small-holder farmers in most developing countries which playsvitalpartfor the farming schemes (Fasoyiro *et al.*, 2013).Pigeon-pea varieties available in South-West Nigeria, could be used in supplementing the little starchy staples(Fasoyiro *et al.*, 2009b). The mature, immature seeds and unripe pods of pigeon- pea could be eaten. The seeds are used complete, dehulled or consumed in flour form regularly. Since pigeon-pea is suitable in tropical areasof developing countries where inadequatequality of protein is a

restrictiveissuewith increase in population, suitableprocessing methods that will expand

itsutilisation isdesirable to solve malnutrition and food uncertainty.

On the other hand, breadfruit and pigeon-pea are recognised to contain some anti-nutrients just like some other legumeswhich inhibit digestive processes and effective utilisation of proteins. These anti-nutrients aresaponin, protease inhibitors, lectins, heamagglutinin and flatulence issues (Osabor *et al*., 2009; Alonso *et al*., 1998).Antinutritional Factors (ANF) are chemical substancesexisting in food crops,though non-poisonous but producehostile physiological responses in animal who consumes. Sometimes, theyhinder utilisation of nutrients in leguminiouscrops (Nwokolo, 1996).Nevertheless, these may be removed or lessenusing fermentation and germination (Khorkhars and Cheuham,1986).Also, fermentation and steaming as reported enhance detoxification of breadfruit(Onweluzo and Nnamuchi, 2009).However, fermentation as one of methods for handling and conserving breadfruit is fairly unpopular as introduced in Pacific Islands (Adekanmi *et al*., 2012).Fermentation is one of classic means for organoleptic enhancement,detoxification, nutritional quality,preservative properties and antibiotics production in foods (Oyewole and Isah, 2012).Fermentationhassignificantparts in reduction of anti-nutrients,nutrientaccumulation and anti-microbial actions;giving fermented products pleasingsmell and quality. This isowing toenzymesmetabolic actions andraw materials microorganism (Oyarekua,2013).

Fermentation technology for various homes use and industries cannot be overstressedbecause ofrole in diet, wellbeing and economy sinceexistenceofmankind.Previous works shown thatseveral authors have worked on breadfruit and pigeon-pea fermentation but their reports had not addressed molecular aspect of identification and characterisation of organisms and possible applications of the crops and fermentation methods differs. Ojokoh *et al.* (2013) investigated fermentation effect onbreadfruit (*Treculia Africana*) and cowpea (*Vigna unguiculata*)nutrientsand antinutrients using solid state fermentation. The micro-organisms isolated were identified with the aid of traditional/conventional methods. Also, reports of Nwaneri *et al*. (2017) on microbiology and biochemistry of fermented African breadfruit using solid state method, identified organisms with conventional methods and not characterised with molecular methods. Adegbehingbe *et al*. (2017), Adeniran and Ajifolokun, (2015) and several authors'fermented breadfruit and diverse groups of organisms were identified using solid state fermentation.

Influence of processing techniques on properties of pigeon-pea (Pele *et al*., 2016), fermentation ofpigeon-pea and millet as complementary food (Mbaeyi-Nwaoha and Obetta, 2016)were researched on and microbial properties analysed. Adebayo-Oyetoro *et al*. (2017) co-fermented sorghum and boiled pigeon-pea as weaning food. Fasoyiro *et al*. (2009) and host of authors fermented pigeon-pea seeds for products development using conventional methods.This traditional/conventional method of microbial identification are prone to errors (Petti*et al*., 2005). However, molecular methods by means of 16S rRNAsequencing presents current state-of-art in identification and characterisation of micro-organisms especially dominant lactic acid bacteria in fermented breadfruit and pigeon- pea using submerged fermentation method.16S rRNA sequencing developedas moreimpartial, precise and dependableprocedure for bacterial proof of identity. Also, it has additionalability of defining taxonomical relationships among bacteria (Clarridge, 2004).Breadfruit in addition with pigeon pea identified as an essential high-yielding food crop in a lot of tropical regions and they have greatcommercialstandards andrecognised for their capabilities to influence food security.Several authors fermented breadfruits seeds, whileenhancementhave been attained innutritive value and legumequality bygermination,dehulling,fermentation,heat treatment (Forster*et al.,* 2011;Oloyo, 2004), limited work completed in the area of breadfruit fermentation, production of composite from fermented breadfruit –pigeon-pea and production ofcookies. Also, not much has been completed on starter culture development from such fermentation.With growing situation on food insecurity, concerns for diet, general health and the way millions of people are chronically undernourished, it is vital to know nutritional status of fermented flours for further utilisation.It is therefore thestudy objectiveto produce and evaluate fermentationinfluence on nutrients, anti-nutrients in breadfruit and pigeon-pea flours soas toinvestigate prevalentLAB potential as starter culture.

1.2 Problem Statement

Breadfruit is known to be highly perishable and processing to flour is one of the methods of preservation (Ragone, 2011). However, the flour is deficient in protein (Adebayo-Oyetoro *et al*., 2012) and contained anti-nutrients such as oxalate, phytate, alkaloid, e.t.c., thus limiting its utilizations. Substitutions of flours with protein rich legumes have produced value added composite flours (Ojokoh *et al*., 2013).

Pigeon-peasare lesser known crop that contained about 23-26% of protein(Onweluzo and Nwabugwu, 2009).The study ofUgwu and Oranye (2006) and Adegbehingbe*et al*.(2017)revealed decrease in anti-nutritional factors of breadfruit flour and seeds through fermentation. In-depth knowledge of microbial activities through fermentation process of breadfruit and pigeon-pea composite flours and their effects on flour quality and products are desirable.

1.3 Justification of study

Due to high level of post-harvest losses (60-80%)of breadfruit (Steve *et al.,* 1995), occurrence of anti-nutrients,insufficiency of protein in this lesser known crop as well as increase in food insecurity in Nigeria, it has become imperative to transformbreadfruit to storable form, reduce its disadvantages and enhance it with legume to improve its nutritive value.Hence, substitution of fermented pigeon-pea flour with breadfruit for production of breakfast meal and pizzelle cookies will improve the nutritional status of the products. Research outcomewillincreasebreadfruit and pigeon-pea utilisation; cropgrowers willprofit and new research areas will be opened.

1.4 ResearchObjectives

The key objective was to characterise fermenting organisms and determinechemical,functional, pasting, sensory properties of fermented breadfruit and pigeon-pea products.

Specifically, this study intended to:

i. improve shelf life of breadfruit and pigeon-pea through processsing into flour for better utilisation.
ii. determine chemical,functional,pastingproperties of breadfruit and pigeon-pea.
iii. detect,then quantify level of antinutrients in breadfruit and pigeon-pea flours.
iv. determine and identify mostdominant LAB in breadfruit and pigeon-pea using 16SrRNA gene amplification and sequencing approach.
v. examine consumer's acceptability of breakfast meal and pizzelle cookiesprepared from breadfruit-pigeon-pea composite.

CHAPTER TWO

LITERATURE REVIEW

2.1 Breadfruit description

Breadfruit (*Artocarpus communis*) (*synonym Artocarpus altilis*) belongs to tropical tree*moraceae*family (Orwa *et al*., 2014). A*rtocarpus*resulting from Greek words; artos means bread and carpus means fruit. Breadfruit invented from *Artocarpus camansi Blannco* and *Artocarpus mariannensis Trecul*.Breadfruit plants are monoecious flower growing on similar trees.This family has more than 1000 species and about 50 genera tropical trees and shrubs. The tree height is about 26m, clear stem of 6m, 0.6 – 1.8m breadth supported.Nevertheless, some varieties mightcertainly notexceed ¼ or ½ of these sizes. Breadfruit tree allows a host of slight flowers, rod-shapedpoint with 12.5-30cm lengthy and 2.5-3.75cm thick. The male greatly set on a drooping, which is yellow first and brown latter. At the upper surface, the flowers are bright-green and glossy. Some flowers might becloudy, yellowish,covered with tiny stiff hairs underneath and conspicuous yellow veins. (Morton, 1987). Some of species in this class hasedible fruits and seeds, for example, jackfruit (*Artocarpus heterophyllus*) and (*Treculia africana*) both are the seeded form generally known as breadfruits (Zerega *et al.,* 2004).Breadfruitskin is changing in colour from yellowish-green to brown and mighthave 20-30cm diameter. It isfewerroundedshape with denseroughbut resembling wax in appearance. The fruit is hard in the green stagewithwhiteinner, starchy and slightly fibrous. It is mildwhen fully ripe; cream coloured or yellow and pale inner, with sweet-smelling. Also, a humidlight yellow /colourlessflesh surrounds a central withslightuniquearoma inside (Yamaguchi, 1983).The seeds are round in shape, irregularly oval, pointed at one another, dull-brown with blackerstrips and length is approximately 2 cm.Breadfruit has twoimportantvarieties which areregular wild varietyplanted in some regions with little fleshy tissue and seeds; then cultivated that isextensively grown which iswithout seedvariety,sometimes,few establishedseeds found in seedless cultivars. Seeds are thin,dark-brown andeatable,with skin thickness of around 0.5 mm (ICRAF, 2010).

However, breadfruit is periodic and sobountiful that it cannot all be consumed fresh during its season. This is because the trees regularlyyieldhugeproduces at a particular period of year

andsafeguarding harvested fruit is a concern as a result ofquick deteriorationafter harvesting(SPC, 2006; Adepeju *et al*., 2011).As a result of insufficient routine techniques of processing to use all the breadfruits harvested,farmers usuallyhelpless as their harvestedbreadfruits wasting. To avoid waste, numerousprocedures of conserving breadfruit have been developed. Breadfruit was well-kept in numerous diverse ways before the Europeans came to the Pacific, these include fermenting and drying (SPC, 2006). The significance of breadfruits notwithstanding, are underutilized anddeserted but the unexploited potential needs to be harnessed (Quartermain, 2006; Omobuwajo, 2007).Underutilisation is as result of societal stigmatisation, thinking breadfruit is for poor and slaves, since is being considered as lesser known crops. All these factors headed to itsabandonment (Appiah *et al*., 2011a; Akanbi *et al*., 2009,Spore, 2007).

2.2 History and Distribution of Breadfruit

Breadfruitdrew to the Tahitian cultivars offered by Captain Bligh to St. Vincent Island,also Jamaica around 1972,then spread to whole of Caribbean (Kerr, 2009). This was presented on a search for inexpensive, high-energy food for West Indies and MauritusBritish slaves in 1796. The trees were disseminated through root cuttings, air-layering of plants above ocean distances and native range by Polynesiantraveller.It arrived Africa in 1899 throughdeterminationsof Camayenne botanic garden in Guinea andblow-out furtherto some regions of West Africa. The seedless types of *Artocarpus communis* are extensivelydispersedin Eastern Polynesia and parts of Caroline Islands with variety of cultivars.New Guinea Island, South East Asia and Philippines have spiny seeded breadfruit similar to *Artocarpus communis*.Breadfruitusuallyimplantedviacountries likeGhana,Sierra Leone, Jamaica and Nigeria (Macrae *et al.,* 1993).

Breadfruit is believed to beintroduced to Ife Wara, South West, Nigeria from Caribbean before turn ofcentury, thenblow-out to neighbouring town and villages(Adewusi *et al*., 1995). Breadfruit is a widespreadregularnutritiveceremonial dinner food in Ile-Ife, Osun State, about 80km away from Ibadan. This is for producing a type of pounded yam called “Iyan Jaloke” or “Gberefuru” and common in other parts of Osun State ofNigeria where it is cooked and consumed as yam. It is presumed that one breadfruit tree in a farmhouse can supply dinner to a family of four fora year (Anonymous, 2010).

2.3Harvesting and Yield

Breadfruits plucked whenever is matured through appearance by minor droppingsap on surface. Harvesting is carefully done to retainfruit quality. The harvesters use to climb the trees and pluck fruit through the forked stick. Although this might cause bruising/piercing but well-thought-outhealthier than takingfruits via hand asfragmented pedicel drips latex (Morton, 1987).Harvesting is best done before the build-up of the field heatin the early hours of the day. This is done by mounting the tree end-to-end. In presence of harvesting device, breadfruit must not allow to fall on ground so as to avert mechanical injuryof the fruit. Breadfruit can be plucked whenstrong, notcompletely ripe and each weighs between 1-5kg (Omobuwajo, 2003). This is because they are commonly consumedunripe, when breadfruitfleshy tissue is white and soft (Brouk, 1975). The fruit matures 1 to 3 days after harvest and can be used within 5 days of harvesting and should not be left in the sun or wind. The fruit yield per tree differs depending on area. Breadfruit tree has an abundantfruitful ability, the average size is between 400 - 600 fruits per year (NTBG, 2009).The fruit termed as vital crop of countlessprofitableworth (Soetjipto and Lubis, 1981). Breadfruit yieldsare higher to staples likecassava and yam (Singh, 2009). Breadfruit yieldabout 50 - 150 fruits per year inSouth Pacific and average production is 150- 200 fruits in South Indiaper annum and yielddiffers from wet and dry areas.

2.4 Composition and Nutritive Value

Breadfruit is an outstandingnutritional staple thatrelates favourably with starchycommoditiesgenerallytaken in tropical countries with number higher than 120 species(Camille *et al.,* 2011). This crop standsamonguppermostproducing plants. Breadfruit remainsabundantinpotassium,fiber, calcium and magnesium (Ragone, 2007). Itis a vital food with nutritive valuesbut high in starch (Jeffrey *et al*., 2006). It is valued food reserve of high-calorie diet (starch - 68%,protein - 4%, fat -1% on dry basis) withsubstantialquantitiesof minerals and vitamins,particularly the B-Vitamins. Breadfruit is 25% carbohydrates (110kcal/100g) and 70% water. Studiesshowed that breadfruit (*Artocarpus communis*) is a leading source of dietary carbohydrates; matured ones have about 84% carbohydrate and starch having above 60% total

carbohydrate. These carbohydrates, operated as simple sugars such as fructose and glucose by the body are freelyused to improve the energy generation process in the body (Oladunjoye *et al.,* 2010). Studies by Ekpenyong (1985) and Makinde *et al.* (1985), indicated considerable variations in nutrient contents of Africa breadfruit. Breadfruit (*Artocarpus communis*) has been reported by several authors as good source of nutrients (Orwa *et al*., 2009; Adewusi *et al*., 1995). Breadfruitshave yeast odour and fresh bread texture, then vitamin C, Vitamins B1 (100ug) averagely present as well as small amounts of zinc and thiamin (100 µg/100 g). The quantity of pro-vitamin A carotenoidwhich is vitamin A precursor, differswith ripeness. Dry breadfruit hasrelated nutrientsquantities asraw breadfruit, excludingvitamin C and thiamin that are less stable (Zerega *et al*., 2004). Breadfruit remainsrespectable fibre basisalsovitalfor healthy gut. Diet rich in fibre aidsin regulating blood sugar, decrease lipids in blood (risk of heart disease) and weight control.

Breadfruit is an excellent fruit for a healthy, optimally working heart because of availability of potassium. Potassium is a crucialconstituent of the body liquidswhich control heartbeat rate and body's pressure levelefficiently.Ithas calcium and betterbasis of vitamin C (Ragone, 1996). Calciumis use for healthy bones in the body and also usefulwhenblood levels drop. Calciumis essential in muscle contraction, nervefunctioning and blood clotting. Breadfruits havenecessary vitamins and antioxidants like xanthin, which work to defend the body from the devastating attacks of bacterial and viral agents. Besides, they also inhibit free radical substances from harmful the body's cells. In effect, they combine their efforts to diminish the risks of osteo arthritis, rheumatoid arthritis, several cardiovascular diseases and cancer. Vitamin B_9 (folic acid), recognised as folate, is a vital constituent forcells functioning,reproduction and normal growth. Vitamin B_9 plays importantpart in procedureson cell division. 14 micrograms vitamin B_9could originate in raw /fresh breadfruit (100g). Vitamin B_9offers 4% endorseddailyworth and folate has been known in reducingAlzheimerincidence and cognitive decline. VitaminCissturdyabsorbent antioxidant; then, eating fruits abundant in vitamin C aid togrowbattlecontrary tocommunicable agents and harmful searches free radicals. Water-soluble vitamin,riboflavinfunctions in redox reactions,as co-enzyme and antioxidant in energy metabolism. This vitamin like othersassist the body in converting carbohydrates, fats and proteins into glucose as body fuel.

Breadfruit is well compared with rice as a result of nutrients availability, a portion of seeded variety could meetvitamin C daily requirements, while comparing with vitamin C and other nutrients in rice which are very low(NBTG., 2014, Ragone, 1997).Breadfruit has reasonably high level of potassium, iron,calcium, niacin, riboflavin and pro-vitamin A (Graham and De-Bravo, 1981). Iron is an amplecomponent andbiologically important foreachactive organism.It playssignificantpart in procedures that unceasingly take place in molecular level, particularlyduring heamoglobin formation. In 100graw breadfruit, 0.54 milligrams of iron can be found and provides 3% dailyendorsedrate for average adult. Magnesium is an important mineral that showed positiveinfluence in energy creation, healthy immune system regulationand muscle functioning. It regulatesblood glucose level andassists in protein creation. 100g raw breadfruit has 25mgmagnesium, which is 6% daily valuesuggested for an adult.Magnesium is needed for bone, crucial to heart function, insulin secretion and its function.The fruit is also containingsmallquantity of fat and sodium.Though, breadfruit remainsan excellent vitamin,carbohydrate and minerals bases but contains small protein and fat (Rincon, 2007; Adebayo-Oyetoro, 2012).Protein content of breadfruit ranges from 3-5% (Appiah *et al*., 2011; Qulai *et al*., 2013) with poor amino acid quality (Golden and Williams, 2001).

Table 2.1: Raw Breadfruit Nutrients (Nutritional value per 100g)

Nutrients	Quantity
Energy	431kJ
Carbohydrate	26.04 g
Sugar	10.56g
Fibre	4.7 g
Fat	0.22 g
Protein	1.03g
Water	70.65 g
Lutein and zeaxanthin	22 µg
Thiamine (vit. B_1)	0.11 mg
Riboflavin (vit. B_2)	0.03 mg
Niacin (vit. B_3)	0.9 mg
Pantothenic acid (B_5)	0.457 mg
Vitamin B_6	0.1 mg
Folate (vit. B_9)	14 µg
Choline	9.8 mg
Vitamin C	27.8 mg
Vitamin E	0.1 mg
Vitamin K	0.5 µg
Calcium	17 mg
Iron	0.54 mg
Magnesium	25 mg
Manganese	0.06 mg
Phosphorus	30 mg
Potassium	490 mg
Sodium	2 mg
Zinc	0.12 mg

Source:Foodand Calorie Counter (2009)

Table 2.2: Breadfruit Amino Acid Profile

Amino acids	Composition (g/100g)
Threonine	0.24
Aspartic acid	1.55
Serine	0.14
Glutamic acid	0.52
Proline	0.09
Glycine	0.40
Alanine	0.33
Cysteine	0.03
Valine	0.19
Methionine	0.21
Isoleucine	0.10
Leucine	0.22
Tyrosine	0.06
Phenylalanine	0.15
Lysine	0.03
Histidine	0.18
Trytophan	0.39
Arginine	0.10

Source: Golden and William (2001)

2.5 Significance / Breadfruit Utilisation

Breadfruit is principally a carbohydrate sourcearound the region where it is produced and eaten in Nigeria. It has been found nutritivelymore thanconservativecalories basessuch as yam, cocoyam, cassava (Orwa *et al*., 2009;Omole *et al.,* 1978).Breadfruit produced into different forms in food industries for utilisation.There are reports on the production of starches and flour from breadfruit (Bakare *et al*., 2012).Akanbi *et al*., (2009) processed raw breadfruit toindustrial starch. Processing of breadfruit into flour and other finished products have been identified as ways of reducing postharvest losses and improvebreadfruit utilisation (Ajani *et al*., 2012; Oulai., 2014).The utilisation of breadfruit for composite flour statedthrough some investigators. According to Olaoye and Onilude (2008),using breadfruit flour as composite for bread production and confectioneries can assist in minimising wastages associated with breadfruit and increase its output. Flour produced from dried breadfruit is sometimes partiallyreplaced wheat flour for bread production in Barbadosand found more nourishingto wheat in lysine and somevital amino acids (Spore, 2007). Typically,breadfruit is usually eaten when mature with texturehard, thenenjoyable substitute starchy crops.Mature fruit could boil, steamed or baked,in addition,it could replace potatoes in many recipes. The immaturefruits couldboil, pickled, then have flavour identical to artichoke hearts. Sliced fruit couldfryfor chips or French fries' production (Morton, 1987).

Breadfruit known as substitute forcarbohydrate diet and its starch may be produced into differentformulae for industrial use (Deivani and Subhash, 2010).Adegoke (1985)suggestedbreadfruit flour as filler in pharmaceutical to replace conventional tuber-crop flours. Esuoso and Bamiro(1995)studied the likelihood of making bread through wheat and breadfruit flour. Olatunji and Akinrele *(*1978*)* recommended breadfruits as composite flourconstituent, with no pronounceddeviationsof dough rheological properties andvalue. Non-alcoholic beverages produced using breadfruit flour as adjunct in malted sorghum (Ilori and Irefin, 1997).Chin-chin and cake made from breadfruit and wheat (Ajani *etal*., 2012). Olaoye *et al*., (2007)baked biscuit using breadfruit flour and study established usage ofripe breadfruit in production of cakes,sweet delicacies, cookies and energy bars.Breadfruit can use to preparewide-range appetizers, beverages,casseroles, fritters, croquettes, pancakes, chowders main dishes, breads, pastries, pasta and desserts (Ragone *et al*., 2012). Also, breadfruit could be crushed to

producehummus,vegetarian burgersbut mature unripe isideal as vegetable and useable in curry, stews, dumpling formulas. Mayaki *et al*., (2003) assessed breadfruit in traditional stiff porridge foods by processing into yam flour- like and pounded yam flour-like products while Omobuwajo (2003) produced breadfruit into three snack food items specifically biscuits, prawn cracker and chips and establish the acceptability in terms of overall quality. Andrew (2011) worked on nutritional and morphological variety of breadfruit; Ragone (2007) reported on breadfruit diversity, conservation and potentials while Jones *et al*., 2011 investigated on novel foods from breadfruit for food security. Adepeju *et al*., (2015) worked on development and evaluation of wheat-breadfruit cookies. Furthermore,acceptable bread from breadfruit and wheat composite flour produced by Giami *et al*., (2004). Adebowale *et al*., (2008) also produced instant yam breadfruit flour while Oladunjoye *et al*., (2010)establishedsubstitution of breadfruit meal for maize in poultry diet if properly produced.

Ragone and Cavaletto (2006) evaluated sensory of breadfruit value and nutritivestructure of 20 cultivars of breadfruit. Also, Ojokoh *et al*., (2013) researched on the fermentation effect of nutrients and antinutrients compositionsin Afrcan breadfruit and cowpeaflour blends and discovered development in the nutritionalquality and effectivedecline in the anti-nutrient contents.Ojokoh *et al*., (2014) investigated breadfruit and cowpea fermented with *Lactobacillus plantarum*as complementary foods for infants and established its potentials for management of protein-energy malnutrition. Ishaya and Oshodi (2013) evaluated attributes of composite bread producedfrom breadfruit (*Artocarpus altilis*) flour, wheat (Triticum aestivum) and benth seed (*Adenopus breviflorus*) flour. Sensory assessment donediscovered that breadfruit and benth seed could replacewheat as much as 20% lacking substantialalteration in taste, appearance and colour in comparison to commercial bread. Adepeju *et al*., (2014) researched on complementary food using breadfruit.The complementary food developed from breadfruit, soybean and groundnut floursanalysed.This related with existing ones to know its acceptabilitybased on texture,dietary bulkand caloric density. Results showed that the formulations had better functional properties in term of water binding capacity, gelation, bulk density, swelling capacity, viscosity and calorific density.These similarobservations were made by Ijarotimi and Aroge (2005) on nutritive composition of weaning food produced from breadfruit- soybean flour. The weaning food showed high energy values and satisfactorily meet Recommended Dietary Allowance (RDA) for children at 60% inclusion. Also,breadfruit has been dehydrated usingtunnel and freeze.The

drying andwaste from these proceduresestablishes an extremely digestible stock feed (Morton, 1987).

Investigation on microbiological including sensory properties of gari from cassava andbreadfruit co-fermented done by Adeniran and Ajifolokun (2015).The result showed six bacteria species isolated from fermentedpulp andestablished breadfruit (20%) co-fermented with cassava producesnewinvention.This equates favourably with cassava gari based (100%) on microbial and sensoryfeatures.Okoye and Obi (2017) reported that the use of germinated breadfruit seeds for the composite flour in cookies production can help to minimize post harvest losses.

2.6Breadfruit Bio-deterioration

Breadfruit is a vital staple food in some tropics. However,the main factor limiting breadfruit is poor storage, as the fruit experiencesquickphysicaldeclineonce harvesting and reduced yield owing to diseases (Adepeju *etal*., 2011). Adebayo and Ogunsola (2005) noted that most of difficulties in food encounted by developing countries can be ascribed to enormous postharvest wastages. Due to short shelf lives, breadfruits are usually used in 5 days after harvesting. Nevertheless, breadfruit might take as much as 10days before getting to markets in cities after harvesting, this result tomassive losses owing to bio-deterioration.Amusa *et al*., (2002) investigated aetiology of breadfruit bio-deterioration in storage and its effects on fruit nutrients.*Aspegillus niger, Rhizopus stolonifer, Botryoduplodia theobromate, Mycorellosiellafulva, Penicillum spp and Aspergillus flavus*associated to deterioration of fresh breadfruitkeptinsidelaboratoryfor 9 days.

Breadfruit trees infected by soft-scales, mealy bugs and branches also infested by ants after fruiting. Anthracnose and Phytophthora palmivora usually attack the fruit, undeveloped breadfruit trees have destroyed with disease caused by *Rosellinia spp*. Also,Phomopsis, Dothiorella and Phylospora affect the stem and decay breadfruit.Some symptoms detected on unwholesome treesare fruit rot and tip dieback. Fruit flies have been observed to damage breadfruit. Paul and Chen (2004) established that breadfruit couldbe preserved from fruit fliesby means of vapour heat treatment or radiation. However,healthy treesand good sanitation could reduce problems caused through diseases. Storage of breadfruit at temperature below 12^{o}Cends

to chilling injury (Ragone, 1997). This showed brown scaled-like stainingskin,water lossincreased, increased vulnerability toorganism'sdeterioration and harmful flavour features. Due to this bio-deterioration problem, one way to prevent postharvest lossesis speedy processing and transformation of the fruit into flour or other finished products that can be easily stored (Oulai, 2014).

2.7Pigeon-pea Description

Pigeon-pea is perennial plant which can live for 3-4 years (FAOSTAT, 2011) and it is short-day high feeling plantof photo-periodic variations (Vales *et al*., 2012). It has two main growth patterns namely determinate type that produces cluster pods at top of canopy andgrowth stops athighpoint which result to less otherwise moreunchanging maturity.Non-determinatetype is the second common growth custom where pods tolerated auxiliary bunches. Broadly, latter typecouldstand biotic and a-biotic pressures as a result of intrinsicability to renew. The traditional cultivars are landraces, tall and take about 180-280 days before maturity. Pigeon-pea hasnumerous local names in diverseregions of the world (Saxena, 2008). In Barbados, pigeon-pea seeds grown to feed pigeons andin India, pigeon-peacommonlyrecognised as'red gram, tur or arhar'. Pigeon-pea seeds contain14% seed coat, 85% cotyledonsand 1% embryo withvariationin food nutrients (Faris and Singh, 1990).

2.8 History and Distribution of Pigeon-Pea

Pigeon-peagrew in Asia,then distributed to West Africa in 2000 B.C., where it became second mainhub of origin (Van Den Beldt, 1988). It was taken to West Indies and usedto feed bird (Van der Maesen, 1986). Pigeon-pea grown extensivelyaround 14 Countries in over 4 million ha. Foremost pigeon-pea growers around globe are:Tanzania, India, Uganda,Kenya, Malawi, Mozambique andEthiopia.Puerto Rico,Dominican Republic andWest Indies. Also, Latin America,Burma,Philippines in Asia,Australia, IndonesiaandThailand (Sinha, 1977).

2.9Harvesting and Yield of Pigeon-Pea

Pigeon-peas typically planted throughout the rainyperiod and harvested in dry season in Nigeria. Harvesting usually done after140 days of planting when the pods begin to turn green and plump.The fruit of pigeon-pea is in form of pod, 2-9 seeds/pod and is flat, greencolour, occasionallycovered with hair andmarkedwithdim purple. Pigeon-pea seeds are extensively variable in colour and weigh 4-25g/100 with round or lens shaped (Sheldrake, 1984). The yields of top growth fresh pigeon-pea range to 35 tons/acre, withapproximately 700 lb/acre, these makepigeon-pea utmostyielding legume food. Uganda produces the highest pigeon-peanextby Nepal and India (Ghadge *et al*., 2008).

2.10Pigeon-Pea Composition and NutritiveValues

Pigeon-pea described to haveprotein (20-22%), fat (1.2%), carbohydrate (65%) and(3.8%) ash (FAO, 1982). Wild types of pigeon-pea establishedasencouragingbases of highprotein and numerousgenotypes hadremainedtechnologically advanced with protein.Protein genotypes havealmost 20% above normal (Saxena *et al*., 1987). They containmeaningfullyamino acids (about 25%), specificallymethionine and cystine(Singh *et al*., 1990). The seed has lesserdietary fat and is a respectable amino acidsbasis (Elegbede, 1998). Ithasadditionalfat, minerals,extravitamin A and supplementaryvitamin C toregularpigeon-peas (Foodnet, 2002; Odeny, 2007).Pigeon-pea hasappreciableamounts of protein withsignificantamino acidslikelysine, methionine and so on.However,undeveloped seeds usuallysmall in nutritive values, but havesubstantialquantity of vitamin C (100 g serving, per 39 mg)with slight complex fat content. Pigeon-pea is better in basis of nutritional minerals likepotassium,calcium, phosphorus, magnesium, iron, and sulphur. Pigeon-pea an excellent basis forwater-soluble vitamins, particularlyriboflavin,thiamine,cholineand niacin (Sinha, 1977).

2.11Importance of Pigeon-pea

Pigeon-pea recognizedas foodintended for animal and human consumption.Younghulls, undeveloped seeds and developed seeds might be eaten. Pigeon-pea seeds can be usefulcomplete, de-hulled or milledinto flour. Caribbean eat seedsas green undevelopedpeas, but regularly preparedinto dried split-pea (dahl). Pigeon-pea is rich in organic nitrogen, help in increasing organic matter in the soil and improve structure and superiority (Adu- Gyamfi *et al*.,

2007).The peas help in improving soil quality for long term use as green manure, cover orside streetcrop (Bodner *et al.*, 2007). Pigeon-pea havecapacityin decreasing root-knot nematodeslevelofsubsequentproduceonce use as manure (Daniel and Ong, 1990). Pigeon-pea use effectivelybeneathfarms asshelter crop in improvingpossessions of soil, reducingunwanted plantrivalry and food for raiders (Venzon *et al.*, 2006). In addition, itpossesses different minerals, vitamins, proteins and carbohydrates (Khandelwal *et al*., 2010).Pigeon-pea flour found appropriate incookies, bread and chapattis preparationowing to high content of protein, iron also phosphorus (Harinder *et al*., 1999). Biochemical changes investigated during production of fermented pigeon-peafor making moinmoin by Oyarekua (2011) whileFasoyiro*et al*., (2009) processed local spice (dawadawa)using fermented pigeon-pea. The existence of nutritive fibre in pigeon-pea providespossible health aids in avoidance of chronic diseases and considered as functional food. Thepigeon-pea flour is outstandingconstituentto snackand commendedcomponentin increasing pasta nutritional statusdeprived ofdisturbing sensory belongings (Torres *et al*., 2006).Other likelypigeon-peausagesin Africa for human intakeinclude noodles processing, tempeand fermented products (Mugula *et al*., 2003).In some other parts of the world, pigeon-pea flour usedin place ofstabilizerforsoups and rice. Entire dry seed could be heatedunaccompaniedotherwise together with vegetables. Over 90% crop used upby means of dehulled while immature pigeon-pea could be used as vegetable and nutritious than drypeas. Green pigeon-pea can be frozen, canned, occasionally very young pods harvestedbefore seeds developedand cooked like French beans in curries. Pigeon-pea can be used for fresh sprouts, ketchup and numerous extruding products (Saxena *et al*., 2002). Also, bearing in mind therapeutic importance for human beings, legumes are suitable for controlling cardiovascular disease and diabetes (Hu, 2003).

According to Natural Resources Conservation Service (NRCS), pigeon pea producesapproximately10.5 tons/acre dehydratedsubstance, also50 lb Nitrogen per ton as a green manure. Total nitrogen obtainable from a summer pigeon-pea planted at Florida estimated to be 250 lb/acre,availability of this portion crop, demonstrating the nitrogen unconfined over aperiod of time (Valenzuela and Smith, 2002). Pigeon-pea can be classified asfodder/shelter crop (dried peas, green vegetable peas). Amaefule and Nwagbara (2004) worked on pigeon-pea meal-based diets for pullets. However, combination of pigeon-pea with cerealsmake balance human diet.Dried peas mightstay sprouted temporarily and heatedto make diverseflavour using green or

dried peas. Germinationimproves digestibility of dried pigeon-pea throughdecreaseinimpenetrable sugars stay incooked peas. Fragmented pigeon-peas and most currentgrains, has importantprotein basis intypicallyveganfood. In some regions, undevelopedhulls are eaten as vegetable in dishes likesambar whileEthiopian cooked and eaten young shoots and leaves as well aspods (Asfaw, 1995).

Table 2.3: Raw Pigeon-pea Amino Acid Profile

Amino acids	Composition (g/16 gN)
Lysine	7.79
Histidine	3.66
Arginine	5.86
Aspartic acid	11.56
Threonine	3.12
Serine	3.59
Glutamic acid	9.23
Proline	3.17
Glycine	3.07
Alanine	3.79
Cystine	1.19
Valine	5.85
Methionine	1.19
Isoleucine	3.47
Leucine	6.78
Tyrosine	2.63
Phenylalanine	6.15
Tryptophan	ND

Source: Akande *et al*., 2010

2.12Fermentation

Fermentation means way forbreaking down compounds through microbial enzymes ormetabolic procedure whereby carbohydrates besideslinked compoundscorrodedthrough discharge of energy withoutexterior electron acceptors (Adegoke, 2004 and Adams, 1990).It is aprocedurefor production of foods throughsupport of micro-organisms that own enzymes likelipases,amylases and proteases which hydrolyzecarbohydrates, proteins and lipids existing in croptoimprove flavour, smell and texture (Steinkraus, 1997; Nout and Motarjemi, 1997). Fermentation in food also meansalteration of simple carbohydrate to alcohol and carbondioxide or carbon-based acids by means ofbacteria and yeast vianon-aerobic circumstances (Williams and Dennis, 2011). Itis astandard method for improvement of organoleptic and protective properties, nutritive quality, decontamination and antibiotics production in foods (Oyewole and Isah, 2012).Itoffers low-costprocess of producing and conserving food. It improvesnutritive and healthinessfood value. Theprocedure is widely practice in Africaat industrial and household levels (Mensah, 1997).Conventionally, fermentation is use for preparation of product like beverages by yeast fermentation.Fermentation produced vinegars through*acetobacter,* yogurt,then pickles also preparedby fermentation via*Lactobacilli*(Steinkraus, 1997). Fermentation useswantedresultby*in situ* preparation of preciseuseful bioactive compounds, this could be achieved by removal of undesirable compounds or conversion into desirable compounds (Hugeholtz and Smid, 2001).

Although, weight of micro-organisms is generally small in food, but their impact on nature of food,particularlytaste and other organoleptic propertiesare weighty (Okafor, 2009). Conventional lactic acid fermentation is mostactive and suitable process for dietaryenhancement of cereals (Eneche, 2009). Thisactsasvital role foralleviating antinutrients, rising nutrient concentration and anti-microbial actions. This gives fermented productpleasingtaste, smell,consistencyas a result ofenzymes metabolic actions andraw materialsmicrobiota (Oyarekua, 2013). Fermentation can also be defined as enzyme induced chemical change in foods; these enzymes may be formed by microorganisms and play important role in human growth as oldest method of food conservation (Potter and Hotchkiss, 1998). Fermentation uses microorganisms for transformation of raw materials into valuable products. In case fermentation processaltered for improvement of taste andsmell, resultsto improveddiet, maintenance original constituents and anti-nutrients purification

(Beaumont, 2002). Some important conditions for fermentation are substrate, micro flora and

environmental (processing). Substrates for food fermentations can be plant or animal origins; these can lead to the following,non-alcoholic foods, alcoholic drinks, vegetableand animal. Accessibilityto fermentable carbohydrate, organic nitrogen and minerals remainsignificant in food fermentations. Microorganisms added either as starter cultureor as epiphytic micro-flora are very vital in the food fermentations. Micro-organisms in food variedowing to inherent and external factors thatdisturbuseful properties, preparation,consumption,then, storage (Dullon, 2004). Fermentation could increaselegumes phenolic content such as pigeonpea,then, enhance antioxidant activities.

Fermentation can be classified based on raw material (solid or liquid). It can proceed naturally (natural fermentation) or through the starter cultures (pure culture fermentation). Fermented foods have been used upextensively since primitiveeras around the world; manyfoodsown its processing andfeatures to fermentative actions of micro-organisms; example of food products issauerkraut,fermented sausages and cheese (Ojokoh *et al*., 2013; Arimat*et al.,* 2014). Fermentation serves numerous purposesin developing countries; improves food taste, enhancesfood digestibility, preserves fooddeprivationfrom toxic organisms and improvenutritive value (Achi, 2005). It is valuable for increasing shelf life of some fresh foods,aroma production and flavour in food as well as covering of putrid flavours. It is less expensive in the developing countries than anothermeans of food protectionlike canning or cooling. Fermentation, is also, used for medical motives and as food replacements(Anteneh *et al*., 2011).Fermented foodsconstitute significant componentstoAfricannutritions (Oyewole, 1997). Current use of fermentation in food processing stressedpreparation ofhealth benefit foods and betternutritivevalue. It`s presentlyuse in reducing anti-nutrientslike phytate and tannin. Also, usein increasing bioavailability of vital nutrient such as iron (Moneim *et al*., 1995; Towo *et al*., 2006).Fermentation is use to reducenatural toxins occurrence such as cyanide in cassava (Nout and Motarjemi, 1997; Kobawila *et al*., 2005)and to decline non-digestible carbohydrates by reducingundesirable effects like abdominal distention and flatulence. Lactic acid bacteria and yeast accountablein fermentations (Adeleke *et al*., 2010; Adenike *et al.*, 2007),also, these microorganismscontrolfoodfermentation (Guasch-Jane*et al*., 2006; Robert and William, 2008).Spontaneous fermentation suitable in influencing nutrient density, microbial activities,raw material enzymatic activities andthis leads to enhancement of flavourand texture of product.

Table 2.4: SomeTraditional Nigerian Fermented Foods

Fermented Food	Raw material (Substrate)	Micro-organisms	Uses
Gari	Cassava pulp	*Leuconostoc sp.* *Streptococcus sp.* *Corynebacterium manihot* *Geotricum candida*	Key meal
Fufu	Whole cassava roots	*Lactobacillus sp.* *Leuconostoc sp.*	Meal
Ogi	Maize, sorghum, millet	*Lactobacillus plantarum* *Streptococcus lactis* *Saccharomyces cerevisiae* *Rhodotorula sp* *Candida mycoderma* *Debaryomyces hansenii*	Breakfast cereal, weaning food
Iru (Dawadawa)	African locust bean (*Parkia biglobosa*) Soybean	*Bacillus subtilis* *B. licheniformis*	Condiment
Ogiri (Ogili)	Melon seed (*Citrulluslanatus*) Fluted pumpkin (*Telfairia occidentalis*) Castor oil seed (*Ricinucommunis*)	*Bacillus spp.* *Escherichia spp.* *Pediococcus sp.*	Condiment
Ugba (Ukpaka)	African oil bean (*Pentaclethramacrophylla*)	*Bacillus licheniformis* *Micrococcus spp*. *Staphylococcus spp*.	Delicacy usually consumed withstockfish or dried fish
Palm wine	Palm salp	*Saccharomycesspp*. Lactic acid bacteria Acetic acid bacteria	Alcoholic drink
Burukutu/Pito/Otika	Sorghum, millet, maize	*Saccharomyces spp*. Lactic acid bacteria	Alcoholic drink
Shekete	Maize	*Saccharomycesspp*	Alcoholic drink
Agadagidi	Plantain	*Saccharomycesspp*.	Alcoholic drink

Source: Aworh (2008)

2.12.1Fermentation Methods

2.12.1.1Liquid Substrate or Submerged Fermentation (LSF)

Thistechniqueis appropriatefor microorganism like bacteria whichneeds moisture. Bioactive compounds discharged into fermentation broth and make use of freeliquid substrates like molasses and broths.Submerged fermentation is the growth of micro-organisms in fully liquid system (FAO, 1992). Submerged fermentationmostlyuses for extraction of secondary metabolites that require liquid. The substrates are utilized rapidly; hence constant replacement with nutrients are required.Purification of products are easierusing this technique and is an advantage to other type of fermentation(Subramaniyam and Vimala, 2012).

2.12.1.2 Solid State Fermentation (SSF)

SSFmeansgrowingof microorganisms under preciseenvironmentswithout permitted water for preparation of wantedcrops (Pandey, 1992). This can further describe as bioprocess donewithoutwater but viasolid matrix of high-water adsorption.Solid matrix might be bio or inert, butboth conditionsmust haveadequatewetnesstoward sustaingrowing(Singhania *et al*.,2009).SSF suited for fermentation methods involve fungi and micro-organisms with smaller amountmoistness. However, SSF not suitable for fermentation procedureslinking organisms(bacteria)with higherwater- activities(Babu and Satyanarayana, 1996).Examples are industrial enzymes, fuels andenriched animal feeds.

Table 2.5: EvaluationamongSubstrates

FACTOR	Liquid Substrates	Solid Substrates
Substrates	Soluble	Unsolvable Starch, Cellulose, Pectins, Lignin
Aseptic conditions	Uninfected control	Vapour treatment, non-sterile condition
Water	Large volume and Effluent	Inadequatewater;little activity Effluent absence
Metabolic Heating	Relaxed temperature control	Smallvolume heat transfer Calmventilation and surface exchange
Ventilation	Solvable oxygen restriction Suitable air essential.	
pH	Easy control	Shielded solid substrates
Mechanical agitation	Adequatemixing	Stationarysituations preffered
Scale up	Industrial equipment accessible	New designequipment necessary
Inoculation	Calm inoculation nonstop	Spore inoculation, batch
Contamination	Risks for single strainbacteria	Risks for fungi at low rate
Energetic consideration	High intense	Smallintense
Volume of Equipment	High and expensiveequipment	Small and expensiveequipment
Sewage and pollution	High pollutingsewages	Absencesewages, minor pollutant

Source: Raimbault, 1998

Table 2.6: Merits and Demerits of Submerged Substrate Fermentation

Advantages	Disadvantages
Measure of process parameters is easier than solids	High cost due to the expensive media
Bacteria and yeastconsistentlyspreadall over the medium	Expenses for equipment are higher
High-water content for bacteria	Consumptions of energy are higher
Inoculum portionis generally small	The procedure is very delicate
Lower asset costs	Agitation is regularlyimportant
Better process control	Accidentalpollution
Reduced fermentation period	
Decreased floor space supplies	
Purification of products is easier	
Lesseremployment costs	
Simpler processes	
Easier upkeep of aseptic situations on industrial measure	

Source: Subramaniyam and Vimala (2012)

2.13FermentationSubstrates

Fermentationsubstrateextremelydiffers from one another;therefore, it is vital to select right

substrate. Fermentation methodsneedenhancement for separate substrate, because organisms react in a different way to substrate. Consumption of nutrients vary in each substrate, and also productivity. Notablesubstrates for submerged fermentation include sugars, syrup, liquid materials,sewage/wastewater, fruits and vegetables juiceswhile that ofSSF include wheat bran, hay,paper pulp, bagasse, coconut coir, rice straw,artificial media, fruit and vegetable wastes (Pandey *et al*., 1999).

2.14Organisms responsible for food fermentations

The greatest group of microorganisms involved include;

Bacteria
Yeasts
Moulds

2.14.1 Bacteria

They belong to bigcluster of single-celled or multicellular organismsthroughabsenceof chlorophyll, availability of simple nucleus and reproducingfast by simple fission. Some are spherical, rodlike, spiral or filamentous (Walker, 1988).The vital bacterianecessary infermentations include lactobacillaceae,they can generate lactic acid from carbohydrates. Additionalsignificantorganismforfruits and vegetables fermentationsis*acetobacter* species acetic acid manufacturingacetic acid.

2.14.2 Yeast

They are unicellular organisms replicate asexually viabudding. Commonly, yeasts are bigger than bacteria and they performedimperativepart in food business.Yeasts made available enzymes that help inneeded reactions such as bread leavening, production of alcohol then invert sugar.They have valuable and non-valuable effects in foods and are broadlydispersed in species. Yeasts remain presentinair, soil,intestinal tract of animals, orchards and vineyards.Useful yeastdesirable for food fermentations are from *Saccharomyces* family. Example is *Saccharomyce cerevisiae*.

2.14.3Moulds

They arevitalmicroorganisms in food industries responsible for preservation and spoilage.Some

mould manufacturesunwantedcontaminants, then add to food spoilage.*Aspergillus* species are regularlyanswerabletoobjectionablevariations in foods. They originate in dietsoften andpermit high absorptions ofsugar and salt. Nevertheless, some mouldstransmit flavouressential quality to foods and produceamylaseenzyme for bread making. Moulds from genus*Penicillium*linked toripening and cheesesaroma. They have highestcollection ofenzymes and mightinhabit, thenbreed on different kinds of foods(Mountney and Gould, 1988).

2.15Conditions for Bacterial Fermentations

There are six requirements that are essentials for bacteria fermentation(Steinkraus, 1996;Mountney and Gould, 1988).

2.15.1 Temperature

Bacteria acceptdiversetemperatures thatdeliversenormouschoice of fermentation.Some bacteria performbestat 20 to 30ºC, while thermophilesdesireadvancedat 50 to 55ºCand colder optima temperaturebetween 15 to 20ºC. Lactic acid bacteria performed at 18 to 22ºC, for example*Leuconostoc* species,thatresponsible for fermentation hasideal temperaturebetween 18 to 22ºC while temperaturehigher than 22ºC suitable for*lactobacillus* species.

2.15.2 Concentration of Salt

Lactic acid bacteria stand higher salt concentrations and thesegivebenefit over other less accepting species. Thistolerates fermenters to startbreakdown thatmanufactures acid, thenhinders growth of unwantedbacteria. Leuconostoc well-known for higher salt acceptance,alsoresponsible forgreater number of lactic acid fermentation.

2.15.3 Water activity

The quantity of obtainable waterforbacteriadenoted wateractivity(a_w). Usually, bacteria needequitablyhigher water activity (0.9 or more) to live.Fewspecies can bear water activitylesser,typically, fungi and yeastdominate with minor water activities.

2.15.4 pH

pH of a substrate measuresthe degree of acidity.Best pH for somebacteria is close to neutral

point (7.0) and there some bacteria that are acid tolerant,then ready tolive at decreasedpH. Prominent acidtolerant organisms include *Lactobacillus*, *Streptococcus*species, and they are important in dairy and vegetables fermentation.

2.15.5Oxygen

Some fermentative organisms are anaerobes, although someneedair for breakdown.*Lactobacilli*are microaerophilicprecisely; they manufacture inoccurrence of lessenquantities of oxygen. Aerobic fermentations, oxygen volume is limiting factors. Thisdecideskind, thenquantity of organic productsattained,quantity of substrate used upand energydischarged from reaction.

2.15.6Nutrients

Bacteria require nutrients for breaking down of substances and differ in their specificity todiverse substrates.The bacteria for fermentation needstarches,either simple sugars, for example glucose, fructose or compoundsugarslike starch or cellulose. Energy necessitiesforbacteriaishuge and restrainingsubstratequantityobtainable toinvestigate development.

2.16 Lactic Acid Bacteria

LAB comprisesorganismsunited throughphysical, morphological, and metabolicfeaturesassemblage. Bacteria can be grouped into not producing spores, not respiringgram-positive rods that generateacid as keyproduceincarbohydrates fermentation. Some group of *Lactobacillus*categorizedas non-spore forming,facultative anaerobes (Batt, 2000).LABhad been eateninside several fermented productslike dairy foods. Lactic acid bacteriacatch attention of international research for crucialpart in most fermented diets,capability to makeseveral antimicrobial compounds promote probiotic possessions(Temmerman*et al*., 2003).Lactic acid bacteria remainmicro-organisms that controlfood fermentations (Robert and William, 2008; Guasch-Jané *et al*., 2006). They are essential because of their role in mostfood industries in place of starter cultures. Metabolic and enzymatic actions of LAB produce volatile substances, these lead toflavour and texture improvements (Kleerebezemab *et al*. 2000). LABis food grade retaining,recognised-as-safe (GRAS) position,also coulddischarge exopolysaccharide (EPS).Exopolysaccharide, economically essentialsincetheyconveypurposefulresultin

foods,alsousefulhealthiness effect to end user (Welman and Maddox, 2003).LABis discriminating, non-sporulating,acid tolerant,cytochromedevoid and not-respiring rod gram-positive.Theyrelatedthrough metabolism and physicalfeatureswhichcreate acid asmain metabolism produce(Holzapfel *et al*., 2001). Lactic bacteria, also among significantmicrobes in food fermentations; the bacteria enhance taste and quality of fermented produce. Also,hinder spoilage organismsviacreating inhibiting constituents,thenhugequantities of acid. Human diets caneither beplants or animal origin fermenting through lactic acid bacteria, meanwhile, bacteria have properties thatcan be of benefit to food processing or alteration. LAB had used for food and feed fermentation since prehistoric days, and utilisationsstillon-going in food and feed industriesin place of starter cultures (Boonmee *et al*. 2003).As fermentation agents, LAB mostly used in manufacture of fermsented products like cheeses (*Lactococcus spp*.), yoghurt (*Streptococcus spp.*, *Lactobacillus sp*),sauerkraut (*Leuconostoc sp*.), sausages, refined butter,sour cream, vegetables and meats (Arimah *et al*., 2014). Occurrence of LAB in food and feedwith their longer shelf lives, complementsas generally recognisedas safe aimedatingesting (Aguirre and Collins, 2008). They establishsignificant group of organisms, mainly infood processing industry. Keyfunction of LAB isto metabolize glucose, fructose and citrate to produce acids(lactic, acetic), ethanol and mannitol. LAB has potentials as food additives and usefulingredients forwellbeing and economic profits (Welman and Maddox, 2003).

LAB remainscriticalbecause of their role in most fermented food industries as starter culture.Fermented dairy products are enjoyingacceptance increase as suitable, nourishing, natural and healthy foods (Kalliomäki *et al*., 2001). Metabolite by LABhaveseveralmanufacturingclaimsin food, textile, pharmaceutical industries as preservative, acidifying agent and flavour.They can be usefulfor acid-acetaldehydeproduction (Åkerberg and Zacchi, 2000). Lactic Acid bacteria producesdiversity of compounds like formic acid, acetone,ethanol, hydrogen peroxide, bacteriocins and diacetyl as antimicrobial. These discussprotectivecapacity as natural cheap wayin overcomingmicroorganisms'distributionsame niche (Oliveira *et al.,* 2008).Capability of LABloweringpH offermented foods lead to spoilage hang-up andthereforeextend shelf-life. LAB contribute to production ofacid (acetic, lactic, carbonic) and protectionthroughcreation of massivecollection of antimicrobial and proteins (Elliason and Tatini, 1999).Lactic acid bacteria and theirproducts act as bio-preservatives to increase foodsshelf-life(Ayad*et al*., 2004) and reduce risks of foodborne diseases (Konings *et al*.,

2003). Hence, presence of LAB might confer necessarypotentials and escalatefermented products safety (De martinis *et al*., 2002).LAB could produce anti-microbial constituents such assweeteners, complex sugar,pungent compounds, vitamins and valuable enzymes with probiotic characteristicswhich mightbe of help in promotion of food industries. Probiotics recommended for patients getting radiation treatment, those withrepeated thrush, vaginal or urinaryinfections. Also, people suffering in irritable bowel syndrome and travellers,guardfood murdering. Some LAB species characterized throughlactose transformation,enhanced fermented dairy productsdigestibility and their preservations (Abdel basset and Djamila, 2008;Weinberg *et al*., 2007).This ability of LAB led to its usage as starter culture in manyfermentations processbecause it protects the lifespan of itsmany foods by inhibiting the growth of other harmful pathogens and also help in maintaining palatability of the food (Jeevaratnam, *et al*. 2005).

2.16.1 LAB Classification

LAB has classified on acid production into different genera/species throughfermenting sugars at specific temperatures(Schleifer *et al*, 1995; Parvez, *et al*., 2006). Conventional method of LAB classification was built on physical andchemical characteristics, in recent times, molecular characterization has become important deviceinproofing LAB identity. Characterization includes: 16S rRNA sequencing, amplified DNA profiling, PCR-based fingerprinting, Soluble protein patterns (Salminen, *et al*.,1998)and species differentiation via multiple PCR assay by means ofprecise recA derived primer (Torriani,*et al*., 2001).

2.16.1.1Homofermentative and Heterofermentative

The LAB isdivided into heterofermentative and homofermentative organismsbased oncapability of fermenting carbohydrates (Kuipers, *et al*.,2000). Homofermentative bacteria;*Streptococcus,Lactococcus*produce lactate from glucosewhile LABlike*Leuconostoc*, *Wiessella,lactobacilli*create lactate, ethanol and carbon dioxide from glucose (Salminen,*et al*.,1998)

2.16.2 Taxonomical Classification

Recently taxonomic classification of LAB comes under *Phylum* of *Firmicutes*, class *Bacilli*and order Lactobacillales which comprised of various different genera but major ones are*Lactobacillus,Enterococcus,Melissococcus, and Vagococcus* (Fig.1).However, largest genus of this group is the *Lactobacillus* and it consists of more than 80 recognized species. Examples are *Lactobacillus plantarum,acidophilus, Lactococcus cremoris, Bifidobacterium bifidum, Lactobacillus Casei,rhamnosus, delbrueckii, bulgaricus, fermentum,reuteri, Lactococcus lactis, Bifidobacterium infantis, Bifidobacterium breve, Enterococcus faecalis, Enterococcus faecium, Bifidobacterium adolecentis*, and *Bifidobacterium longum* (Canchaya,*et al*., 2006; Salminen,*et al*.,1998).

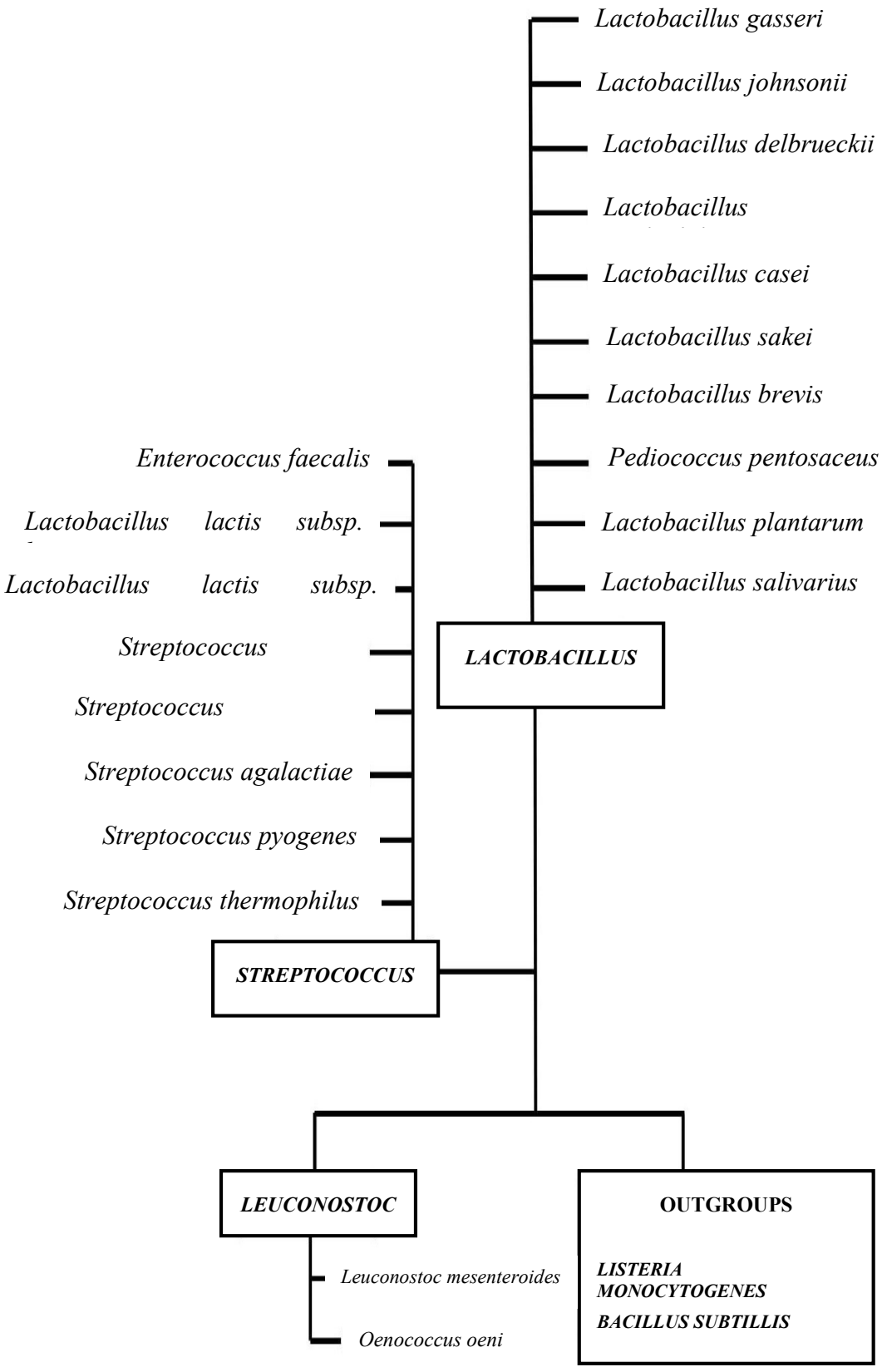


Fig. 2.1. Differentiation of Species according to the recent Taxonomy
Source: Rahul *et al*., 2018

CHAPTER THREE

MATERIALS AND METHODS

3.1 MaterialSources

Maturedhealthy breadfruits (*Artocarpus communis*) were procured from breadfruit farmer at Ile-Ife, Osun State. Brown pigeon-peawas obtained from a farm in Ago-Aare, Oke-ogun area of Oyo-State. Additional ingredients andcomponentsbought from Inqaba Biotechnology outlet at IITA, Ibadan, Oyo State.

3.2 Methods

3.2.1 Fermented Breadfruit Production

Newly harvested matured breadfruits were washedby means of tap water, peeled and sliced manually with stainless knives,non-essential matters were removed (Awoyemi, 2012) with some modifications. Breadfruitslices were put inside the tap water on ratio of 2:1 (w/v) inside low density transparent bucket and covered for 24, 48, 72, 96 and 120 h. Breadfruit slices were allowed to ferment spontaneously at28±2^{o}C (ambient temp.) and 37±1^{o}C (Model No. KJ-9022A Incubator). However, on completion of each fermentation period, water discarded. The pulp wasdrained and dried inside cabinet dryer for 17 h at 55^{o}C.Dried pulpwas grinded into flour and siftedthrough 0.25 mm (Model BS 410, Endecotts, Limited, U.K), British standard sieve(Akusu and Wordu, 2016).Breadfruit flour waspackaged inside low density polyethylene materials for advance use (Fig. 3.1).

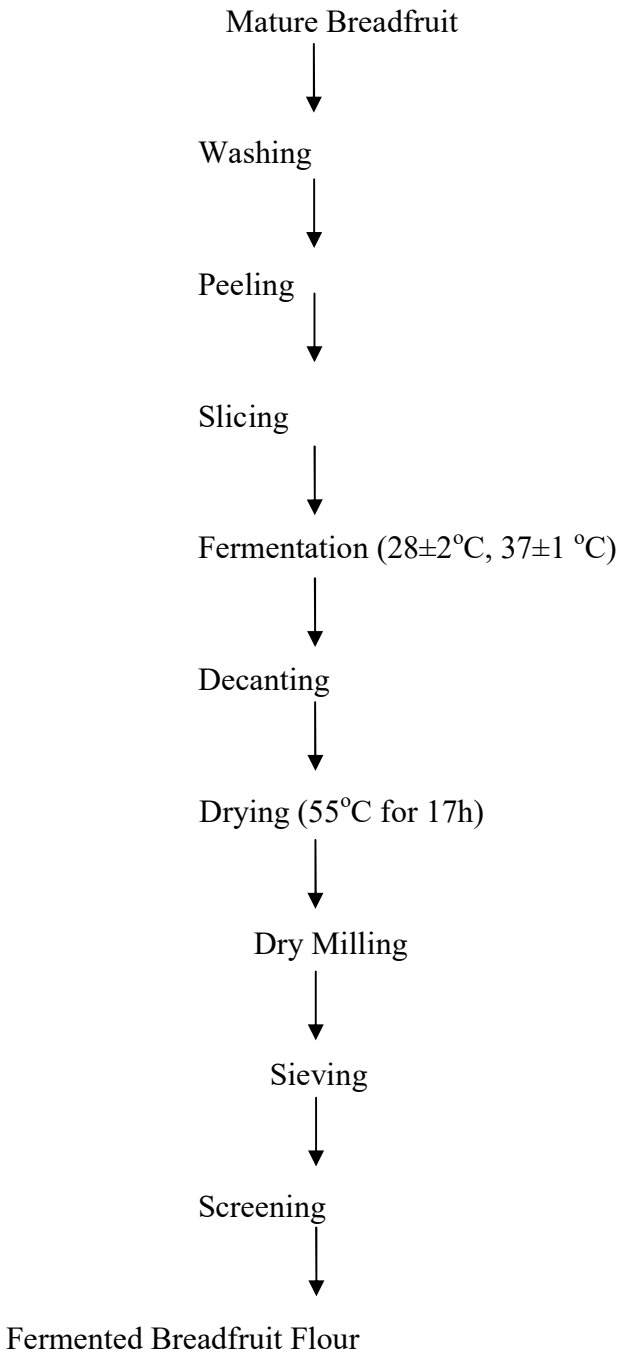


Fig. 3.1: Production of Fermented Breadfruit Flour

Source: Awoyemi (2012)

3.2.2 Production of Breadfruit Flour (Unfermented)

Breadfruit flour was obtained through technique described (Ajani*et al*.,2012). Healthy breadfruit waswashed carefully, peeled and sliced (1cm thick)usingstainless-steel knives.Washed, sliced, breadfruit soaked in 5% sodium metabisulphite solution toinhibit enzymatic browning. Sulphited chips blanched inside water bath (Clifton) for 5minanddehydrated for 16 h at 55^{o}C by a cabinet dryer. The dried-up chips were pulverizedviahammer mill and sieved through 0.25 mm (Model BS 410 Endecotts, Limited, U.K), British standard sieve (Akusu and Wordu, 2016). Quality flour was packaged inside thick (0.04mm) low density polyethylene materials forfurther use (Fig. 3.2).

3.3 Fermented Pigeon-pea FlourProduction

Fermented pigeon-pea produced using Echendu *et al*. (2004) method with little modifications. Wholesome light brownish pigeon-pea seeds hand-picked and washedusing tap water. Cleaned pigeon-pea seeds were poured inside the tap water at ratio 2:1 (w/v) using transparent covered buckets for 24, 48, 72, 96 and 120h respectively. These ferments spontaneously at28±2^{o}C (ambient temp.) and 37±1^{o}C (Model No. KJ- 9022A Incubator). As the fermentation periodis terminated, the fermented pigeon-pea seeds were dehulledusing pestle and mortal. Dehulled seeds then washed, drained and dried inside cabinet dryer for 8 hat 55^{o}C. Dried pigeon-pea seeds were milled and sifted through 0.25mm(Model BS 410, Endecotts, Limited, U.K), British Standard Sieve (Akusuand Wordu, 2016).Samples werepacked inside thick gauge (0.04mm) low density polyethylene forusage (fig. 3.3).

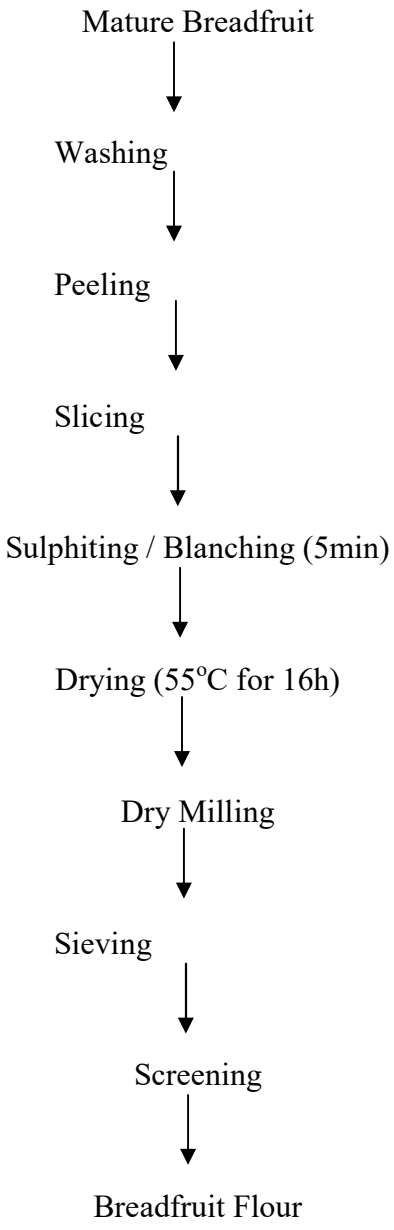


Fig. 3.2: Breadfruit FlourProduction

Source: Ajani *et al*.(2012).

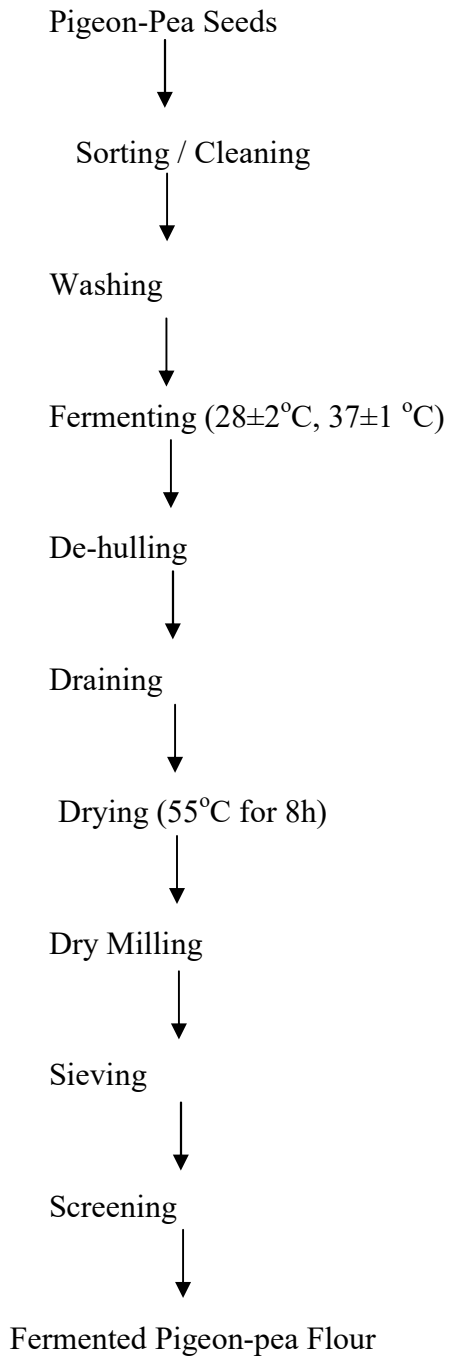


Fig. 3.3: Fermented Pigeon-pea FlourProduction

Source:Echendu *et al*.,(2004)

3.4 Breadfruit-Pigeon-Pea Breakfast Meal Formulation

Breadfruit-pigeon-pea meals were prepared from the followingmaterials; breadfruit: pigeon-pea flours ratios(100:0, 90:10, 80:20, 70:30, 60:40 and 50:50). The breakfast meal was formulated from 24h fermented breadfruit and pigeon-pea flour at 28±2°Cand 37±1^{o}Crespectively. All the (breadfruit flour, pigeon-pea flour, egg yolk, sunset yellow, glucose, salt, potable water) were weighed and mixed properly (Table 3.1). The soft paste was poured inside boiled water (100^{o}C),thenagitated briskly with turner till mixturecooked. Powdered milk; vanilla flavor, condensed milk flavor and sugarwere added to balance the taste using the method ofTai Situ et *al*. (2009) with littleamendments.

3.5 Formulation of Breadfruit-Pigeon-pea Pizzelle Cookies

The pizzelle cookie samples were prepared from theseratios of breadfruit flour: pigeon-pea flour (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50). The cookieswere formulated from 24hfermented floursat 28±2°Cand 37±1^{o}C. Breadfruit flour, pigeon-pea flour, salt were measured and mixed together in a medium mixingbowl. Beaten eggs were added to melted butter, then, chocolate powder, sugar and vanilla were mixed together carefully to form stiff dough. Dough was then dropped inside Salton pizzelle maker (Model WM-6, made in China) and closed for 1min at 125^{o}C to produce dry and firm cookie (Okpala and Chinyelu, 2011) with little modifications. The recipe is as showed in Table 3.2 whileflow chart showing the productionprocedure as shown in Figure 3.4

Table 3.1: Ingredients for Production of Breadfruit-Pigeon-pea Breakfast Meal

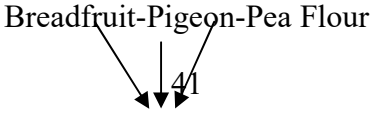
Ingredients	Quantity
Composite flour	100g
Sugar	40g
Powdered Milk	40g
Vanilla Flavour	2 ml
Salt	0.1g
Glucose	0.2ml
Egg Yolk	0.2ml
Sunset Yellow	0.1g
Condensed milk flavor	5 g
Water	90-95 ml

Source: Tai Situ *et al*. (2009)

Table 3.2: Ingredients for Breadfruit-Pigeon-pea Pizzelle Cookie

Ingredients	Quantity
Composite flour	100g
Salt	3g
Egg	54ml
Butter	30g
Chocolate powder	20g
Sugar	90g
Vanilla Flavour	5ml

Source: Okpala and Chinyelu(2011)



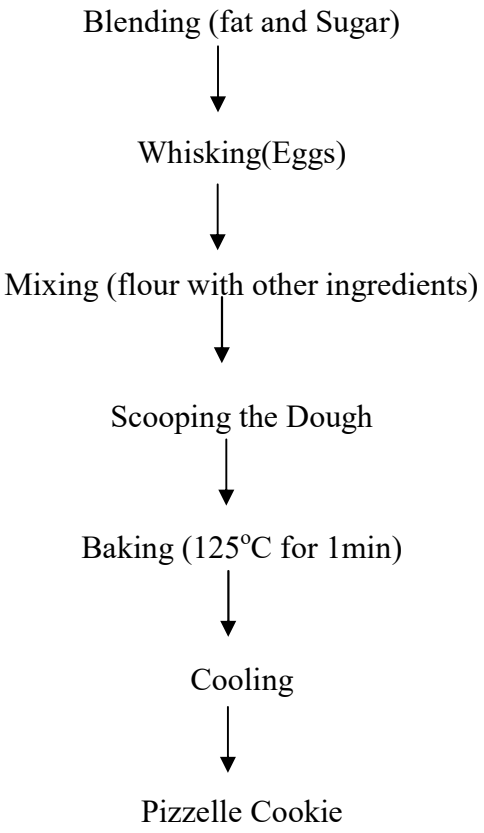


Fig. 3.4: Production of Pizzelle Cookie

Source:Okpala and Chinyelu(2011)

3.6 Flour ChemicalComposition

3.6.1 Moisture

Moisture contentdetermined viatechniquedesignatedthroughAOAC(2012). 5g each of samples were measured insidepreviouslyweighed and clean dehydratedpetric dishes. The weighed samples were placed inside ventilatedoven (Fisher Scientific Co.USA, Model 655F)at 105^{o}C. Later at 6h, samples were moved to cool inside desiccator and final weights were taken. Moisture content determined by equation (1)

Calculation involved

Percent moisture (MC) = $\frac{w_1 - w_2}{w_1 - w_0}$ x 100% ---------- (1)

% dry matter = 100 –MC

w_1= Dish + initial sample weight

w_2 = Dish +final sample weight

w_0 = Initialdish weight

3.6.2 Protein determination

Protein content determined throughtitrimetric techniqueusing AOAC (2005). About 0.20g of the flour sample was put inside tubes for digestion. Selenium tablet(catalyst),then 10 ml H_2SO_4solution waspoured inside the tube each.Digestion was doneby means of kjeldahl-digesting methodtill samples became clear. Digested samples permitted to cool,thenwatered downthroughpurifiedliquid. NaOH solutionwas added to the samples and later dispensedinto 25 ml mixed indicatorflasks (14% boric acid and bromocresol indicator). The sampletitratedopposed to 0.01 N HCl solutions. Titrationinside empty sample equally determined and the percent protein content was assessed.

3.6.3 Fat determination

Fatdetermination carried out by means of AOAC (2000) protocol: One gram of each flour sample put inside free extraction cap,thensealed. Thimble located inside extractor and fitted with reflux condenser. 250ml soxhlet flask which has earlier dry was allowed tocool inside desiccator andevaluated. Soxhlet flask packedup to ¾ volume of ether (b.pt.40^{o}C-60^{o}C), then extractor and condenser positioned on boiler. Heater wasworking continously using runningwaterfor 6 h. Ether escapes continually observed and heater adjusted suitably for boil mildly.

Ether left to drain offforsome times (10-12 min.) until short siphoning. After which, ether in the extractor cautiously drained. Thimblewith the sample removed,thendryon glass clockat bench top. Extractor and condenserwere exchanged,then purification continued till flask dried. Flask with fatseparated, extractor cleansed and dry toconstant weight in an oven. Dry soxhlet flaskpreliminary weight was regarded as W_1,then the finaldried flask weight and oil/fat (W_2), percent fat/oil gotby equation (2).

% Crude Fat (ether extract)=$\frac{W_2-W_1}{s_w}$ x 100 ---------- (2)

w_1 = Flask weight

w_2 = Flask and oil weight

s_w = Sample weight

3.6.4 Ash determination

2g sample was preciselyevaluated into pre-ignited andporcelain crucible measured beforehand, sited inside muffle furnace (Gallenkamp, England), thenkindled for 2 hat 600^{o}C. Later,crucibles cooled after ashing below 200^{o}C insideheater for 20 m, thencool further to room temperature inside desiccator. Crucibles and content weighed, the ash was reported as percentage ash content, as shown in equation 3 (AOAC, 2002).

Calculation:

Ash content (%) = $\frac{w_2-w_0}{w_1-w_0}\ x\ 100$ ---------- (3)

w_0= Crucibleweight

w_1 = crucible and sample weight before incineration

w_2 = Crucible + sample weight after incineration

3.6.5 Fibre determination

Fibre content was determinedby means of AOAC (2006) technique: the sample (2g) weighed into fibre flash then 200ml of H_2SO_4 added from hot 1.25% into the mixture. The pre-heated digester apparatus placed on beaker,thensamplesimmered and refluxed for 30min.Sample sievedvia Whatman GF/A paper by means ofpurified water till filtrate neutral. Filtraterelocated from Whatman GF/A paper into beaker with aid of 1.25% hot NaOH thatbring to better state of 200mls. Beaker thenpay backto digestion apparatus,boiled, refluxed for 30 min., filtered andrinsed. Whatman GF/A paper movedfiltrateinto crucible,then dryover night at 100^{o}C.Sample wasallowed to cool inside dessicator and weighed (weight A). Sample placed inside furnace for 6 h at 600^{o}C,cool in dessicator and re-weighed (weight B). Loss in weight throughburningsignifies crude fibre weight.

$$\% \text{ Fibre } = \frac{Weigh\ \ A - Weight\ B}{Sample\ weight(g)} \times 100 \qquad \text{----------} \qquad (4)$$

3.6.6 Carbohydrate determination

Sample carbohydrate carried outvia difference,through subtracting sum of values obtained from analysis from 100 (James, 1995).

% CHO=100 –(Protein+ Fat+ Ash + Fibre + Moisture)

3.6.7pHdetermination

Samples measured with pHmeter each day through means of the methoddesignatedby AOAC, 2005. pH meter was switch on and permitted for 5 minto warm up before standardizationvia pH 4 and pH 7 buffer solutions to ensure sensitivity and accuracy of the measurement. pH was measured through dipping meter electrode into each buffer solution with thorough rinsing with distilled water. The values of samples (initially prepared through dissolving 10g dry sample into 10 ml distilled water) were taken separately by dipping the pH meter electrode into sampleswater, then rinsed thoroughly with distilled water after each dip. Then, valuesread out from the display unitaccordingly.

3.6.8Total Titratable Acidity

Acidity determinedthrough titration method. 10 ml aliquot sample was titratedalongside0.1M NaOHby means ofPhenolphthalein indicator described by AOAC (2005).

3.7 Functional Properties

3.7.1 Bulk Density

Techniquereferred tovia Oladele and Aina (2007)adoptedin bulk density determination.50 g sample placed inside 100 ml measuring cylinder.This cylinderbeaten repeatedly on laboratory table tillpersistentcapacityachieved.

Density calculated as below:

$$\text{Density (g/ml or g/cm}^3\text{)} = \frac{\text{weight of sample}}{\text{volume of sample after tapping}} \qquad (5)$$

3.7.2 Water Absorption Capacity

Absorptionresolved at 37 °C, then 60 - 90°C by combination of AACC (1995), Rutkowski and Kozlowska (1981) and Sosulski (1962). 2 gsample spread insidedistilled water, thenhomogenised every 30 svia glass rod. Centrifuging was done at 4000 x g for 20 minutesafter mixing for five times. The supernatantcautiouslypoured, thenthe pellets of tube were permittedat 45anglesaimed at 10 m to drain and weigh. WAC stated as percentage riseof sample weight.

3.7.3 Oil Absorption Capacity

SamplesOACassessedvia Eke and Akobundu (1993) technique. 1g of sample (M_0) blended with 10 ml vegetable oil via 20 ml separator tube. The liquid mixtureblendedinsideblender for 2 minutes,permittedfor 30 min at 28°C to stayandlaterseparatedon 4500 rpm.Supernatantemptied,alsothrew away;thenobserving oil dripsdetached and tube weighed (M_1). Oil absorption capacity determinedaccording to the following equation:

$$OAC\ (\%)\ =\ \frac{M_0 - M_1}{M_0} \times\ 100 \qquad \text{---------} (6)$$

3.7.4 Foaming capacity and stability determination

Foaming ofsamples determined through Coffman and Garcia (1977) method. 3g sample put insidedry,clean, graduated measuringcylinder. 30 ml distilled waterpoured to each softly levelled sample,thenvolumes noted; the cylinder spun andstand for 120 min whereasvolume variations documentedafter 10 min. Foaming capacity and stabilityvalues enumerated as follows:

$$FC(\%) = \frac{V_t - V_0}{V_0} \times\ 100 \qquad \text{---------} (7)$$

$$FS(\%) = \frac{FC}{FC_0} \times\ 100 \qquad \text{---------} (8)$$

V_0 (ml) = original sample volume, V_t= total volume after trials

FC_0= foaming capacity at zero minute

3.7.5 Gelation Capacity

Samples gelation capacity doneviaCoffman and Garcia (1977) procedure throughminormodification. Suitablesuspension 2, 4, 6, 8, 10, 12, 14 16 and 20% w/v set insidepurified water (5ml). Test tubescomprising suspensionsboiled for 1hr inside water bath (Gallenkamp). Tubes

with contents cool at 4°C,then gelation capacitycalculated as absorption when sample upturneddo not drop.

3.8 Pasting properties determination of the Flour Sample

Pasting properties of the samples were determined viaa Rapid visco analyser (RVA). The samplemoisture content determined to acquire preciseweight of sample and water volume necessary for test.Flour sample (2.5g) was mixed with distilled water in acanister fitted into the rapid visco analyzer. The slurry was heated to50-95°C with holding of 2 minutes. This was followed by cooling to 50°C,holding for another 2 minutes before reading the various values measured on a computer (AOAC, 2006).

3.9 Anti-nutritional FactorsDetermination

3.9.1 Phenolic

The phenol assessedby means of Folin-Ciocalteau reagentassay (McDonald *et al*., 2001) byminoramendment. Calibration setthrough mixing solution of gallic acidwithFolin – Ciocalteau,thenNa_2CO_3.Mixture permitted to stayfor 30 minat 20^{o}C, thencolour developed through absorbance anddocumented at 765 nm via UV-VIS spectrophotometer. One millilitre of each of extract solution in methanol blended with above reagents andabsorbance determined phenolicafter 30 min.Phenol acquired from equation: y = 0.00048x + 0.0055, then gallic acid equivalent via formula ; $T = \frac{cV}{M}$; where T = total phenolic , C = concentration of gallic acid recognisedafter calibrated , V = extract volume and M = sample extract (0.052g).

3.9.2 Flavonoid

Colorimetric method used to prepare flavonoid of flour samples (Nguyen and Eun, 2011). Solution extract of 0.25mlflour samplepouredinside purifiedH_2O (1.25 ml), 0.075 ml sodium nitrite poured into mixture,then incubated for 5 minwith additional10% aluminium chloride (0.15 ml).Mixture permitted at ambient temperaturefor 6min to stay,1M NaoH (0.5ml)poured, thenblendedvia distilled water (0.275 ml). Mixturequantifiedusing spectrophotometer instantlyat 510 nm. Quercetin adopted for calibration. Flavonoidstated as;

$$conc\left(\frac{ug}{ml}\right) \times vol. \times \frac{df}{wt\ of\ sample} \quad \text{----------} \quad (9)$$

3.9.3 Phytate

Phytate extraction carried out from sample using modified technique of Harland and Oberleas (1977).Thisstandard methoddepends onalteration of free phytic acid and liberated organic phosphorus viacolorimetric measurement. A 2g sample take out 40 ml of 2.4% HClbelow constant shaky at 25^{o}Cused for 3 h. Thenextracts sievedby Whatman paper(No. 1). It was evaluatedthrough spectrophotometric with absorbancewavelengthof 640 nm as sketched in AOAC (2005). Phytic evaluated fromorganic phosphorus throughpresumptuousofa molecule phytic comprising6moleculesphosphorus andprocessedby way ofequationunderneath (AOAC, 2005).

Phytate (mg/g) = $conc\left(\frac{ug}{ml}\right) \times vol. \times \frac{df}{wt\ of\ sample}$ ---------- (10

3.9.4 Tannin

Tannin determinedusing quantitative technique as described in food quality control manual (AOAC, 2005). A 0.5g sample measured inside conical flask,thenmixed with 10ml (80% ethanol). Mixture shaken and permitted to stay for 1 h, also,1ml extract transferred intoanother tube using pipette. Then,5ml purified water, two drops Fecl, 0.1M Hclincluded and mixed properly. Also, 4 droplets potassium ferrocyanideincluded, then absorbanceread at 620nmvia spectrophotometer.

3.9.5 Oxalate

1 g sample measured into 1000 ml conical flask. H_2SO_4 (0.75 M) pouredand solutionprudently stirred occasionally with stirrer for 1h and mixturesievedvia filter paper. Sample filtrate collected, thenanalysedat (80-90°C) using 0.1M $KMnO_4$till colourpink appeared and persevered in 30 seconds (AOAC, 2012).

3.9.6 Hydrogen cyanide

Hydrogen cyanide done withalkaline picric colorimetric procedure by Balogopalan *et al.,*(1998) and Onwuka (2005). A 5g of sample dispersed inside 50cm^{3}purified water (1:10 w/v),thenpermitted staying overnight onambient temperature. Sample sieved and extract used for analysis. A portion (1 cm^3) extractmixed with 4 cm^3 of alkaline picrate solution and boiled for 5 min with water bath. Absorbance of developed reddish brownread via UV spectrophotometer at 490nm wave length. Cyanide solution (KCN)standard treated as explained above and read off in spectrophotometer. Before each reading, blankreagentdisplayed zeroon instrument. HCN determinedvia formula as stated in equation 11.

Calculation

$$\text{HCN (mg/l)} = \frac{(1000)}{W} \times \frac{au}{as} \times \frac{0.5m \ \ /l}{10(au/as)} \qquad \text{----------} \qquad (11)$$

W = sampleweight

as = absorbance standard

au = sampleabsorbance

c = concentration standard (g/cm^3)

3.9.7 Alkaloids

20 ml of 10% acetic acid pouredinside 5g flour and kept for 4 hr. Samplesieved,then fitratethickenedvia evaporation usingwater bath filled to¼ original volume. Drop of conc.NH_4OHpouredinside extract until precipitation completed. Completedaqueous solution allowed to calm down and precipitate collected. Precipitate wash awaythrough dilute NH_4OHthensieved, and filter paper left to dry in the oven at 60^{o}C. The residue (alkaloid) dried and weighed. Sample weight (W), Filter paper(W_1),paper weightwhen it dries to constant weight (W_2) Harbone (1998).

$$\% \text{ Alkaloids} = \frac{w_2 - w_1}{W} \times 100 \qquad \text{----------} \qquad (12)$$

3.9.8 Saponin

Protocol used for quantification of saponinimprovedthrough Obadoni and Ochuko (2001). A 20 g sample dispensedinside glass beakercontained 200 ml ethanol (20%). The mixture heated at 55^{o}C for 4-5 hinside the water bath with constant shaking. Suspension was sieved and extracted again throughfiltrate using 200 ml ethanol (20%). Collected extract evaporatedthrough heating at 90^{o}C. The thickened extract inside separating funnel transferred, then diethyl ether (20ml)poured slowly and strirred. Ether and liquid layer obtained;solventtransfer to another flask while ether discarded. Cleansing process recurrent twice,then 60 ml n-butanol pouredto resultant concentrate. Purifiedextracts via butanolwashed twicethrough sodium chloride solution (10 ml of 5%). Layer with $NaCl_2$castoff and final extractheat slowly till vanishing occurred. Later,filtratedehydrated inside oven to constant weight,thenpercentage saponinevaluated.

3.10 Microbiological Analyses

3.10.1 Samples Fermentation

50 ml sterile waterwas added to 5g of breadfruit chips and pigeon pea; samples were properly mixed and fermented for 24, 48, 72, 96, 120 h at 28±2°C and 37^{o}C respectively.

3.10.2 Microorganisms Culturing

Samples wereexposed to microbial analyses inorder toobservevibrantvariations ininhabitantsof organisms responsible for fermentation. Harriagan and McCance (1976) procedure engaged. 1gsample aseptically measured with weighing scale and cautiouslyput into 9 ml saline / peptone water. 1 ml suitable dilutions (10^1, 10^2, 10^3) combined with molten agar, andmedia triplicateson plate using pouring method. Subculturing done with Nutrient Agar; MRS agar used to detect lactic acid bacteria,thenhatchedat 35 °C for 48 h using anaerobic containers while total viable count was determined next to incubation. Isolates classificationbased on biochemical and morphological tests as descibedby Holt (1994) and Shen *et al*. (1999).Lactic acid bacteria strains were characterized using techniquessuggested by some authors (Charteris *et al*., 2001:Sharpe, 1979, Harrigan and McCance, 1976).

3.10.3 Lactic Acid Bacteria Sub-culture

Nutrient agar wasused in the experiment and prepared agreeing to producer'sdescription.Weighednutrient agar poured inside the flask filled with measured saline / peptone water and allow the bubbled to dissolve properly before usage. Conical flask shielded by means of cotton wool, enclosed by aluminium foil and mediasterilised for 15 min at 121^{o}C duringautoclaving. Medium allowed cooling before pouring 15.0ml into sterile petri dish for solidification. Sub-culturing was done through streaking on theplates and plateshatched in reversed position at 37^{o}C for 24 h.Uncontaminated bacterial isolatesacquiredthrough sub-culturing and pure coloniesscrutinised cellullar morphology, gram staining, catalase test, oxidase test, sugars (glucose, fructose, sucrose, lactose, maltose, melibiose, rafinose and ribose). Cultures preservedin nutrient glycerol for storage on 4^{o}C (Gerba and Pepper, 2004).

3.10.4 Morphological and Biochemical Characterisation of Bacteria

Cultural characteristics (colour, shape and size) on the growing medium and cellular morphology were doneby means ofmicroscopy,thengram staining as described by Feng *et al*., 2011. Cellular morphology as welldetectedbeneathJCM-5000 electron microscope(Nikon, Japan) below ×5,400.Biochemical and physiological analysisaccomplished using the methods described by Holt (1994) and Shen *et al.* (1999).

3.10.5 Gram's Staining Method

A little part of each grown bacterial clusterpicked with sterile inoculating loop. This transferred into sterile water on clean slide, smeared properly and heat secure. Slide was placed on rack over a sink and smear was flooded for 1 minby means of crystal violet.Temporarilywash awayvia tap water, then later waterlogged with grams' iodine (mordant) for 1 min before excess mordant washed off beneath a running tap water. The stain decolorized through 95% alcohol aimed at about

20 secs andwashedby means oftap water. Finally, smear counter stained by safranin for 20 secs, and the excess dye was washed off through tap water,thenpermitted to dry. Immersion oil poured

on the smear,then examined below oil immersion lens at x100 using a light Microscope. Gram-positivebacteria seemed purple whilenegative was pinkish in colour (Roberts and Greenwood, 2003).

3.10.6 Catalase

Catalase test noticesoccurrence or non-occurrence enzyme ofindividually isolate. Enzyme speed updisruption of H_20_2towarddischarge oxygen and water.3 droplets newly prepared hydrogen peroxideincluded in loop-full 18-24 h culture bacterial isolate on clean, grease-free slide.Sterilisedpurified liquidhelpedin place ofregulator.Gaseffervescence specifiescatalase-positive reaction whereasabsenteeism or latefoams formation shows negative reaction (Jideani and Jideani, 2006).

3.10.7 Oxidase

Oxidase test conductedby means of plate method. Culture of test bacterium grown on a nutrient agar and was flooded with a fleshly prepared 1% solution of tetrametyl-p-phenylenediamine hydrochloride and purple colour observed within 10 min. Colony that rapidly developed a purple colour was recorded as oxidase positive while the one without blue-purple colour referred to as oxidase negative (Jideani and Jideani, 2006).

3.10.8 Sugar Fermentation

Sugar testsintended to detectchange in pH when fermentation occurs in a given carbohydrate. Fermentation of carbohydrates produces bacteria while acids lower pH medium, thenchangephenol redto yellow from red. Whenever gas is formed as derivativeof carbohydrate, durham tube in medium produceeffervescecollection (Etok *et al*., 2005). Test conducted by inoculating peptone medium (10ml) with 0.1ml of 24 h old grown test bacterial isolate. Incubation carried out for 24 h at 37^{o}C thus, fermented sugar turned the medium to yellow colour showing positive reaction while medium holding unfermented sugar retained the original (red) colour and was noted as negative reaction. Acid production in some cases was accomplished by CO_2 evolvement made visible in the closed part of Durham's tube as vacuum or space owing to the movement of the medium in the inverted Durham's tube.

3.11 Bacteria IsolatesIdentification using 16SrRNA Gene Sequencing

3.11.1 DNA Extraction

Bacterial isolates developed overnight transferred to eppendorf tube,thenspunned at 14,000rpmfor 2 min. Supernatant threw away, thenDNA pull outby means of ZYMO kit (ZYMO Research; Inqaba Biotech, South Africa). DNAenumeratedthrough Nano Drop Spectrophotometer (Thermo.2000, USA), then stored at -20°C pending use. DNAlater re-drooped inside sterile distilled water (100µl). The concentrations samples measured and DNAconcentration wasestablished. DNA purity checked using agarose gel (1.0 %).

3.11.2 DNA Electrophoresis

Gel electrophoresis can use todefinesuperioritythenreliability ofDNA by means of fractionation on agarose gel (1.0%). Gel producedthroughsoftening, then boiling 1.0g agarose in buffer solutions (100ml 0.5 X TBE).Produced gel permitted to cool at 45^{o}C,10µl ethidium bromide (5mg/ml) mixed together,thenpoured inside theelectrophoresis chamber inserted withcombs. 3µl DNA, 5µlpurified water and 2µl (6X)dyecombinedwith solidified gel then loaded. Electrophoresis done for 2 h at 80V andDNAintegrityimaginedthensnapped throughUtra Violet light (Thottapilly *et al*., 1999).

3.11.3 I6SPCR Amplification of Lactic Acid Bacteria

16S universal primer was used for PCR amplification andcompleted with MJ investigation thermal cycler (PTC-200 model).5'AGAGTTTGATCCTGGCTCAG3' (Forwardprimer) and 5'ACGGCTACCTTGTTACGACTT3'(Reverse primer). PCR mix involved 1µl from buffer (10X), 0.4µl from $MgCl_2$(50mM), 0.5µl of dNTPs (2.5mM), 0.5µl of 5mM buffer for forward primer, and reverse primer was 0.5µlof 5mM buffer. Then 0.05µl of TaqDNApolymerase and purified water (5.05µl). Polymerase chain reactiondelineationadoptedopening denaturation at94^{o}C (3 min), then94^{o}C of 30 cyclesat 60 secs, 56^{o}C at 60 secs, 72^{o}C at 120 secs,final extension of72^{o}C at 5 min, then 10^{o}C hold (Williams *et al*., 1990).

3.11.4 PCRPurification

Amplicon further cleansedusing 2M Sodium Acetate wash technique before sequencing.1µl 2M Na Acetateadded to 10µl of PCR product, pH 5.2, then 20µl ethanol. The mixture reservedat -20^{o}C (1 h)androtated at 10,000 rpm (10 min),later wash away with 70% ethanol then dried. The mixture re-suspended inside sterile water (5µl),thenset asidefor sequencingat 4^{o}C.

3.11.5 PCR Sequencing

Forward I6S was used as primer for the reaction.PCR mix comprises ofbig dye terminator mix (0.5 µl), 5X buffer (1µl), 16S primer (1µl),distilled water (6.5µl)with 1 µlPCRproduct. Polymerase chain reactionsequencing is rapid;preliminary thermal rise to 96^{o}C (1min.),then 25 cycles, thermal slope to 96^{o}C (10 secs), thenincline to 50^{o}C (5secs) and rise to 60^{o}C (4 min.)Later, upgradeto 4^{o}C and hold (Williams *et al*., 1990).

3.11.6 Sample PreparationThrough Gene Sequencer

Sample preparation through cocktail mix contained9µlHi Di Formamide blend by means of 1µl refined sequence equal to 10µl.Samples loaded in Applied Biosystem machine (ABI 3130xl) and sequence in form of Adenine, Cytosine, Thymine, and Guanine gene obtained.

3.11.7 Sequencing and Statistical Analysis

Sequences obtained associatedwith 16SrRNAsample in Genbank and homology ofsequences analyzedthrough National Centre for Biotechnology Information program BLAST server, then construction of phylogenetic tree was done using CLC software (NCBI).

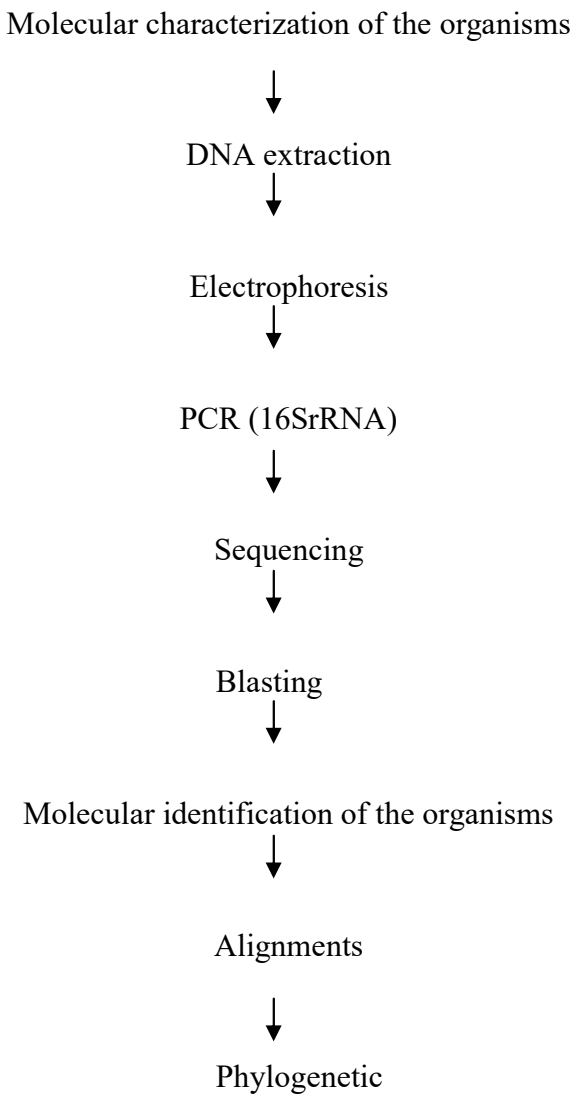


Fig. 3.5: Identification of Microorganism using PCR Assay

3.12 Sensory Evaluation

3.12.1 Sensory Properties of Pigeon-Pea-Enriched Breadfruitbreakfast meal and Pizzelle Cookie

Sensory assessment done on fermented breadfruit-pigeon-pea breakfast meal and cookie. Semi-trained panelists that are conversant with the products and without anyprevious information on the coded test productswere used. Sampleswere coded and offered to 50-members semi trained panelists who assessed appearance, aroma, taste,thentotalsuitabilityof breakfast meal while crispinesswas evaluated for cookie inaddition to the stated quality parameters in meal. Qualitycharacteristics were measuredvia Hedonic scale (Larmond, 1977).

Like extremely 9
Like very much 8
Like moderately7
Like slightly6
Neither like nor dislike5
Dislike slightly 4
Dislike moderately3
Dislike very much 2
Dislike extremely 1

Data gottenexposedtoward Analysis of Variance

3.13 Statistical Analysis

Data were recorded in triplicate,thenthe meanswere calculated. Sensory testing was subjected to analysis of variance(ANOVA) and means were separated by Duncan New Multiple Range test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 ChemicalCompositions for Fermented Breadfruit Samples at Different Temperatures and Time

Chemical composition of fermented breadfruit were as shown in table Table 4.1. Moisture contentvariedbetween 9.27– 8.13; protein4.17 – 3.63, fat 1.00 – 0.77, ash 2.90 – 2.73, crude fibre 3.47 – 3.03, carbohydrate 79.20 – 81.67%at28±2°C and 37±1^{o}C respectively. With reverence to moisture content,values foundpresently are similar to the finding by Appiah (2011) and Adepeju *et al.,* (2015) butmarginallylesser tothoseacquired by Ojokoh *et al*., (2013). Moisture content of samples fell within tolerableboundary of less than 10%. The value shown to be for flour suitability(Onimawo and Akubor, 2012). This is supplied by the recommendation of CIAT (2001) and CODEX Alimentarius Commission (1995). Low moisture contents attained from breadfruit flour in this study would improvestorage strengththroughreducing the mouldiness and additionalunwantedbiological reactions (Onimawo and Akubor, 2012).

Protein content of the unfermented sample agreed with the work done by Amusa *et al*., (2002) while fermented breadfruit samples were slightly lower than control, the different treatments could be responsible for the decrease and might be result of leaching the nutrients into water through the fermentation process. The values achieved were found to be in agreement by Okaka(2005) whoreported decrease in nutrients of root and tuber samples through processing. Obasi and Wogu (2008) stated that protein reductionin maize might beas a result of soluble protein loss in soaking. As proteins play functional roles in food formulations,breadfruit flour protein may be useful for reducing protein-energy malnutrition and applicable in food formulation systems of breakfast mealand other complementary foods. Similar values were obtained for fact with that of Adepeju *etal*. (2011). Presently, decline in fat contents of breadfruit ispossibly due to existence of lipolytic enzymes that decomposed lipids to glycerol and fatty acids (Oyarekua, 2011). Ajani *et al*., (2012) testified lowcrude fat of breadfruit. Fat contentshows an importantpart in storage of foodas higher fat remain objectionable for baked products.Fat can encourage rancidity in foods, causeunfriendly and odorous growth (Ihekoronye and Ngoddy, 1985).

Ash contents in unfermented and fermented breadfruit achieved were higher than (2.37- 2.38%) told by Appiah (2011a), Ijarotimi and Aroge (2005) respectively. The ash content of breadfruit slightly decreasedasfermentation progressed as detailed by Ejigui *et al*.(2005) onyellow fermented maize. The previous studythrough Obizoba and Atii (1991) showed that soaking diminished ash content in sorghum. Availability of ash at a vicinity of 3.00% suggests that breadfruit flour could be good source of minerals.

Breadfruit fibreobtainedfrom the present study were (3.47 – 3.27, 3.47 – 3.03%).The reduction in crude fibre maybe due to enzymatic breakdown of fibre throughfermentation via bacteria. Ofuya and Nwajiuba (1990)findings established over 35% cellulose loss, duringsolid-state fermentation of cassava peel. Appiah *et al*., (2011a) testified reduction in fibre content (3.12 – 3.00%) for fermented breadfruit. Based on Ihekoronye and Ngoddy, (1985), fibre is recognised to support digestive system of human. Shankar and Lanza(1991)also reported the beneficial effects of fibre inaverting cancer. Breadfruit havereasonably higher fibre than wheat.This make breadfruit entice good tolerability by a lot of people as well as health Organization.

Carbohydrate contents of fermented and unfermented breadfruit in this study were (79.20 – 80.93, 79. 20 – 81.67%). The results showed increased in carbohydrate contents at28±2°C and 37±1 ^{o}C. Adepeju *et al.,* (2011) obtained (79.46%) for unfermented whole breadfruit while Ijarotimi and Aroge (2005) obtained (81.27%) from unfermented breadfruit which was a bit higher. The results obtained from fermented breadfruit in this study seems to be differentfromAppiah (2011a) resultsthat reported reduction in carbohydrate contents for fermented breadfruit (79.24% - 76.71%). The methods used as well as sample variety, may be responsible for these results. Excessivecarbohydrate suggests thatbreadfruitcould be good energy basis asessential food (Roberts-Nkrumah, 2005). Breadfruit flour might findrequest as thickener,suitablefor formulationsof diabetics and hypertensive patients needingsmall sugar diet.

Table 4.1: Chemical Composition of Fermented Breadfruit at Diverse Temperature and Time

Fermentation Period (Hr)	Temp. (°C)	Moisture Content (%)	Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate % (By difference)
0	0	9.27±0.03^{a}	4.17±0.03^{a}	1.00±0.06^{a}	3.47±0.03^{a}	2.90±0.06^{ab}	79.20±0.06^{e}
24	28±2	8.53±0.09^{ab}	4.10±0.06^{ab}	0.93±0.03^{abc}	3.33±0.09^{abc}	2.97±0.03^{a}	80.13±0.12^{d}
	37±1	8.20±0.06^{cd}	3.83±0.03^{cde}	0.97±0.09^{ab}	3.27±0.03^{bc}	2.83±0.09^{abc}	80.90±0.15^{bc}
48	28±2	8.57±0.03^{ab}	3.93±0.09^{abc}	0.97±0.01^{ab}	3.40±0.06^{ab}	2.97±0.09^{a}	80.17±0.12^{d}
	37±1	8.27±0.15^{bc}	3.80±0.06^{def}	0.97±0.03^{ab}	3.23±0.09^{cd}	2.89±0.06^{ab}	80.83±0.22^{bc}
72	28±2	8.43±0.09^{c}	4.00±0.06^{abc}	0.90±0.02^{abc}	3.30±0.06^{bc}	2.77±0.09^{bc}	80.60±0.12^{c}
	37±1	8.30±0.06^{bc}	3.70±0.06^{ef}	0.90±0.06^{abc}	3.13±0.09^{de}	2.83±0.03^{abc}	81.13±0.09^{b}
96	28±2	8.33±0.09^{bc}	3.83±0.03^{cde}	0.87±0.03^{abc}	3.40±0.06^{ab}	2.87±0.09^{abc}	80.70±0.10^{c}
	37±1	8.13±0.09^{d}	3.73±0.09^{ef}	0.77±0.03^{c}	3.10±0.06^{de}	2.73±0.09^{c}	81.53±0.10^{ab}
120	28±2	8.43±0.03^{c}	3.77±0.03^{def}	0.80±0.06^{bc}	3.27±0.09^{bc}	2.80±0.06^{abc}	80.93±0.09^{bc}
	37±1	8.17±0.09^{d}	3.63±0.09^{f}	0.77±0.09^{c}	3.03±0.03^{e}	2.73±0.03^{c}	81.67±0.09^{a}

Meanswithdifferent subscripts within each rowsweredifferent significantly at 5% level

4.2 Chemical Composition of Fermented Pigeon-pea Flour at DiverseTemperature and Time

The results of the chemicalanalysis for fermented pigeon-pea shown in Table 4.2. Moisture of flour ranged between 8.83 – 9.23%, 8.83 – 8.72% for 28±2^{o}C and 37±1^{o}C respectively. Moisture increasedmarginally with increased in fermentation periods at28±2^{o}C while decreased at 37±1^{o}C. Theresults similarto previous findings (Fasoyiro *et al*., 2013; Appiah *etal*.2011a). Observed decrease in moisture content is an improvement because low moistureenhances necessaryquality of flour. Also, low moisture content in foods do increase in product shelf life and delay microbial development.

Based oncomposition of pigeon-pea, the following protein values obtained:24.77 – 22.27%, 24.77 – 4.53% at 28±2^{o}C and 37±1 ^{o}C respectively. Several authors have reported decrease in protein throughfermentation of legumes. Granito *et al*.,(2002) established reductionof *P*. v*ulgaris* flours and revealedlinkon volume of water used all through fermentation and protein decrease. Similar observations reported through Fasoyiro *et al*., (2012); Akande *et al*., (2010). Slight reductionofprotein obtained in this work maybe as a result of heat treatment and possiblyprotein hydrolysis by micro-organisms with the announcement of amino acids desirable for freshmixture (Oyarekua, 2011). Protein content of pigeon pea makes it a worthycomplement to breadfruit as a quality plant food. Mensah and Tomkins (2003) indicated that when legume proteins complement other food crop, anexcellent protein can beachieved.

Fat content of the flour increasedasfermentation proceededbecause of temperature variations (Table 4.2). Fat values ranged from 1.43 – 2.77% and 1.43 – 5.37% at 28±2^{o}C and 37±1^{o}C respectively. The values obtained for unfermented peas were comparable to the findings of Adepeju *et al*., (2015) whilehigher values were attained for fermented samples as the fermentation period increased. Fats remainimportant in diets because of palatability,increasein satiety andmaintainingaromas (Aiyesanmi and Oguntokun, 1996). Similarly,it isessential inbiological,organisationaloperative andtransport of vital fat-soluble vitamins in the body system.

Ash contents of pigeon-pea flourstudiedvariedfrom 3.67 to 3.87% and 3.67 to 1.26% for the treatments. The value obtained for sample at28±2^{o}Cweresimilarwith resultrecorded byFasoyiro *et al*., (2013), Mbaeyi-Nwaoha and Obetta (2016) during pigeon-pea processing (1.02-4.01%)

with different methods and evaluation of fermented pigeon-pea (3.10 – 3.56%) respectively. Ash revealsmineral volumepresentin food.Lowest ash in 37±1 ^{o}C samples show leaching of minerals into soaking water which leads to loss of minerals in flour.

Fibre contents ofsamples were between 1.40 – 1.77%, 1.40 – 1.50%at28±2^{o}C and 37±1^{o}C respectively.Arawande and Borokini (2010) reported crude fibre between 0.97 to 1.10% for Jack beans. The values obtained were marginally lower than thoseof Adebowale and Maliki (2011) during pigeon-pea fermentation andmaybe due tovarietalchanges together with theprocessing methods. Aziah *et al*. (2012) also noted 2.85% and 3.70% crude fibre in chickpea and mungbean flours. Legumes arevital sources of fibre next to cereals (Perez-Hidalgo *et al*., 1997) andhasaversepossible for diabetes,cardiac diseases,colon cancer, overweightness and additional diseases (McPherson, 1992).Codex Alimentarius Commission (2000) stressed the importance of fibre in food and advised that fibre for weaning foods should not exceed 5%. Low contents obtained in this study suggest its suitability in infant formulations.

Carbohydrate contents of fermented pigeon-pea flours in this study ranged from 59.90 – 61.70%, 59.90 – 82.54% at28±2^{o}C and 37±1^{o}C respectively. Oyarekua (2011) and Ghadge *et al.,* (2008) achievedrelated values in fermented pigeon-pea production and instant whole pigeon-pea. The moderate carbohydrate contents of flour samples suggest itsusefulness in solving problem related toenergy malnourishment.

Table 4.2: Chemical Composition of Fermented pigeon-pea flour at Diverse Temperature and Time

Fermentation Period (Hr)	Temp. (°C)	Moisture Content (%)	Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate % (By difference)
0	0	8.83±0.03^{c}	24.77±0.09^{a}	1.43±0.09^{h}	1.40±0.06^{cd}	3.67±0.03^{c}	59.90±0.20^{gh}
24	28±2	8.63±0.09^{bc}	23.83±0.12^{b}	1.73±0.03^{g}	1.67±0.09^{ab}	4.03±0.09^{a}	60.10±0.12^{gh}
	37±1	7.90±0.75^{d}	15.37±0.09^{e}	5.37±0.15^{a}	1.33±0.09^{de}	1.58±0.04^{f}	68.45±0.94^{d}
48	28±2	9.23±0.09^{a}	24.47±0.09^{c}	2.50±0.06^{e}	1.47±0.03^{bcd}	3.40±0.06^{d}	58.93±0.12^{h}
	37±1	8.58±0.07^{bc}	12.47±0.12^{f}	3.40±0.12^{c}	1.13±0.09^{ef}	2.32±0.01^{e}	72.10±0.23^{c}
72	28±2	9.07±0.09^{ab}	23.80±0.06^{b}	2.77±0.03^{d}	1.53±0.07^{cd}	3.80±0.06^{bc}	59.03±0.17^{h}
	37±1	8.70±0.09^{bc}	11.77±0.09^{f}	4.63±0.12^{b}	1.50±0.06^{bcd}	1.26±0.03^{h}	72.14±0.56^{c}
96	28±2	8.67±0.03^{bc}	23.47±0.03^{c}	1.63±0.09^{gh}	1.77±0.03^{a}	3.87±0.03^{b}	60.33±0.09^{f}
	37±1	8.72±0.03^{bc}	8.50±0.12^{g}	2.90±0.06^{d}	1.17±0.03^{ef}	1.38±0.01^{g}	77.73±0.42^{b}
120	28±2	8.47±0.09^{bc}	22.27±0.09^{d}	2.13±0.09^{f}	1.77±0.07^{a}	3.67±0.09^{c}	61.70±0.12^{e}
	37±1	8.28±0.14^{bc}	4.53±0.12^{h}	2.37±0.09^{ef}	0.97±0.03^{f}	1.31±0.02^{g}	82.54±0.31^{a}

Means with dissimilar subscripts within columnsweredifferent significantly at 5% level

4.3 Chemical Composition of Fermented Breadfruit Enriched with Pigeon-Pea at 28±2^{o}C for 24h

The results for composite flour proximateof fermented breadfruit enriched with pigeon-pea shown (Table 4.3). 100% fermented breadfruit had highest moistureof 8.53% and moisture for enriched breadfruit flour were between 7.47 to 7.08%.Ijarotimi and Aroge, 2005 observed reduction in moisture (5.76 – 4.22%) of soy- breadfruit blends as the substitution of soybeanrises.Lowermoisture content obtained in samples can be ascribed to drying of raw materials to safe moisture level which occurred during sample preparation. Hence, this will reduce the activity of micro-organisms in the products and provide high stability. Drying and other forms of heat treatment reduce moisture content of foods to safe level and thus increasing their shelf life (Falade and Ogunmelu, 2014).Significant increase observed in protein as substitution level through fermented pigeon-pea increased. Protein increased from 4.10 – 10.30% in the composite flour blends.Similar trends (4.20 – 8.05%) observed via Adebayo-Oyetoro *et al*. (2012) and Akinjayeju (2004) that reported rise in protein content of breadfruit enriched with pigeon-pea.Otunola *et al*., 2006; Plahar and Hoyle (1991) also detected increase in protein of flour with better legume substitution through suplementation of pigeon-pea, groundnut and cowpea with maize, millet and sorghumin foodpreparations. Protein of blends improved due to legume inclusion. Higher amount of protein in breadfruit-pigeon-pea blends can be due to inclusion of pigeon-pea flour noted as good protein basis in comparable with legumes like cowpea and groundnut(Oshodi *et al*., 1985; Fasoyiro *et al*., 2010).High protein in enriched flours would improve nutritional status for developing country(Nigeria) where individualsbarelymanage to pay for proteinous diets due to highprices (Falola *et al*.,2011).

Crude fatof enriched fermentedblends ranged from 0.93 – 1.58%. Fat contents were not significantly increased as pigeon-pea substitution increased.Lowest content was observed in 100% fermented breadfruit (0.93%),then 50% pigeon sample had highest value (1.58%).Okafor *et al*. (2018) observed increase in fat content (7.60 – 10.15mg/100g) during fermentation of maize co-fermented with pigeon-pea flour.Similar work supportedthroughAdebayo-Oyetoro *et al*. (2012) and Appiah *et al*. (2011) during breadfruit and pigeon-peaprocessing. The improvement in fat maybe due tolipolytic enzymes actionduring fermentation which make fatty acids available (Modu *et al*., 2013).Fatincrease in fortified maize-pap and other products were reported by Mbata *et al*. (2009).Fat values though increased with levels of supplementation but

low compare to others. This suggests that the blends will be stable and rancidity may not have adverse effect easily.

Values obtained for ash content remained significantly different (2.97-4.80 %), increased in ash content observed can be as a result of increase inclusion level of pigeon-pea flour.The value increasedfrom 2.97% in the control to 4.80 %, the noticeable increase in ash content with increase in pigeon-pea substitution was a proof that the samples were high in mineral.Appiah *etal*. (2016) and Oyarekua (2011) reported that breadfruit and pigeon-pea are rich in minerals.Adebayo-Oyetoro *et al*. (2012) discovered ash increaseas pigeon-pea level increased (1.48 – 1.92%).The increase in crude ash withpigeon-peasubstitution is related todiscoveries by Atobatele and Afolabi (2016)during cookiesevaluation from soya bean and maize blends.The increase in ash content of enriched flour exhibited that blends might be micronutrients-dense.

Fibre content of enriched fermented breadfruit varied between 3.33 and 1.19%. Fibre content reduces with increase in pigeon-pea inclusions.The present result is in correlation with fibre content (1.65 – 1.46%) reported for African breadfruit-pigeon pea fortified flour (Adebayo-Oyetoro *et al*., 2012), then Omoniyi et *al*.,(2016) onpotatoalsosoybean blends (4.62-3.76%).Fibre increases stool substancein body by acting as vehicle for faecal and donates to health of gastro-intestinal as well as metabolic in man (Atobatele and Afolabi, 2016).

Carbohydrate content of fermented breadfruit flour at 28±2^{o}C for 24h (80.13%) was higher than enriched samples which ranged from 79.61% to 75.05%. The results showed significant reduction of carbohydrate substitution as the substitution progressed. Similar reduction in carbohydrate were observed by Abiodun *et al*. (2016) after enrichment of Gari with melon. Similar trends were noticedduring enrichment of maize with soybean and enrichment of breadfruit with pigeon-pea byBalogun *et al*. (2016) and Adebayo-Oyetoro *et al*. (2012).This maybe due toincrease in protein and other food components of the enriched samples. Hence, the flour blends may be good in reducing energy-protein malnutrition.

Table 4.3: ChemicalComposition of Fermented Breadfruit Enriched with Pigeon-peaat 28±2°Cfor 24h

Sample	Moisture Content %	Protein %	Crude Fat %	Ash %	Crude Fibre %	Carbohydrate % (By difference)
240	8.53± 0.15[a]	4.10± 0.10 [f]	0.93± 0.04 [d]	2.97± 0.07[f]	3.33± 0.15[a]	80.13± 0.20[a]
241	7.47± 0.35[b]	6.47± 0.07[e]	1.00± 0.02[d]	3.48± 0.01[e]	1.97± 0.01[b]	79.61± 0.25[b]
242	7.29± 0.20[b]	7.71± 0.20[d]	1.13± 0.05[cd]	3.70± 0.03[d]	1.77± 0.02[c]	78.40± 0.37[c]
243	7.19± 0.30[c]	9.14± 0.02[c]	1.29± 0.01[c]	3.76± 0.02[c]	1.43± 0.01[d]	77.19± 0.27[d]
244	7.12± 0.31[c]	9.76± 0.07[b]	1.44± 0.02[b]	3.98± 0.01[b]	1.38± 0.01[d]	76.32± 0.28[e]
245	7.08± 0.14[c]	10.30± 0.03[a]	1.58± 0.01[a]	4.80± 0.01[a]	1.19± 0.01[e]	75.05± 0.14[f]

Means with different subscripts within row is different significantly at 5% level

Legend:

240 – 100% fermented breadfruit at 28±2°C (24h)

241 - 90%: 10% fermented breadfruit flour: Pigeon-pea flour at 28±2°C (24h)

242 - 80%: 20% fermented breadfruit flour: Pigeon-pea flourat 28±2°C (24h)

243 - 70%: 30% fermented breadfruit flour: Pigeon-pea flour at 28±2°C (24h)

244 - 60%: 40% fermented breadfruit flour: Pigeon-pea flour at 28±2°C (24h)

245 - 50%: 50% fermented breadfruit flour: Pigeon-pea flour at 28±2°C (24h)

4.4 Chemical Composition of Fermented Breadfruit Enriched with Pigeon-pea at 37±1^{o}C for 24h

The sample moisturevariedfrom 8.20% to 7.04% as indicated in Table 4.4.Enriched flour moisture had significance differences.Results show that addition of pigeon pea flour caused moisture reduction of blends.Present study is similar to work carried out by Rita *et al*. (2010) and Edema *et al*. (2005)in wheat-soy composite cake.Hence, low moisturedetectedremains good pointer for potential longer shelf-life. Smith (1972) reported that total moisture of sample should not surpass 14%. However, the moisture content falls within the acceptable moisture level that could extends product shelf-lifedue to little water activity.Enrichedbreadfruit proteinat 37±1^{o}C for 24h varied from 3.83 – 7.22%. There are improvements in protein values with increased pigeon pea flour substitution. Increase in protein is comparable to findings through Olaoye *et al*. (2006) and Rita *et al*. (2010).Protein increase as a result of pigeon-pea substitution is expected due to pigeon-pea richnessin protein.Besides, this finding confirms ealier reports on the beneficial effect of vegetable protein (Agbede and Aletor, 2003).The breadfruit – pigeon pea flour may alleviate disease such as kwashiorkor as a result of high carbohydrate intake.

Fat increase marginally as pigeon pea substitution increased (0.97- 1.81%).Ijarotimi and Aroge (2005) noticed similar trend (6.77 – 16.30%) during substitution of breadfruit with soybean flour. Ajani *et al*. (2016) also observed increased in fat contents (1.20 –1.63%) during enrichment of gari with soybean and groundnut. Otunola *et al.* (2007) established fat increase in fortified maize-ogi with okra seed and bambara groundnut. The low fat in this samples are indication that the enriched blends will be suitable in terms of stability.

The finding of increase in ash content (2.83 – 4.03%) in the enriched fermented breadfruit flour in this studycomparable to Ajanaku *et al*. (2013)reportedfor fortified samples.Ash determined mineral of a particular food; higher ash leads tobetter mineralof food (Ukegbu and Anyika, 2012).Ash endorsednutritional allotment in foodremainsintact.

Fibreenriched breadfruitranged between 3.27 – 2.44%. Highest value recorded for 100% fermented breadfruit which is control.In this work, crude fibrefound reduced as level of substitution increased. The reduction maybe as the fermented breadfruit flour reduced;then replacing by pigeon-pea flour with lower fibre tobreadfruit, percentage nutrients in that flour might have became lower.Uzopeters *et al*.(2008) informeddecrease in kokoro fibre flour

replacedwith defatted groundnuts and soybean.Fibre consist of indigestible carbohydrate in plantscell.

Carbohydrate content of enriched fermented breadfruit varied from 80.90% to 77.18% and there was decreased in values with pigeon-pea inclusion. Jimoh and Olatidoye (2009)reported decrease in carbohydrate throughaddition ofsoybean andAdebayo-Oyetoro *et al*. (2012)testifiedto the declineof carbohydrate from 74.82% to 68.46% insorghum enhancedthroughwalnut (45%)and ginger (5%).RecommendedDietetic Allowance (RDA) for carbohydratefoods is ≥60mg/100g (FAO/WHO, 1998).

Table 4.4: ChemicalComposition of Fermented Breadfruit-Pigeon-pea Composite at 37±1^{o}Cfor 24h

Sample	Moisture Content %	Protein %	Crude Fat %	Ash %	Crude Fibre %	Carbohydrate % (By difference)
370	8.20± 0.10^{a}	3.83± 0.06^{f}	0.97± 0.14^{d}	2.83± 0.16 ^{e}	3.27± 0.07^{a}	80.90± 0.26^{a}
371	8.14± 0.18^{a}	4.42± 0.19^{e}	1.11± 0.02^{d}	3.63± 0.03^{d}	2.44± 0.02^{d}	80.26± 0.46^{b}
372	8.01± 0.07^{c}	4.93± 0.06^{d}	1.43± 0.01^{c}	3.74± 0.01^{c}	2.49± 0.01^{d}	79.40± 0.07^{c}
373	7.32± 0.12^{d}	5.57± 0.17^{c}	1.49± 0.04^{c}	3.86± 0.02^{c}	2.56± 0.02^{c}	79.20± 0.29^{d}
374	7.23± 0.20^{d}	6.47± 0.24^{b}	1.62± 0.06^{b}	3.92± 0.03^{b}	2.65± 0.02^{b}	78.11± 0.O5^{e}
375	7.04± 0.07^{e}	7.22± 0.08^{a}	1.81± 0.02^{a}	4.03± 0.01^{a}	2.72± 0.01^{b}	77.18± 0.16^{f}

Means with different subscripts within row is different significantly at 5% level

Key:

370 – 100% fermented breadfruit at 37±1^{o}C (24h)

371 - 90%: 10% fermented breadfruit flour: Pigeon-pea at 37±1 ^{o}C (24h)

372 - 80%: 20% fermented breadfruit flour: Pigeon-pea at 37±1^{o}C (24h)

373 - 70%: 30% fermented breadfruit flour: Pigeon-pea at 37±1^{o}C (24h)

374 - 60%: 40% fermented breadfruit flour: Pigeon-pea at 37±1^{o}C (24h)

375 - 50%: 50% fermented breadfruit flour: Pigeon-pea at 37±1 ^{o}C (24 h)

4.5pH of Fermented Breadfruit and Pigeon-pea

Table 4.5 showspH values for fermented breadfruit flour at28±2^{o}C and 37±1^{o}C. The pH values obtained ranged from 6.26 - 4.64 (wet samples), 6.25 - 4.79 (dry samples) and 6.26 - 4.44 (wet samples), 6.25 - 4.52 (dry samples) for 0-120 h of fermentation period respectively. The study observed decreased in pH values of wet breadfruit sampleswith increase in fermentation period while slightriseobserved in dry samples but lower than control. Ojokoh *et al*., (2013)statedrelated reduction in pH through fermentation in breadfruit and cowpea; this could be attributed to the production of lactic acid by *Lactobacillus plantarum*.Adepeju *et al.* (2014) also stated similar trend through the production of complementary diets from breadfruit. pH values showlow acidity in samples and important for some functional properties.

Table 4.6 showsfermented pigeon-peapH values at 28±2^{o}C and 37±1^{o}C respectively. The values ranged from 6.90 to 5.45(wet samples), 6.90 to 4.68(dry samples) and 6.90 to 3.81 (wet samples), 6.90 to 4.97(dry samples). Values of pigeon-pea pH decline as fermentation period proceeded, even though the values for dry pigeon-pea flours were marginally higher than wet ones. Afoakwa *et al*.,(2010) observedpHreductionall through fermentation period of pigeon-peawhichis possibly caused by the activities of lactic acid bacteria.Oyarekua (2011)noticeddecline in pHthroughout the fermentation of pigeon-pea flour whileAmoa-Awua and Jakobsen (1995) reported similar pH reductionin the fermentation of cassava.

Table 4.5: pH of Fermented Breadfruit at Different Temperatures and Time

Temp.	Duration (Hour)	Wet (Sample)	Dry (Sample)
28±2°C			
	0	6.26 ± 0.01^{a}	6.25 ± 0.01^{a}
	24	5.24 ± 0.02^{c}	5.46 ± 0.01^{b}
	48	4.86 ± 0.01^{d}	5.46 ± 0.01^{b}
	72	4.71 ± 0.02^{e}	5.05 ± 0.01^{e}
	96	4.70 ± 0.02^{e}	4.89 ± 0.02^{g}
	120	4.64 ± 0.02^{f}	4.79 ± 0.01^{h}
37±1°C			
	24	5.52 ± 0.01^{b}	5.34 ± 0.01^{c}
	48	4.87 ± 0.01^{d}	5.13 ± 0.01^{d}
	72	4.72 ± 0.01^{e}	4.91 ± 0.07^{f}
	96	4.46 ± 0.01^{g}	4.85 ± 0.03^{g}
	120	4.44 ± 0.01^{g}	4.52 ± 0.01^{h}

Means with similar subscript within row is similarsignificantly at 5% level

Table 4.6: pH for Fermented Pigeon-peaat Different Temperatures and Time

Temp.	Duration (Hour)	Wet (Sample)	Dry (Sample)
28±2°C			
	0	6.90 ± 0.05^{a}	6.90 ± 0.05^{a}
	24	6.50 ± 0.02^{b}	6.07 ± 0.01^{b}
	48	6.05 ± 0.05^{c}	5.16 ± 0.02^{c}
	72	5.95 ± 0.05^{d}	5.15 ± 0.01^{c}
	96	5.70 ± 0.07^{e}	4.80 ± 0.01^{e}
	120	5.45 ± 0.05^{f}	4.68 ± 0.02^{f}
37±1°C			
	24	3.81 ± 0.01^{i}	4.97 ± 0.07^{d}
	48	3.83 ± 0.01^{i}	4.99 ± 0.02^{d}
	72	3.98 ± 0.03^{gh}	5.13 ± 0.01^{c}
	96	4.01 ± 0.01^{g}	5.16 ± 0.01^{c}
	120	4.07 ± 0.02^{g}	5.49 ± 0.01^{bc}

Means with similar subscript within row is similar significantly at 5% level

4.6 Total Titratable Acidity of Fermented Breadfruit and Pigeon-pea

Results of titratable acidity forbreadfruit and pigeon-peaat 28±2^{o}C and37±1^{o}Crespectivelyrangedfrom 0.03 - 0.24 (%), 0.03 – 0.23 (%) and 0.01 – 0.24 (%), 0.01 – 0.19 (%) as presented in tables 4.7 – 4.8. The results showed significant decrease in pH through fermentation with equivalentrise in acidity. The rise in acidity might be ascribed tolactic acid bacteriaactionthrough fermentation process. This results to production of organic acids and additional metabolites initiating souring or acidification of the product (Afoakwa *et al*., 2010, Adesokan *et al*., 2011). Adegbehingbe *etal.,*(2017)reported comparable observation while fermenting uncut andmilled breadfruit seeds(2.34% to 3.60%), (2.43% to 3.12%).

Okigbo (1980) reported acid production during cassava fermentation which assumed responsible for product steadiness, flavour growth and cyanide elimination. Sefa-Dedeh *et al*. (2004) noted that the acid produced during fermentation of maize had antimicrobial effects on some pathogens. Mensah *et al*. (1990) also established that high titratable acidity of fermented cereals reduced the occurrence of diarrhoea in infants. Thus, based on data obtained in this study, kind of acid produced through fermentation of breadfruit and pigeon pea can have antimicrobial effects on some pathogens andlessen diarrhoea in infants if consumed.

Table 4.7: Total Titratable Acidity of Fermented Breadfruit at Different Temperatures and Time

Temp.	Duration (Hour)	TTA (%)
28±2°C		
	0	0.03 ± 0.01^{i}
	24	0.08 ± 0.03^{h}
	48	0.09 ± 0.01^{g}
	72	0.10 ± 0.01^{f}
	96	0.13 ± 0.01^{e}
	120	0.24 ± 0.02^{a}
37±1°C		
	24	0.07 ± 0.01^{h}
	48	0.09 ± 0.00^{g}
	72	0.16 ± 0.03^{d}
	96	0.19 ± 0.01^{c}
	120	0.23 ± 0.01^{b}

Values within same columns with different alphabet(s) weredifferent at 5%

Table 4.8: Total Titratable Acidity for Fermented Pigeon-pea at Diverse Temperatures and Time

Temp.	Duration (Hour)	TTA (%)
28±2°C		
	0	0.01 ± 0.00^{j}
	24	0.12 ± 0.02^{h}
	48	0.15 ± 0.01^{g}
	72	0.20 ± 0.03^{c}
	96	0.21 ± 0.01^{b}
	120	0.24 ± 0.04^{a}
37±1°C		
	24	0.11 ± 0.02^{i}
	48	0.15 ± 0.01^{g}
	72	0.17 ± 0.01^{f}
	96	0.18 ± 0.01^{e}
	120	0.19 ± 0.02^{d}

Means with similar subscript within row is similar significantly at 5% level

4.7 Functional Properties for Fermented Breadfruit Samples at Different Temperatures and Time

Table 4.9 shown fermentedflours functional properties. In fermented breadfruit, loose and packed densities variedfrom 0.38 – 0.43, 0.38 – 0.50g/ml; 0.42 – 0.49, 0.42 – 0.55g/ml at28±2^{o}C and 37±1^{o}C. Adepeju *etal*.,(2011)relatedresults fromprocessed whole and pulp breadfruit flours. The flour densitieslikenedto 0.40-0.55 g/cm^{3}achievedinbreadfruit, soybean and tigernut (Ijarotimi and Aroge, 2005), also 0.55 g/cm^{3}fermented maize (Mbata *et al*., 2009a). Rise indensity is necessaryforpackaging benefit, for instance greater amount might be filled within constant capacity (Fagbemi, 1999). Density measuresflourweight (Oladele and Aina, 2007). Specified suplementary food tosmalldensitybecause it promotes digestibility among children who have immature digestive system reported(Mbata *et al.,*2009). In this respect,breadfruit flour can be appropriatein weaning food formulations and also havepossible usage as breakfast meal ingredient.

Water absorption capacityfor fermented breadfruit ranged from346.05 – 226.60% and 346.05 – 224.75%, respectively at different treatments. It ismaximumwater quantity food material can take,thensustainbelowpreparationsituation which is connected to dryness and penetrability of material. Table 4.9 shows water absorption capacity of samples which varied as fermentation period increases,significant decrease observed in values of fermented breadfruit treatments. The change may be ascribed to the variance in their carbohydrate contents (Adepeju *et al*., 2011). Water Absorption Capacities (WAC)for fermented breadfruit achieved were higher than one detailed for unfermented breadfruit (Adepeju *et al*., 2011) but were related to 227% described for fermented bambarra groundnut through Fasasi *et al.,* (2007). WAC enable food producersknow quantityof liquid needed during production,in that way improved handling features. Results suggest that breadfruit flourmight foundsuitablerequests in food preparations like breakfast meal, cake and other confectionery products.

Oil Absorption Capacity (OAC) for breadfruitcontrol (256.70%) slightly lower than fermented breadfruit samples. There were minimal increase in the values at 28±2^{o}C (256.70 – 276.65%) and 37±1^{o}C (256.70 – 286.40%).This may be attributed to the proteins denaturation and

dissociation (Qulai *et al*.,2014).The higher the denaturation, the higher the Oil Absorption Capacity.OAChelpsin food preparations and allows a sign of aroma-holdingcapability to flour (Narayana and Narasimga, 1982). Oil also makes flour suitable in food preparations (Odoemelam, 2003) and good oil capacities in flour (Table 4.9) suggestssuitabilityfor food preparations involving mixinglike confectioneries where oil is theessentialcomponent (Banigo and Mepba, 2005).

Foaming Capacity of breadfruit ranged from 12.70 – 2.96%, 12.70 – 2.00 % at 28±2^{o}C and 37±1^{o}Cseparately. Foaming Capacities decreased throughuse of treatmentsparticularly at 37±1^{o}C, observing that fermentation reduced the foaming rate. The foaming capacities acquired for breadfruit flour were equivalent to previous report on breadfruit cultivars and treatment effects on breadfruit (Oulal *et al.,* 2014; Appiah *et al.*, 2011a). Foaming capacity attribute to proteinsolubility, inorder words, foaming has to do with soluble proteins (Narayana and Narayasimga, 1982).Foaming properties may be suitable in food systems to enhance texturaluniformity, appearance of foods, leavening features in confectionery products.

Foaming stability of the flour samples ranging between 0.17 – 0.07% and 0.17 – 0.00%. Samples fermented at 28±2^{o}C have better foaming stability than other samples. Similar results onfoaming capacity and stability increase on sample concentration had been informed (Vani and Zays, 1995). Nwoji (2005) established increased in foaming capacity of germinated flour while heat treatment decreased the foaming stability. Yasumatsu *etal.* (1972) established higher foaming stability in native proteins than denatured protein. Foaming is useful for texture improvement, consistency and food appearance (Akubor and Eze, 2012).

Least Gelation Capacity (LGC) values for breadfruit were (4 – 6%) and (4 – 6%) at 28±2^{o}C and 37±1^{o}C respectively (Table 4.9). The values were virtuallyequivalent but statisticallydifferent throughdiverse temperatures and time. Values acquiredlinked well with the prior report of Fasasi *et al*. (2007). Gelation capacities obtained is lower in tolegume seed flour with (12%) (Aremu *et al*., 2007),then lupin seed (14%)(Sathe *et al*.,1982). Lower gelatingcapacity sample, givesimproved gelating ingredients (Adepeju *et al.*, 2014; Akintayo *et al.,* 1999). Thus, breadfruit and pigeon-pea flours may serve as good gelating and thickening agents.

Table 4.9: Functional Properties of Fermented Breadfruit at Different Temperature and Time

Fermentation Period (Hr)	Temp. (^{o}C)	LBD (g/ml)	PBD (g/ml)	WAC (%)	OAC (%)	FC (%)	FS (%)	LGC (%)
0	0	0.38±0.01^{f}	0.42±0.01^{f}	346.05±3.95^{a}	256.70±3.10^{bcd}	12.70±1.59^{a}	0.17±0.01^{a}	4.00±0.01^{b}
24	28±2	0.38±0.02^{f}	0.40±0.00^{g}	249.05±2.15^{cd}	276.65±4.85^{ab}	5.62±0.78^{b}	0.07±0.01^{b}	4.00±0.03^{b}
	37±1	0.36±0.01^{g}	0.40±0.02^{g}	301.15±27.35^{b}	286.40±4.10^{a}	4.58±0.08^{d}	0.05±0.01^{c}	4.00±0.01^{b}
48	28±2	0.36±0.01^{g}	0.38±0.03^{j}	237.85±0.95^{d}	271.30±11.00^{abc}	4.81±0.89^{c}	0.00±0.00^{e}	4.00±0.03^{b}
	37±1	0.50±0.02^{a}	0.55±0.01^{a}	281.05±9.05^{bc}	284.50±1.30^{a}	4.17±0.17^{f}	0.00±0.00^{e}	4.00±0.01^{b}
72	28±2	0.35±0.02^{h}	0.40±0.03^{g}	230.10±0.50^{d}	256.70±1.30^{bcd}	4.66±0.74^{e}	0.00±0.00^{e}	6.00±0.05^{a}
	37±1	0.36±0.02^{g}	0.40±0.01^{i}	256.80±1.60^{cd}	280.75±1.55^{a}	3.27±0.27^{h}	0.00±0.00^{e}	6.00±0.05^{a}
96	28±2	0.42±0.01^{c}	0.44±0.01^{d}	229.00±5.60^{d}	251.40±0.20^{cd}	3.85±0.85^{g}	0.00±0.00^{e}	6.00±0.02^{a}
	37±1	0.40±0.02^{e}	0.45±0.00^{c}	250.35±5.05^{cd}	280.90±8.50^{a}	2.88±0.96^{j}	0.01±0.00^{d}	6.00±0.01^{a}
120	28±2	0.43±0.01^{b}	0.49±0.01^{b}	226.60±3.20^{d}	251.80±0.70^{cd}	2.96±0.04^{i}	0.00±0.00^{e}	4.00±0.03^{b}
	37±1	0.41±0.00^{d}	0.43±0.01^{e}	224.75±3.75^{d}	257.45±1.65^{bcd}	2.00±0.01^{k}	0.00±0.00^{e}	6.00±0.01^{a}

Means with similar subscript within row is similar significantly at 5% level

Legend:

LBD=LooseBulk Density

PBD =PackedBulk Density

WAC=WaterAbsorption Capacity

OAC = Oil Absorption Capacity

FC=Foam Capacity

FS =Foam Stability

LGC =Least Gelation Capacity

4.8 Functional Properties for Fermented Pigeon-pea at Different Temperature and Duration

Mean values obtained forpigeon-pea functional illustrated belowinTable 4.10. Loose densities of pigeon pea flours varied 0.57 - 0.72 and 0.57 - 0.68g/ml whereascrowded densities were between 0.67 – 0.83 and 0.67 – 0.78g/ml at28±2^{o}Cand 37±1^{o}C respectively. Bulk densities of fermented pigeon-pea samples increased with fermentation periods. This may be attributed to soaking or absorption of water. Similar results were reported by Oppong (2015) during production of cowpea flour (0.7and 0.82g/ml). Appiah (2011b) documented0.80, 0.79,0.69 g/cm^{3}of Tona, Adom and Nhyira cowpeas respectively. Densities of treated products dictate featuresof itspackaging. Wilhelm *et al*. (2004) established thatproducts densities influences volume,durability of packaging material and texture. Higher mean values recorded in this workindicatessmall packaging priceas flour particles are weightier,and can occupy a lesser amount ofgapfor each unit mass. Akpata *et al*. (1999)documented higher density in rice (0.914 g/cc).

Water Holding Capacity in flours were between223.1 - 318.5% and 223.1 - 277.6%at 28±2^{o}C and 37±1^{o}Crespectively.Adebowale and Maliki, 2011; Oyarekua, 2011 notedsimilar observations during fermentation of pigeon-pea.Fermented values compared higher to unfermented, whichis similar to the report ofFasoyiro *et al*.(2010). WAC considered to be vital in protein viscous foods, examples arebaked products, doughand so on. WACis necessary in food classifications to enhanceproduceevenness and arrangement (Osundahunsi *et al*., 2003). Therefore, flour maybe beneficial in food preparations.

Oil Absorption Capacity (OAC)for pigeon-pea flours were 203.55 – 213.10% and 203.55 – 208.55%. The oil absorptioncapacities improved as the periods of fermentation increased in this current study. It was observed in treated samples than in raw, which is similar to Igene *et al*. (2005) findings on processed winged bean flours. Also,Elkhalifa *et al*. (2005) statedriseofoil absorption during sorghum fermentation. Higher oil absorption of 214% and 196% were reported for unripe banana flour and brown rice flour respectively (Anuonye *et al*., 2012).Proteins nature and higher protein contents of flours also contribute expressivelyto oilholdingpossessions of food constituents (Ravi and Sushelamma, 2005).Better absorption of oil in pigeon-pea flours couldascribed to high protein contents of the samples. OAC isvaluablefor structure connectionsin

food as well as increasing shelf life of meat products and confectioneries.

Foaming capacity infermented pigeon-pea flours were in ranges of 22.50 – 9.25, 22.50 – 8.20 % at 28±2^{o}C and 37±1^{o}C respectively. As fermentation periods and temperatures increased, foaming capacity reduced. In this current study,non-fermented sample have higherfoaming properties and the observation was comparable to the report by Adebowale and Maliki (2011). Decrease of foaming capacity in pigeon-pea ascribed to increase infat through fermentation period (Igbabul *et al*., 2014). The reduction in foaming might be clarifiedbased on presence of globular proteins that make denaturing surface difficult (Okpala *et al.*, 2013). Foaming formation remainsprotein type, pH, processing methods, thickness and surface pressure role. Foaming capacity determine flour ability to foam; which is dependent on stretchy protein whichdecline water surface (Asif-UI-Alam *etal*., 2014).

Foaming stability of fermented pigeon-pea for the two samples were lesser than unfermented pea. Results varied from 80.60 to 0.0%, 80.60 to 0.0% at 28±2^{o}C and 37±1^{o}C respectively. Similarly, Adebowale and Maliki (2011) observed decline in foam stability through increase in fermentation periods. Also, a study by Akubor *et al*., (2013) recorded 80% foam firmness for African star apple kernel.Thesemight be due to decline in protein through fermentation since protein absorptionproduces protein-protein relations at air-water bond.Also,encouragescreation of complex filmswhichprovide high viscoelastic opponent to foamsfusion thatrises stability (Adebowale,2003).Enujiugha and Akanbi (2005) also stated that inherent protein producesbetter stability than denatured protein.

Gelation Capacity of fermented samples from pigeon-pea presented in (Table 4.10) ranged from 6 – 4%, 6 – 10% for the two treatments and highest values recorded for samples at 37±1^{o}C. The gelation variation of pigeon-pea ascribedcomponentssizeslike lipids andproteins, carbohydrates. This suggestconstituent'scollaborationmight besignificant topigeon-pea functional properties (Kaur *et al*., 2007). Values for fermented pigeon-pea flours are related to reports of pigeon-pea (4%) through Onimawo *et al.,*(1998), thensoybean (10%) (Alfaro *et al*.,2004).

Small gelationdetectedpresentlycould be benefitted using flour asadditive gel-foaming materials in food products,as low gelation linked to oxidized amylose and amylopectin.High gelation capacities however, mightremainas a result ofimproved interaction occurred among binding pressures as absorptionrises (Ikegwu *et al*., 2009).

Gelation Concentration measures leastquantity of flour desirablefor gel formation in measured water. This differs from one flour to another, depending onproportions of ingredients structure likecarbohydrate and protein (Abbey and Ibeh, 1988). The increase in protein concentration boostscontact among binding forces which increases gelling capacity (Lawal, 2004). Thus, thelesser the gelation capacity, the better the gelling capacityinthe flour (Usman *et al*., 2016).

Table 4. 10: Functional Properties for Fermented Pigeon-pea Samples at Different Temperature and Time

Fermentation Period (Hr)	Temp. (^oC)	LBD (g/ml)	PBD g/ml	WAC (%)	OAC (%)	FC (%)	FS (%)	LGC (%)
0	0	0.57±0.03^{f}	0.67±0.01^{g}	223.10±3.50^{h}	203.55±1.95^{bcd}	22.50±0.10^{c}	80.60±0.20^{a}	6.00±0.05^{b}
24	28±2	0.67±0.07^{c}	0.77±0.05^{d}	218.05±0.05^{h}	205.60±1.40^{bcd}	19.25±0.15^{d}	75.35±0.15^{b}	4.00±0.02^{c}
	37±1	0.68±0.02^{b}	0.78±0.02^{c}	277.60±1.10^{ef}	179.25±11.65^{e}	23.35±0.25^{b}	50.25±0.25^{c}	6.00±0.05^{b}
48	28±2	0.67±0.02^{c}	0.77±0.07^{d}	267.75±2.75^{fg}	205.70±1.60^{bcd}	18.40±0.20^{e}	12.50±0.30^{f}	4.00±0.09^{c}
	37±1	0.63±0.01^{d}	0.71±0.01^{e}	218.05±0.01^{h}	192.70±0.80^{abcd}	25.30±0.10^{a}	40.40±0.20^{d}	6.00±0.03^{b}
72	28±2	0.67±0.02^{c}	0.80±0.05^{b}	282.40±2.20^{de}	208.10±2.00^{abc}	15.60±0.20^{f}	10.45±0.45^{g}	6.00±0.08^{b}
	37±1	0.57±0.01^{f}	0.69±0.01^{f}	188.15±0.25^{cde}	192.70±0.80^{abcd}	25.30±0.10^{a}	40.30±0.30^{d}	10.00±0.11^{a}
96	28±2	0.67±0.01^{c}	0.77±0.07^{d}	291.90±0.80^{bcd}	210.60±0.50^{ab}	12.45±0.25^{g}	0.00±0.00^{h}	6.00±0.07^{b}
	37±1	0.61±0.04^{e}	0.71±0.02^{e}	269.45±2.25^{fg}	204.40±1.40^{bcd}	9.40±0.30^{h}	33.45±0.15^{e}	6.00±0.01^{b}
120	28±2	0.72±0.02^{a}	0.83±0.03^{a}	318.50±1.60^{a}	213.10±5.30^{a}	9.25±0.15^{h}	0.00±0.00^{h}	6.00±0.09^{b}
	37±1	0.61±0.01^{e}	0.71±0.01^{e}	269.45±2.25^{fg}	208.55±0.55^{abc}	8.20±0.20^{i}	0.00±0.00^{h}	4.00±0.03^{c}

Values within same columns with different alphabet(s) were statistically different at 5%

Legend:

LBD=Loose Bulk Density

PBD= PackedBulk Density

WAC=Water Absorption Capacity

OAC= Oil Absorption Capacity

FC= Foam Capacity

FS=Foam Stability

LGC=Least Gelation Capacity

4.9 Pasting Properties for Fermented BreadfruitSamples at Different Temperatureand Time

Effects of fermentation on breadfruitpasting presentedbelow(Table 4.11). Thisresults tomixture of procedures; gelation from grainbreak to successive polymer arrangement,as a result of mechanical shear throughstarch heating and cooling (Otegbayo *et al*., 2006). Breadfruit flour observed in this study demonstratedsubstantial higher peak viscosity 458.33 – 489.29 RVU and 458.33 – 512.00 RVU respectively. Peak viscosity is maximum viscosity attainablethrough cooking of flourand this established bystarch-water suspension for the period of heating (Adebowale *et al*., 2005). Highflourviscosity maybe linked to quantity of starch; amyloseproportion to amylopectin,then resistivity to swelling of starch particles (Adepeju *et al*., 2011). Peak viscosity describedasextent of starch impairment; extreme starch weakening result tohigher viscosity (Sanni *et al*., 2001), indicates high binding volume of the thickener granules. However,pastes viscosity denotesthickeness level on cooking viaadvancedstarches swelling influence. Nevertheless, increased in values were observed as the fermentation time increased at 28±2^{o}C and 37±1^{o}C (Table 4.11). Otegbayo *et al*. (2006) noticed higher values in pounded yam; this implies that at higher peak viscosity, breadfruit might form thicker pastes on cooking. Peak viscosity associated to cooking easinessand showing pastestrength in gelatinization throughapplications in food processing (Opata *et al.*, 2007). Also, it is the highest holding strength indicatingcapacity of starch crumbs to upholditsconcentratedform when paste held in 2 min. 30 sec. at 95°C.

Table 4.11 showed result of breadfruit samples trough whichvariedfrom 335.85 – 411.71RVU;335.85 – 386.25RVU respectively. The same trend was observed by Awolu (2017) for pearl millet based composite flour. It is leastthickness valueof temperature phase for Rapid Visco Analyzer,then measures pastecapacity to resistdisintegrateby cooling (Ayo-omogie and Ogunsakin, 2013). Trough thickness is capacity of starch particlestowardstay undisrupted when breadfruit starch exposed to holding duration of continuous higher temperature and shear stress.

Breakdown viscosity of breadfruit varied from 122.29 – 8.12RVU and 122.29 – 47.71 RVUat 28±2^{o}C and 37±1^{o}C, respectively. Breakdown viscosity measures the degree of starch disintegration. Also, measures flouraptitude to survive heat and stress in course of cooking (Adebowale *et al*., 2005). Small breakdown results to highersteadinesspaste(Hugo*et al*., 2000). Breakdown viscosity of breadfruit samples reduced as fermentation time increased inall the

treatments. This indicates that the samples have moderately good hot paste stability. Oduro *et al.* (2000) affirmed starchthroughlittle stabilityor small collapsehave very feeble cross-linking insideparticles.

Considering Table 4.11, final viscosity of breadfruitvaried significantly from 515.00 to 629.42 RVU and 515.00 to 572.42RVU at 28±2^{o}C and 37±1^{o}C, respectively. The values obtained were high which showed that breadfruit formedfirmer gel after cooking and cooling owing to excessive carbohydrate in flour. Decrease inviscosities of someflours probably due to biochemical variations (breakdown of starch into sugars) through fermentation (Otegbayo, 2014). Final viscosity determinesstarch capacity to form sticky paste after boiling, then chilling (Maziya-Dixon *et al*., 2007).Breadfruit setback viscosity valuesrange between178.96 – 225.33 RVU, 178. 96 – 195.70RVU at 28±2^{o}C and 37±1^{o}C, respectively. Setback is stageonpasting curveforcooling starchesat 50°C. Greater setback viscosity, greatertendency to retrogradate.Itcontains reunion, reallocation of starch particles. Similarly, liquidearlier bound in visco-elastic on loose process discussed as synergetic. Higher setbacklinkedbyunified paste andstatedasimportantfornative product like pounded yam which needs highthickness,thensteadiness(Kim*et al*., 1995; Lawal, 2004).

Setbackof carbohydratediets connected withtexture of variousproducts. High setback for breadfruit samples in this study suggest that the flour will form cohesive gruels on cooking. Setbackdescribed assignal that thickener has high propensity ofbackwardthroughunfreezing circles (Ikujenlola and Fashakin, 2005). Thus, breadfruit flour might be valuable as ingredient in products like breakfast meal where starch retrogradation is wanted.

Peak time of breadfruit samples ranged between 5.50 – 7.00 min, 5.50 – 5.73min at 28±2^{o}C and 37±1^{o}C (Table 4.11). Time to achieve peak viscosity is significantly lower than (22-38 min) described for dried fufu by Sanni and Jaji (2003), then Shittu *et al*., (2001) through pupuru processing (37-43 min), in same range (5.47 – 7.00min, 3.62-4.27 min) for pearl millet composite flour and toasted tapioca respectively (Awolu, 2017; Adebowale *et al*., 2008). It is the period at which highestthickness was achievedper min. andmeasures cooking period (Adebowale *et al*., 2005; Lawal *et al*., 2004).

Temperature of breadfruit flours at28±2^{o}C and 37±1^{o}C ranging from 78.30 to 81.53^{o}C, 78.30 to 80.25^{o}C and these were higher than values (76-78^{o}C) stated for dried fufu (Sanni and Jaji, 2003). The pasting temperature of breadfruit flours are lesser than boiling; hence flour can become paste in warm water under boiling.Oluwamukomi *et al*., (2005) noted (70.50^{o}C) for fermented maize flour and 79.20 and 80.85^{o}Creported for enriched "gari" semolina withsoy-melon(Oluwamukomi and Jolayemi, 2012). Increase in pasting temperature as fermentation progressed also observed by Afoakwa *et al*., (2010). Pasting temperaturemeasureslowest temperature essentialfor cooking food (Sandhu *et al.*, 2005). Thiscould haveconsequencesin stability of components formula, thenspecify efficiency prices (Newport Scientific, 1998). Flours pasting properties are factorsdefiningaccuracy applicationas functional ingredients andadditionalmanufacturing products.

Table 4.11: Pasting Properties for Fermented Breadfruit Flour at Different Temperature and Time

Fermentation Period (Hr)	Temp. (^oC)	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Setback Viscosity (RVU)	Peak Time (min)	Pasting Temp. (^oC)
0	0	458.33±0.08^{c}	335.85±4.73^{d}	122.29±4.46^{a}	515.00±1.17^{ef}	178.96±3.38^{e}	5.50±0.10^{e}	78.30±0.05^{g}
24	28±2	489.29±0.96^{b}	411.71±2.21^{a}	77.58±3.17^{d}	629.42±2.00^{a}	217.71±4.21^{ab}	5.90±0.03^{b}	79.10±0.10^{ef}
	37±1	512.00±3.00^{a}	386.25±2.00^{b}	125.75±1.00^{a}	558.87±2.96^{c}	172.62±0.96^{ef}	5.27±0.00^{f}	79.10±0.10^{ef}
48	28±2	383.17±0.50^{e}	321.33±5.75^{e}	61.83±5.25^{e}	546.67±3.17^{d}	225.33±2.58^{a}	5.27±0.07^{e}	80.78±0.02^{b}
	37±1	488.92±0.08^{b}	385.25±2.50^{b}	103.67±2.58^{b}	571.67±2.00^{b}	186.42±4.50^{de}	5.63±0.03^{d}	78.28±0.02^{g}
72	28±2	368.62±6.29^{f}	262.58±8.00^{g}	106.04±1.71^{b}	431.04±2.13^{g}	168.46±5.88^{g}	5.77±0.10^{c}	80.15±0.30^{bc}
	37±1	424.42±3.33^{d}	376.71±0.46^{bc}	47.71±2.88^{f}	572.42±1.33^{b}	195.71±0.88^{d}	5.33±0.07^{f}	79.90±0.00^{cd}
96	28±2	309.62±1.79^{h}	248.00±1.33^{h}	61.62±0.46^{e}	390.92±5.25^{i}	142.92±3.92^{i}	5.80±0.07^{c}	81.53±0.02^{a}
	37±1	362.79±0.54^{f}	261.83±0.75^{g}	100.96±0.21^{b}	437.29±0.63^{g}	175.46±0.13^{ef}	5.37±0.03^{f}	79.90±0.05^{cd}
120	28±2	303.96±4.29^{i}	295.83±4.00^{f}	80.12±0.29^{c}	506.79±1.21^{f}	210.96±2.79^{c}	7.00±0.00^{a}	78.63±0.42^{fg}
	37±1	314.54±0.79^{g}	236.33±3.00^{i}	78.21±3.79^{d}	401.12±0.46^{h}	164.79±2.54^{h}	5.73±0.00^{cd}	80.25±0.45^{bc}

Means with diverse subscript within row is different significantly at 5% level

4 .10 Pasting Properties for Fermented Pigeon-pea at Diverse Temperature and Time

Pasting for fermented pigeon-pea are shown (Table 4.12).Peak viscosity forfermented pigeon-pea flours at 28±2^{o}C and 37±1^{o}C ranged between 79.08 – 22.50 RVU and 55.25 – 98.79 RVUrespectively while control was 22.38RVU. Peak is highestattainableviscositythrough cooking of flour and highest viscosity established by starch-water actionthroughheating system (Adebowale *et al*., 2005). Study agreedthroughOloyede *et al*., (2016) findings. It was detected that pasting temperature and peak time reduced faintly at 37±1^{o}C whereas peak viscosity increased as fermentation proceeded. Adebayo-Oyetoro *et al.* (2012) testified decreased in viscosity peakas noticed at 28±2^{o}C as supplementation of pigeon-pea increases in African breadfruit. Peak viscosity depends on solubility, water-holding capability and components structure of food system (Leszek, 2011). It is an indication of starch toexpand before its breakdown. It isregularlyconnectedthrough the product quality and offers a sign ofgelatinous load facedthrough mixing (Maziya-Dixon *et al*., 2005).

Table 4.12 showtrough viscosity for fermented pigeon-pea samples ranging from 78.00 – 26.25 RVU and 53.50 – 88.08 RVU at 28±2^{o}C and 37±1^{o}C while control was 21.96 RVU. Trough viscosity also called holding strength or hot pasteis highest thicknessat constant temperature phase of RVU profile, thendetermines breakdownin cooling (Chinma *et al*., 2009). 21.96 RVU documentedas least for controlwhereas 88.08 RVU was notedas highest for the fermented pigeon pea at 37±1^{o}C.

Breakdown values for fermented pigeon-pea flour were between 0.42 - 2.88 RVU and 0.42 - 10.71 RVU respectively. Breakdown viscosity can be defined as the degree of paste firmness or starch particlebreakdownthrough heat (Dengate, 1984). There were general increase in breakdown values of all the samples with different temperatures and fermentation period. Increase in breakdown values of pigeon-pea as fermentation progresses suggests simple cooking. High breakdown viscosity responsible for low capacity of sample to survive heat and shear pressure inboiling (Adebowale *et al*., 2005). However, sample with relativelysmall breakdown thicknesswould have better stable paste through heating than higher thickness (Farhat *et al*., 1999) even thoughability of thickenerto tolerate heat at high temperature and pressureare vitalforvariousprocedures.

Final viscosity of the fermented pigeon-pea samples at 28±2^{o}C and 37±1^{o}C (Table 4.12) ranged from 31.71 to 100.00 RVU and 31.71 to 123.25 RVU respectively. It is typicallyviewed as pointerfor steadiness ofcooked paste forreal use (Ragaee and Abdel-Aal, 2006). There were increase in final viscosities of all treated samples as fermentation progressed. Oloyede *et al*. (2016) observed related resultsthroughfermented moringa seed flour while Ige (2017) noticed comparable findings in complementary foods prepared from pigeon-pea-maize flours.Increase noted in fermented pigeon-pea might be ascribed to disintegration ofcarbohydratescompound to minor sugars through fermentation (Oloyede *et al*., 2016). Thepasteviscosity is linked to amylose;suggestingflour high inamylose will have highviscosity andvice versa (Goering and Dehass, 1990).

The study revealed setback viscosity of fermented pigeon-pea whichvaried between 22.08 – 10.58 RVU and 13.58 – 35.17 RVUat 28±2^{o}C and 37±1^{o}C. Setback viscosity measures paste stability after cooking. The control had the lowest value (9.75 RVU)whereas one of the samples at 37±1^{o}C had the highest value (35.17 RVU). However, there were increase in setback viscosities of all the samples fermented, with increased in time. The increase in the setback values might berise in hydrogen bondthrough cooling and higher starchamylase (Alais and Linden, 1986). Low setback values showamount of starch retrogradation and syneresis. High setback value reducesretrogradation in cooling of product made fromflour and vice versa (James and Nwabueze, 2014). The low setback in pigeon-pea indicateslowretrogradepropensity. Floursthat havereversing propensities are plusfor products like soups and pastes thatexperiencethicknessloss,also precipitation due to regression (Adebowale and Lawal, 2003).

Pasting / peak time of fermented pigeon-peascrutinised rangedbetween 6.67 - 6.93 min and 6.47 - 5.57 min.while control was 6.73 min. It is use to knowleastperiod and temperature essential for cooking flour (Chinma *et al*., 2009). Peak period isviewed as asign of overall time engaged by eachmixture to achieveowntopmost viscosity, that is, time for pastes to gel during cooking. As fermentation period increased at 28±2^{o}C including control sample, pasting time values increased while samples at 37±1^{o}C decreasedwith pasting temperatures. Hence, flourblends with low peak time would cook faster to higher time.

Pasting temperature of pigeon-pea ranged between 0.00 - 90.43^{o}C, 0.00 - 87.70^{o}C. Pasting temperature providesleast temperature required to cook flour, the costs of energy and other constituent'ssteadiness (Shimelis *et al*., 2006). Sample at 28±2^{o}C has the highest pasting temperature while the unfermented sample shows no pasting characteristic (error). The remarks in this study are comparable to values(84 – 89^{o}C) achieved by Usman *et al*.(2016) on weaning food blends from sorghum varieties. Moorthy (2002) establisheddiversepasting temperatures (61.5^{o}C to 86.3^{o}C) in sweet potato starches, the differences might have caused through interior structure changes in starch that occurred informless and crystallize areas (Crosbie *et al*., 2004). Higher pasting temperatures might be linked to existence of strong bond forces by granule interior and amylopectin's high crystalline nature with amorphous amylase (Ikegwu *et al*.,2009, Opata *et al*, 2007).

Table 4. 12: Pasting Properties for Fermented Pigeon-Pea Flour at Different Temperature and Time

Fermentation Period (Hr)	Temp. (^oC)	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Setback Viscosity (RVU)	Peak Time (min)	Pasting Temp. (^oC)
0	0	22.38±0.13^{h}	21.96±0.13^{i}	0.42±0.00^{i}	31.71±0.13^{j}	9.75±0.00^{h}	6.73±0.00^{ab}	0.00±0.00^{c}
24	28±2	79.08±1.33^{bc}	78.00±1.50^{b}	1.08±0.17^{h}	100.08±1.50^{c}	22.08±0.00^{c}	6.67±0.00^{ab}	88.75±0.05^{b}
	37±1	55.25±3.17^{d}	53.50±2.92^{e}	1.75±0.25^{f}	74.71±2.88^{d}	21.21±0.04^{d}	6.47±0.27^{bc}	88.03±0.02^{b}
48	28±2	42.96±0.79^{e}	41.83±1.08^{f}	1.12±0.29^{h}	59.17±0.50^{f}	17.33±0.58^{e}	6.80±0.00^{b}	88.13±0.08^{b}
	37±1	62.71±0.04^{c}	60.33±0.25^{d}	2.37±0.29^{e}	73.92±0.00$^{e\backslash}$	13.58±0.25^{g}	6.27±0.07^{cd}	87.65±0.40^{b}
72	28±2	35.46±2.21^{f}	33.71±2.46^{g}	1.75±0.25^{f}	48.83±2.50^{g}	15.12±0.04^{f}	6.93±0.07^{a}	88.05±0.00^{b}
	37±1	65.38±3.13^{c}	61.63±2.63^{d}	3.75±0.50^{c}	75.42±3.42^{d}	13.79±0.79^{g}	6.20±0.07^{cd}	87.70±0.30^{b}
96	28±2	27.87±0.79^{g}	26.58±1.08^{h}	1.29±0.29^{g}	37.17±2.42^{i}	10.58±1.33^{h}	6.93±0.07^{a}	88.03±0.07^{b}
	37±1	84.71±2.13^{b}	75.67±1.83^{bc}	9.04±0.29^{b}	104.67±2.42^{b}	29.00±0.59^{b}	5.57±0.03^{e}	86.33±0.08^{b}
120	28±2	22.50±5.17^{h}	26.25±1.08^{h}	2.88±0.38^{d}	44.25±1.75^{h}	18.00±0.67^{e}	6.87±0.07^{b}	90.43±1.68^{a}
	37±1	98.79±1.29^{a}	88.08±0.67^{a}	10.71±0.63^{a}	123.25±0.58^{a}	35.17±0.08^{a}	5.53±0.00^{e}	87.20±0.05^{b}

Values within same columns with different alphabet(s) were statistically different at 5%

4.11 Anti-Nutrient Contents of Fermented Breadfruit Samples at Different Temperatures and Time

Anti-nutrients of fermented breadfruit flour results at 28±2 ^{o}C and37±1^{o}C respectively shown (Table 4.13).

Phenolic contents forfermented breadfruit were between 3.32 - 1.53 mg/g and 3.32 - 1.09 mg/g. During fermentation period of 24h to 120h, anti-nutrients were significantly reduced, for example there were decreased in phenolic in all samples as fermentation time proceeded (Table 4.13). Unfermented sample (control) has the highest content of phenolic while the fermented sample at 120h has the least content. Ojokoh *et al*.(2013) observed similar findings in breadfruit fermentation. Phenolic contents attained werefound lower to the report (Odoemelam and Osu, 2009) through fermentation of breadfruit in different locations. Phenolicsrecommended to have chemo-preventive and cardio-protectiveeffects (Vita, 2005, Dragsted *et al*., 1993). It is also useful indefending human body against oxidative harmthroughunrestricted radicals (Halliwell, 1997).

Flavonoid contents of fermented breadfruit samples studied varied from 1.87 – 0.81 mg/g and 1.87 – 0.33 mg/g. There weredecrease in flavonoid contents of breadfruit flour as fermentation increased at28±2^{o}C and 37±1^{o}C, respectively. Flavonoids reported havingbiologicalpossessionslikeanti bacteria, toxic and inflammatory activities,then frequently function as sturdy antioxidants, unobstructedfundamentalforagers and metallic chelators (Jimoh and Oladiji, 2005). Plant flavonoids are potential dietary cancer chemo-protective and anti-tumor agents (Elangovan *et al*., 1994). Therefore, breadfruit mightoffer the neededdietetic bioflavonoids for cancer prevention and growth of tumor in humans if adequately consumed.

Fermented breadfruit phytate were 0.47- 0.19 mg/g, 0.47 - 0.25 mg/g at28±2^{o}C and 37±1^{o}C,respectively. The values are related to study investigated(Abiodun *et al*., 2016) incassava which phytic acids decreased with fermentation periods. Decrease in anti-nutritional contents may be due to leaching into the water during fermentation and dewatering process. Obasi and Wogu (2008) and Onimawo and Akubor (2005) reported that phytate get reduced in yellow maize through soaking. Wide variety of microflora known to possess phytase activitiesthat are partially accountable in reduction of phytic acid in fermented samples(Ojokoh, 2005). Phytic acid can bind some vital minerals or nutrients of digestive tract and leads toinorganicdeficits.

Hence, acidlevel in breadfruit issmall and may not have any dangerif matched to phytate diet between 10 – 60 mg/g on consumptionfor longer periodresulting to bioaccumulationdecreaseof minerals in single-chambered animals (Thompson, 1993). However, presence of phytate is helpfulsince it might haveencouraging nutritional role as anti-oxidant and cancer mediator (Turner *et al*., 2002).

Tannins are recognised in preventingactions of enzymes likelipase,trypsinand amylase (Griffith, 1979). Oladunjoye *et al*. (2010) reported soaking and other methods reduced tannin.Table 4.13 showed tannin contents of fermented breadfruit that ranged between 6.15 – 4.78 mg/g and 6.15 – 4.65mg/g, respectively. The decrease in values of tannin in breadfruit is similar to the findings on mucuna beans(Udensi*et al*. 2008). Tannin contents in the breadfruit flour were higher than other anti-nutrients (Table 4.13) but the values were however lower in comparison to 13.3, 19.1 and 99.2 g/kg describedinfluted pumpkin, breadnut and cashewnut respectively (Fagbemi *et al*., 2005).Tannin reductionthrough fermentation of breadfruit might be dischargeof polyphenols into the fermentation water lower than concentrationeffect (Uzogara *et al*.,1990). Tannins are polyphenols and water solublethoughtypicallyfound in seed coat (Singh,1988).

Oxalate has harmful consequences on diet,then healthvia calcium absorptionreduction, also assisting kidney stone creation(Nooman and Savage,1999).Fermented breadfruitoxalatesamples varied 0.40 - 0.24 mg/g, 0.40-0.20 mg/g. Reduction in oxalate maybe processing method used and activities of micro-organisms. The values of oxalate recorded are in agreement with those achieved (1.26mg/g – 0.83mg/g) by Obasi and Wogu (2008) through soaking of yellow maize. Therefore, decrease in oxalate through fermentation can have positive influence on consumer's health,improving bioavailability of needed minerals and reducingkidney stones riskamong consumers (Bhandari and Kawabata, 2006).

Hydrogen cyanide (HCN) contents of fermented breadfruit samples ranged between 1.0 - 0.24 mg/100g, 1.0 – 0.09 mg/100g. Results showedsubstantialdecreasein cyanide of all samples fermented. The decreasedetected in samples might be microbial actionsthrough fermentation (Kobawila *et al*., 2005). Hydrogen cyanide content of processed breadfruit seed stated by Nwaigwe and Adejumo (2015) ranged between 0.48 - 1.49mg/100g and Sanni *et al*., (2008) reported that gari from diverse processing points contained 1.8 to 49.60mg HCN/kg.

Consumption of foods immense in cyanide might be injuriousto nervous system (Chung *et al*.,

1998). Smallcyanidefound from fermented samples show breadfruit flour can safely use for food preparationssince level of cyanide is far below dangerousquantity of 1.40mg/100 mg (Oke, 1999). Littlequantities of cyanide informed for processed cookies from blends of sorghum flours and African breadfruit using autoclave (Okpala and Okoli, 2011). Cyanide contents of fermented breadfruit are in line with the standards compulsory for cassava flour and other flours in Colombia and Africa (CIAT, 2001) where it is specified that cassava flour should not have more than 50mg HCN/kg.

Alkaloids found in legumes are responsible for the unpleasant taste and turgidity in humans (Fereidoon, 2014). Table 4.13 presented the fermented breadfruit alkaloid contents. The contents varied from 1.19 - 0.23 % and 1.19 - 0.25 % at 28±2^{o}C and 37±1^{o}C, respectively. Ojinnaka *et al*.,(2013) reportedalkaloid reduction duringbreadfruit soaking for production of cookies. Nwaigwe and Adejumo (2015) autoclaved breadfruit seed and obtained reduced alkaloid values which ranged between 4.00 – 1.33%. The values attained by these authors seemed low and realistic for regularly consumed food (Ezeagu, 2005). Alkaloids affectmetabolic and physiologicalactions in the body;hence they are extensively used in medication (Harbone, 1973). However, some plant alkaloids cause thoughtful intoxications in animals, humans and often mutagenic (Aletor and Adeogun, 1995).

Fermented breadfruit saponin ranged between 0.46 to 0.03% and 0.46 to 0.06 %at 28±2^{o}C and 37±1^{o}C, respectively. General reduction observed in all the samples based on fermentation periods and similar with findings(Ojinnaka *et al*.,2013) in course for producing cookies from breadfruit. The saponin in fermented breadfruit under study was very low and they might not pose threat to human health. Low level of saponinsmight be leaching when breadfruit was soaked overnightthrough fermentation. Soaking has been reported to reduce saponin content. Saponinsreported to possess anticarcinogenic belongings, safeinflectionactions and proliferationrule. Also, it has benefits of inhibiting cancer growth and lowering saturated fatactions (Jimoh and Oladiji, 2005).

Table 4.13: Anti-Nutrient Contents of Fermented Breadfruit Flour

Fermentation Period (Hr)	Temp. (^oC)	Phenolic (mg/g)	Flavonoid (mg/g)	Phytate (mg/g)	Tannin (mg/g)	Oxalate (mg/g)	Cyanide (mg/100g)	Alkaloid (%)	Saponin (%)
0	0	3.32±0.20^{a}	1.87±0.05^{a}	0.47±0.06^{a}	6.15±0.23^{a}	0.40±0.00^{a}	1.00±0.01^{a}	1.19±0.06^{a}	0.46±0.01^{a}
24	28±2	2.78±0.13^{b}	1.51±0.05^{d}	0.30±0.01^{c}	5.93±0.16^{b}	0.32±0.01^{c}	0.40±0.01^{d}	0.92±0.03^{b}	0.28±0.02^{c}
	37±1	2.39±0.07^{d}	1.78±0.01^{b}	0.38±0.01^{ab}	5.60±0.09^{c}	0.34±0.01^{b}	0.87±0.01^{b}	0.83±0.01^{c}	0.32±0.01^{b}
48	28±2	2.58±0.12^{c}	1.47±0.04^{e}	0.29±0.04^{cd}	5.62±0.22^{c}	0.28±0.01^{e}	0.33±0.00^{e}	0.70±0.02^{e}	0.25±0.02^{d}
	37±1	1.93±0.11^{e}	1.61±0.06^{c}	0.30±0.02^{c}	5.49±0.06^{d}	0.29±0.00^{d}	0.67±0.01^{c}	0.80±0.04^{d}	0.19±0.01^{e}
72	28±2	2.26±0.13^{cd}	1.20±0.05^{f}	0.28±0.03^{cd}	5.26±0.06^{ef}	0.27±0.01^{e}	0.29±0.00^{f}	0.50±0.04^{f}	0.03±0.01^{h}
	37±1	1.92±0.12^{e}	1.60±0.06^{c}	0.30±0.07^{d}	5.32±0.05^{e}	0.25±0.01^{f}	0.20±0.01^{i}	0.68±0.02^{e}	0.09±0.01^{f}
96	28±2	1.60±0.18^{ef}	1.12±0.01^{g}	0.23±0.01^{f}	4.92±0.15^{g}	0.25±0.00^{f}	0.27±0.01^{g}	0.25±0.01^{h}	0.05±0.00^{g}
	37±1	1.55±0.16^{g}	1.53±0.08^{d}	0.27±0.03^{cd}	4.84±0.14^{h}	0.22±0.00^{g}	0.16±0.01^{j}	0.27±0.02^{g}	0.08±0.02^{f}
120	28±2	1.53±0.17^{g}	0.81±0.07^{h}	0.19±0.01^{g}	4.78±0.03^{i}	0.24±0.00^{f}	0.24±0.00^{h}	0.23±0.02^{i}	0.05±0.00^{g}
	37±1	1.09±0.10^{h}	0.33±0.06^{i}	0.25±0.01^{e}	4.65±0.05^{g}	0.20±0.00^{g}	0.09±0.01^{k}	0.25±0.01^{h}	0.06±0.01^{g}

Means in each column with different alphabetdiffers statistically (5% level)

4.12 Anti-Nutrients of Fermented Pigeon-peaat Different Temperature and Time

The phenolic contents from fermented pigeon-pea flour ranged0.86 - 0.23 mg/g, 0.86 - 0.48mg/g at 28±2 ^{o}C and 37±1^{o}C, respectively (Table 4.14). Decreased in phenolic contents of the samples at 28±2 ^{o}C and 37±1^{o}C as the fermentation periods increased observed. Related findingsreported by Lasekan and Shabnam (2013) in the fermentation of rambutan seed. Hithamani and Srinivasan (2014) noticed decrease in polyphenol contents during sprouting and pressure-cooking of finger and pearl millets. This could be as a result of phenolics diffusion in cell, then diffused phenolicsoxidation by polyphenol oxidaseactivity (Afoakwa *et al*., 2008). Reduction of phenols is desirable as this anti-nutrient is known to impart poor colour on food due to enzymic browning. However, Bravo (1998) indicated that the nutritionalconsumption of polyphenols is 1 g/ day (US), 23 mg/day (Dutch) and 28 mg/day (Denmark) which means the flour could be good for eating.

Flavonoids are polyphenolic acknowledgedas high antioxidant possessions and free essentialforagingcapability (Scherer and Godoy, 2009).Fermented pigeon-pea flavonoids varied between 0.59– 0.09 mg/g, 0.59– 0.76 mg/g samples at28±2^{o}C and 37±1^{o}C (Table 4.14). As fermentation period increased at 37±1^{o}C,there was increase in flavonoids of fermented samples.Fermentation reported to cause risein flavonoid contents in legumes (Ademiluyi and Oboh, 2011). However, decreased values of flavonoids noticed at 28±2^{o}C might be ascribed to either sample absorption or fermentation periodas experienced in pistachio hulls fermentation(Ehsan *et al.,* 2010). Okorie and Olasupo (2014) also observed decrease in flavonoids when African oil bean seeds were soaked overnight.

Table 4.14 revealed phytate contents of fermented pigeon-pea samples 0.45- 0.08mg/g, 0.45 - 0.34mg/g at 28±2^{o}C and 37±1^{o}C while sample at 28±2^{o}Chas the least value (0.08mg/g).Reduction of phytate as fermentation progresses has also stated(Adeniran *et al*.,2013) throughfermentation of lima and locust beans.The current study acquired values lesser to888 mg/g reported in moth bean also, 51.6 mg/g in prosopis *chilensis* (Vijayakumari *et al*., 1996). Pigeon- pea soaking and boiling reported to showphytate reduction(Igbedioh *et al*.,1994).

Phytatedecline observed in pigeon-pea samples (Table 4.14) indicate that the nutritional status of the processed samples might be of health benefit to the consumers. The reduction in phytate level could attributed to an unsolvable complex being formed among phytates, thenanotheringredient

(Vijayakumari *et al*., 1996).

They are known to reduce digestibility of starches, fats, protein andnormallyexpelledwhen they are bound. Before phytates ingested, they impact digestive enzymes and bind minerals like zinc, iron and manganese in the gut (Raboy, 2001).

At 28±2 ^{o}C and 37±1^{o}C, respectively, the present study discovered tannin from fermented pigeon- pea samples range between 0.91 – 0.13 mg/g, 0.91– 0.14 mg/g (Table 4.14). There weredecreased in contentsas fermentation periods wereincreasing for all the treatments. Decreased in tanninwas observed by Onwuka (2006) during pigeon-pea and cowpeaprocessing. Onilude *et al.* (2014)noticedtannindecrease inside cereal- soybean blends due to malting and toasting. Kinyua *et al*., (2016) reported that fermentation and malting of sorghum as well as pigeon-peas dehulling decreased tannin content. The decrease in tannin occurs due to leaching of tannin ions into water via fermentation and also bypolyphenol oxidase activity in food grain, or microflora activity due to fermentation(Ene-Obong and Obizoba, 1996,Fagbemi, 2005).Reports also established that soaking and fermentation decreased tannins content inraw African yam bean (0.41% - 0.19%) and in some legumes (Nwosu*et al*., 2012, Ikemefuna *et al*., 1991).Tanninbelongs to polyphenol grouptestified as antioxidant throughoxidative stressavertinglinked with heart disease,cancer and inflammation (Tapiero *et al*., 2002).

Oxalate of fermented pigeon-pea (Table 4.14)varied 0.14- 0.09 mg/g, 0.14 - 0.34mg/g at28±2^{o}C and 37±1^{o}C,respectively. Decreased in value of oxalate intreated pigeon-pea could ascribed to dischargeof oxalate to water. Ajayi *et al*., (2011) observed decreased oxalate contents in pigeon-pea, lima and jack beans. However,slight increaseobserved in pigeon-peaoxalate values at 37±1^{o}C but lesser than those of walnut (1.13mg/g) reported by Ogungbenle (2009), sorghum (5.22mg/g) and millet (4.06mg/g) described by NAS (1974) respectively. The oxalate levels in all the samples were within safe level (4-5mg/g). Oke (1969) reported that low levels of oxalates (4-5mg/g) are acknowledged to cause no irritation in the mouth or inhibit with iron or calcium absorption.Dresbach (1980) stated that oxalate decrease to physiological bearable quantityvia processing,improveduseof nutrients for metabolic activities.

Fermented pigeon-pea cyanide contents ranged between 0.60-1.21 mg/100g and 0.08-1.21 mg/100g, respectively. This report agreed with findings (Oluwamukomi and Adeyemi 2015) that observed cyanide reduction in fermentation of soy-melon gari. Adegbehingbe *et al*.(2014) also

reported decreased in cyanide contents of fermented lima bean seeds. Cyanide contents of pigeon-

pea sampleslesser than report for ground bean flour (Chikwendu, 2005) in the present study. The reduction in HCN level through fermentation is due to leaching, as cyanideswater solvable (Tresina and Mohan, 2012). The reductions of HCN in all the flour samples were far beyond the 35 mg/100g lethal value (Oke, 1969).This suggests that fermented samples examined might be good for eating.

Table 4.14 presented fermented pigeon-pea alkaloid contents varied from 0.92 to 0.46% and 0.92 to 0.48% for samples at 28±2^{o}C and 37±1^{o}C. Highest alkaloid content found in unfermented sample (control).However, reduction of alkaloid contents occurred in different treatments with increase in fermentation periods. Udensi *et al*., 2014 and Siddhuraju *et al*., 2002 detectedalkaloid reduction when samples were autoclaved.Nwaigwe and Adejumo (2015) also, established that low level of alkaloid content reduces flatulence in humans.

Saponins are glycosidescomponents, thenstated as natural soapsbased onfrothycharacteristics. Saponins identified for useful and harmful possessions depending on its concentration. The current study revealed saponin contents of samples from 0.64 to 0.26% and 0.64 to 0.34% (Table 4.14).Nwannekezi *et al*., (2017) through different processing methods of pigeon pea flour obtained similar results.As fermentation time increased, saponin contents in fermented pigeon-pea flour decreased with different temperatures.Highest value obtained from unfermented sample while the least was from samples treated at 28±2^{o}C (Table 4.14).

Saponin reduction may be due to microbial degradation (Nwanekezi *et al*., 2017). Onimawo and Akubor (2005) testifiedsaponins trace elements to benutritivelyfavourable because of hypo-cholestorolemic activity (cholesterol lowering). In addition, content reduces heart diseases risk when consuming saponin-rich legumes. Foods rich in saponin are essential innutrition to regulate cholesterol, check peptic ulcer and osteoporosis. Gemede and Ratta (2014) established its applicationsas viral adjuvants and bacterial vaccine (Quillaja saponins).

Table 4.14 Anti-Nutrient Contents of Fermented Pigeon-pea Flour

Fermentation Period (Hr)	Temp. (^oC)	Phenolic (mg/g)	Flavonoid (mg/g)	Phytate (mg/g)	Tannin (mg/g)	Oxalate (mg/g)	Cyanide (mg/100g)	Alkaloid (%)	Saponin (%)
0	0	0.86±0.02^{a}	0.59±0.03^{e}	0.45±0.01^{a}	0.91±0.01^{a}	0.14±0.01^{d}	1.21±0.04^{a}	0.92±0.10^{a}	0.64±0.02^{a}
24	28±2	0.40±0.01^{f}	0.49±0.01^{f}	0.23±0.01^{c}	0.40±0.00^{c}	0.13±0.01^{d}	0.92±0.01^{b}	0.86±0.01^{b}	0.32±0.01^{g}
	37±1	0.71±0.04^{b}	0.60±0.03^{d}	0.36±0.01^{b}	0.46±0.15^{b}	0.22±0.02^{c}	0.19±0.01^{g}	0.91±0.11^{a}	0.52±0.00^{b}
48	28±2	0.33±0.01^{g}	0.33±0.02^{g}	0.19±0.00^{d}	0.33±0.01^{d}	0.11±0.01^{e}	0.80±0.03^{cd}	0.83±0.02^{ab}	0.32±0.01^{g}
	37±1	0.67±0.06^{c}	0.61±0.03^{d}	0.36±0.02^{b}	0.34±0.06^{d}	0.23±0.03^{c}	0.18±0.01^{g}	0.84±0.05^{ab}	0.50±0.01^{b}
72	28±2	0.31±0.00^{gh}	0.28±0.01^{h}	0.16±0.00^{e}	0.28±0.02^{f}	0.10±0.00^{e}	0.77±0.02^{d}	0.66±0.02^{c}	0.29±0.00^{h}
	37±1	0.66±0.02^{e}	0.70±0.01^{e}	0.35±0.01^{b}	0.33±0.10^{d}	0.30±0.01^{b}	0.09±0.00^{h}	0.63±0.05^{c}	0.46±0.01^{c}
96	28±2	0.25±0.05^{i}	0.22±0.01^{i}	0.11±0.01^{f}	0.25±0.02^{g}	0.10±0.00^{e}	0.68±0.01^{e}	0.50±0.03^{cd}	0.28±0.00^{hi}
	37±1	0.64±0.02^{d}	0.73±0.02^{b}	0.34±0.02^{b}	0.31±0.00^{e}	0.31±0.02^{b}	0.09±0.01^{h}	0.57±0.02^{d}	0.44±0.01^{cd}
120	28±2	0.23±0.01^{j}	0.09±0.01^{j}	0.08±0.00^{g}	0.13±0.01^{h}	0.09±0.01^{f}	0.60±0.01^{f}	0.46±0.01^{e}	0.26±0.00^{i}
	37±1	0.48±0.03^{e}	0.76±0.02^{a}	0.34±0.01^{b}	0.14±0.05^{h}	0.34±0.01^{a}	0.08±0.01^{h}	0.48±0.06^{e}	0.34±0.01^{g}

Meanswithdiverse alphabet within eachrowwerestatistically differs(5% level)

4.13Biochemical and Carbohydrates Features of Bacteriain Fermented Breadfruit and Pigeon-pea

Table 4.15 revealed biochemical characteristics of fermented breadfruit- pigeon pea isolates. The isolates from breadfruit and pigeon-peas produced lactic acid bacterianamely *Lactobacillus fermentum and plantarum*while some werebacilli like*Bacillus anthracis, thuringiensis,cereus, pumillus, paenibacillus thuringiensis* and *Alcaligenes*. Some selected isolates (*L. fermentum, L. plantarum, B. anthracis, thuringiensis,cereus, pumillus and paenibacillus thuringiensis*)picked after the characterizationprovided blue-purple colourthrough gram staining; this discovered gram-positive bacteria.*Lactobacillus plantarum*and*fermentum*differentiatedthrough their biologicalcapability todivulge hexoses completely through Embden-Meyerhof path. However, *Alcaligenes* strain was negative to the test.Some bacteria strains identified showedpositive reaction andgenerate acid during fermentation for these sugars: glucose, fructose, gluconate, sucrose, lactose, maltose, melibiose, rafinose and ribose. Although, some were unable to ferment these sugars. In the current study (Table 4.15), the LAB identified wasable to ferment the sugar while bacilli strains fermented glucose only.In food industry, LAB act as valuable and decay organisms; they use in fermented milk production likesour milk, yoghurt,butter and cheese.

LAB is useful for preparationin sausages,sour dough,pickles, sauerkraut, silage beverages such as wine. It canbe found ingenital intestinal,animal and man respiratory tracts (Hammes *et al.*, 1992). LAB and their metabolism productsserve as bio-preservatives, therefore, increasefood shelf-life (Schillinger and Lucke, 1989; Ayad *et al.*, 2004).Theydisplayed many anti-microbial actionsvia production of bacteriocins and compoundslike ethanol, H_2O_2, organic acids, diacetyl, reuterin (Oral jenson Axelsson *et al*., 2008) and decreasing foodborne diseases risks (Konings *et al*., 2003).Therefore,the present LAB might haveneededpotentials and improvesfermented products safety (De martinis *et al*., 2002).LAB capabilityto lowerfermented foods pH leads to inhibition/reduction of food spoilage (Elliason and Tatini, 1999).Studies shown isolated LAB typesof diverseenvironmental niches, for examplesin milk, meat, vegetables, mouth, intestine andmammals vagina.*Lactobacillus plantarum* is used as starter culture and as probiotic LAB in cheese making (Vinderola *et al.*, 2000; Gomes *et al.*, 1995).

Research showed probiotics ability tocontrolimmune responses, lesser biomarkers like faecalactivities,superficial bladder and cervical cancer (MacFarland, 2000).

Additionalbenefits ofprobiotics include improvement of inflammatory bowel disease;contagion control, multi drug-resistance microbes'abolition. Also,blood cholesterolreduction, then anti-mutagenic/anti-carcinogenic activity (Salminen *et al.*, 2005). Previous studies isolated *L. plantarum*during sausage fermentationand sicilian green olive (Randazzo *et al.*, 2004; Parente *et al.*,2001).Sugar isolated conformed to findings of Hedberg*et al*., (2008) and Sharpe (1979), they worked on sugar fermentation in probiotic bacteria. The study also agreed with work done by Ishola and Adebayo-Tayo (2012) on fermented food for bio-molecules production.

Table 4.15 Biochemical and Carbohydrates Characteristics of Bacteria from Fermented Breadfruit and Pigeon-pea

Sample	Gram Reaction	Catalase	Oxidase	Glucose	Fructose	Gluconate	Sucrose	Lactose	Maltose	Melibiose	Rafinose	Ribose
1. *L. fermentum*	+	-	-	+	+	+	+	+	+	+	+	+
2. *L. fermentum 2*	+	-	-	+	+	+	+	+	+	+	+	+
3. *L. fermentum 2*	+	-	-	+	+	+	+	+	+	+	+	+
4. *L. fermentum 3*	+	-	-	+	+	+	+	+	+	+	+	+
5. *B. cereus*	+	+	-	+	-	-	-	-	-	-	-	-
6. *B. anthracis*	+	+	-	+	-	-	-	-	-	-	-	-
7. B. cereus 2	+	+	-	+	-	-	-	-	-	-	-	-
8. B. anthracis 2	+	+	-	+	-	-	-	-	-	-	-	-
9. B. cereus	+	+	-	+	-	-	-	-	-	-	-	-
10. B. thuringiensis	+	+	-	+	-	-	-	-	-	-	-	-
11. L. plantarum	+	-	-	+	+	+	+	+	+	+	+	+
12. L. plantarum 1	+	-	-	+	+	+	+	+	+	+	+	+

Readings done through anaerobic environmentsat 37^{o}C after 24h. Key: + = Positive reaction, -=Negative reaction

Table 4.15 Biochemical and Carbohydrates Characteristics of Bacteria from Fermented Breadfruit and Pigeon-pea (contd.)

Sample	Gram Reaction	Catalase	Oxidase	Glucose	Fructose	Gluconate	Sucrose	Lactose	Maltose	Melibiose	Rafinose	Ribose
13. L. plantarum 2	+	-	-	+	+	+	+	+	+	+	+	+
14. B. anthracis	+	+	-	+	-	-	-	-	-	-	-	-
15. B. cereus	+	+	-	+	-	-	-	-	-	-	-	-
16. B. thuringiensis	+	+	-	+	-	-	-	-	-	-	-	-
17. L. fermentum 3	+	-	-	+	+	+	+	+	+	+	+	+
18. L. fermentum 3	+	-	-	+	+	+	+	+	+	+	+	+
19. L. fermentum	+	-	-	+	+	+	+	+	+	+	+	+
20. L. plantarum	+	-	-	+	+	+	+	+	+	+	+	+
21. L. plantarum 1	+	-	-	+	+	+	+	+	+	+	+	+
22. L. plantarum 2	+	-	-	+	+	+	+	+	+	+	+	+

Readings done through anaerobic settingsat 37^{o}C after 24h. Key: + = Positive reaction, - =Negative reaction

Table 4.15 Biochemical and Carbonhydrates Characteristics of Bacteria from Fermented Breadfruit and Pigeon-pea (contd.)

Sample	Gram Reaction	Catalase	Oxidase	Glucose	Fructose	Gluconate	Sucrose	Lactose	Maltose	Melibiose	Rafinose	Ribose
23. B. anthracis	+	+	-	+	-	-	-	-	-	-	-	-
24. B. cereus	+	+	-	+	-	-	-	-	-	-	-	-
25. B. thuringiensis	+	+	-	+	-	-	-	-	-	-	-	-
26. L. fermentum 2- 1	+	-	-	+	+	+	+	+	+	+	+	+
27. L. fermentum	+	-	-	+	+	+	+	+	+	+	+	
28. L. fermentum	+	-	-	+	+	+	+	+	+	+	+	+
29. L. plantarum	+	-	-	+	+	+	+	+	+	+	+	+
30. L. plantarum 2	+	-	-	+	+	+	+	+	+	+	+	+
31. L. plantarum 3	+	-	-	+	+	+	+	+	+	+	+	+
32. L. plantarum 1	+	-	-	+	+	+	+	+	+	+	+	+

Readings done through anaerobic settingsat 37^{o}C after 24h. Key: + = Positive reaction, - =Negative reaction

Table 4.15 Biochemical and Carbohydrates Characteristics of Bacteria from Fermented Breadfruit and Pigeon-pea (contd.)

Sample	Gram Reaction	Catalase	Oxidase	Glucose	Fructose	Gluconate	Sucrose	Lactose	Maltose	Melibiose	Rafinose	Ribose
33. L. plantarum 4	+	-	-	+	+	+	+	+	+	+	+	+
34. B. cereus	+	+	-	+	-	-	-	-	-	-	-	-
35. B. cereus 1	+	+	-	+	-	-	-	-	-	-	-	-
36. B. cereus	+	+	-	+	-	-	-	-	-	-	-	-
37. L. fermentum	+	-	-	+	+	+	+	+	+	+	+	+
38. L. plantarum	+	-	-	+	+	+	+	+	+	+	+	+
39. L. plantarum	+	-	-	+	+	+	+	+	+	+	+	+
40. B. thuringiensis	+	+	-	+	-	-	-	-	-	-	-	-
41. B. anthracis	+	+	-	+	-	-	-	-	-	-	-	-

Readings done through anaerobic environmentsat 37^{o}C after 24h. Key: + = Positive reaction, - =Negative reaction

Table 4.15Biochemical and Carbohydrates Characteristics of Bacteria from Fermented Breadfruit and Pigeon-pea (contd.)

Sample	Gram Reaction	Catalase	Oxidase	Glucose	Fructose	Gluconate	Sucrose	Lactose	Maltose	Melibiose	Rafinose	Ribose
42. L. fermentum 3	+	-	-	+	+	+	+	+	+	+	+	+
43. L. fermentum 1	+	-	-	+	+	+	+	+	+	+	+	+
44. L. fermentum 2	+	-	-	+	+	+	+	+	+	+	+	+
45. Alcaligenes	_	+	+	-	-	-	-	-	-	-	-	+
46. Bacillus pumillus	+	+	+	+	+	-	-	-	-	+	-	+
47. Paenibacillus thuringiensis	+	+	+	-	+	+	+	+	+	+	-	-

Readings done through anaerobic settingsat 37^{o}C after 24h. Key: + = Positive reaction, **-** =Negative reaction

4.14 Polymerase Chain Reaction,16SrRNA Gene Sequence and Phylogenetic Tree of Breadfruit-Pigeon-pea Isolates

Polymerase chain reaction and gel electrophoresis established as suitable tools for analysis of *lactobacillus* community since they allow the detection of species very rapidly and economically (Burton *et al*., 2003). Strains of more than forty-seven (47) organisms were isolated and screened from breadfruit and pigeon-pea. Plate 4.1, shows the agarose gel electrophoresis of PCR amplicons for fermented breadfruit and pigeon-pea.

The current study presented gene sequencing, phylogenetic trees and alignment derived from 16SrRNA sequence. For this study, figures 4.1 a-cdisplay the breadfruit isolates sequence; fig.4.1 (d) demonstrates phylogenetic tree while fig.4.1 (e) shows the isolates alignment at 28±2^{o}C. However, figs. 4.2 a-d show pigeon-pea sequence; fig.4.2 (e) illustratesphylogenetic tree and fig. 4.2 (f) indicates the isolates alignment at the same temperature (28±2^{o}C). Figures 4.3 a-f reveal breadfruit isolates sequence at the temperature of 37±1^{o}C while fig. 4.3g shows phylogenetic tree and 4.3h shows the alignment of breadfruit isolate. Thus, figures 4.4 a-g show pigeon-pea isolates sequence, fig. 4.4h is the phylogenetic tree and 4.4(i) is the alignment of the pigeon-pea at 37±1^{o}C.Polymerase chain reaction built genomic techniques assumed to have uppermostprobable forquick, dependable and repeatable discovery. Also, establish documentation, classification,thenspecies of same strains (Gomez-Gil *et al.,* 2004). Traditionally, LAB had been categorisedvia phenotypic possessionsincludesphysical tests, sugar formation strategies but molecularmethodsestablished as operative,precisetechnique to ascertain and characterizeflora in multifaceted bacterial groupslike fermented foodsin last 20 years (Kesmen *et al*., 2012). Phylogeneticcentred onsequences,thendisplays relationshipamongbetter-studied

orders. Phylogenetic tree is used to avoid sequence of same clonal isolates, this, dropping cost of DNA sequences. Each cleade represents related organisms, horizontal edges shownshortest and longest groupbranches. Root of universal phylogenetic tree suggests that the bacteria have single ancestor (Prescott *et al*., 2008).

Phylogenetic trees and 16SrRNA gene sequence presented*Lactobacillus plantarum* and *fermentum* as dominating organisms during fermentation of breadfruit and pigeon-pea (fig.4.1 d). This data shows that *Lactobacillus plantarum* and*fermentum* are closely related than other species based on phylogenetic locations. They are heterofermentative *lactobacilli* and can metabolize glucose to a mixture of carbondioxide, lactic and acetic acid.

*Lactobacillus plantarum*recognised as prevalentorganism innumerous natural fermentations (Mugula *et al*., 2003), possiblybecause of ability to tolerate acid, thenbiggercapacity to use substrate(Fleming and McFeters, 1981). *Lactobacillus fermentum* alsoreported todominate fermentation of *fufu* during intermediate and final stages, this producedtypical flavour for the product (Adekoge and Babalola, 1988). *Lactobacilli*testifiedaccountable acid creation,then flavourimprovement in cereals pap and 'gari' (Ngaba and Lee, 1979; Akinrele, 1970). Chen*et al.,* (2010) discovered *L. plantarum* as most essential specie in tomato which is similar to the present study.Representative isolates selected for identification via PCR analyses, bacteria isolated were categorisedviamorphological, biochemical and molecular methods.Biochemical and phylogenetic treesshowedmost characterised LAB belongs to *Lactobacillus spp*, *lactobacilli*arevitaladvocates of lactic fermentation for a very long period (Pang *et al*., 2012; Pang *et al*., 2011). The taxonomic identifications achieved with DNA analyses were completelyreliable with the results of morphological characterization. The results indicated that identificationthrough 16SrRNA is similar to traditional biochemical approaches(Singh and Khullar, 2015).

16S rRNA gene is key among bacteria and hasprecise signature sequences.Saraithong *et al*.(2014) reported 16S rRNAgene sequence for studied bacteria structure in Apis.16S rRNAaccrues mutations quicklythan nuclear rDNA genes,then decode relationships underneath family level (Simon *et al*., 1994).Petti *et al*. (2005)16S rDNA sequencing identified bacteria correctly together with misidentified pathogens by traditional methods.The trait makes the sequence avitalindicator for identification.The 16SrRNA is notable for use but there are others

like 23SrRNA,16S-23S intergenic insert and gyrB (Gomez-Gil *et al*., 2004;Venkateswaran *et al*., 1998).

Gene is satisfactory with interspecific 16SrRNA polymorphisms, essential in adding to discriminative and statisticalreliabledimension (Clarridge, 2004).Also,16S rRNAused extensivelyin determininghuge quantity strains of bacteria andmany deposited sequences for comparison of unknown bacterial strains (Clarridge, 2004). In addition, 16S rRNA measure relationship among bacteria, because ofgeneral gene (Woese, 1987). Universal primers carefully chosen as complementary to conserve regions as shown in figs. 4.1 (i), 4.2fand the sequences (figs. 4.1 a-g, 4.2 a-d; 4.3 a-f, 4.4 a-g, 4.5 a-f, 4.6 a-g) of which variable regions are for comparative taxonomy (Clarridge, 2004; Relman, 1999). Generally,16SrRNA sequences allowbacteriaselectivecomparison at species level and categorising strains at diverse levels (Clarridge, 2004).16S rRNA could be explore in sequences as standard for classification,micro-organisms documentationand also displaysappropriatevariations (Ting *et al*., 2009). Identification of bacteria using 16SrRNA sequence discovered lactic acid bacteria and bacillus *spp*, while dominant organisms are *Lactobacillus plantarum* and *fermentum.* Bacillus sp is common bacterium found plentifully in soil.LABis amongst microorganisms that control food fermentations (Guasch-Jane *et al*., 2006). They aregram-positive whichmakelactic acid keyproduce, then Generally -recognised- as - safe (Konings,2000). This study established that fermented breadfruit and pigeon-pea contain abundant LAB specie which involved in adequate acidification during fermentation process. LAB playsvitalrole in production of quality silage and theydisplay effects on silage quality differently (Yang *et al*., 2010).LABcreatesignificantgroup of organisms infood processing industries, these organisms are responsible for fermentation of most legumes and cereals (Oyarekua, 2011: Amusa *et al*., 2005). LAB hasprobable as food seasonings and functional constituents for health and economy aids (Welman and Maddox, 2003).

On the other hand, bacteria like *alcaligenes faecalis*, *bacillus cereus*, *bacillus pumillus* and *bacillus anthracis*noticed in breadfruit isolates while *bacillus thuringiensis* and *paenibacillus taichungensis* found in pigeon-pea isolates at the same temperature could be as a result of handling. The 16SrRNA gene sequence comparedvia Basic Local Alignment Search devicethrough sequencesdatabase inNational Centre for Biotechnology.Thefindings inthis study using phenotypic and molecular characterization established that organisms recognised as same specie once gene homology developed to 99% (Laurentiu *et al*., 2014; Fry *et al*. 1991).

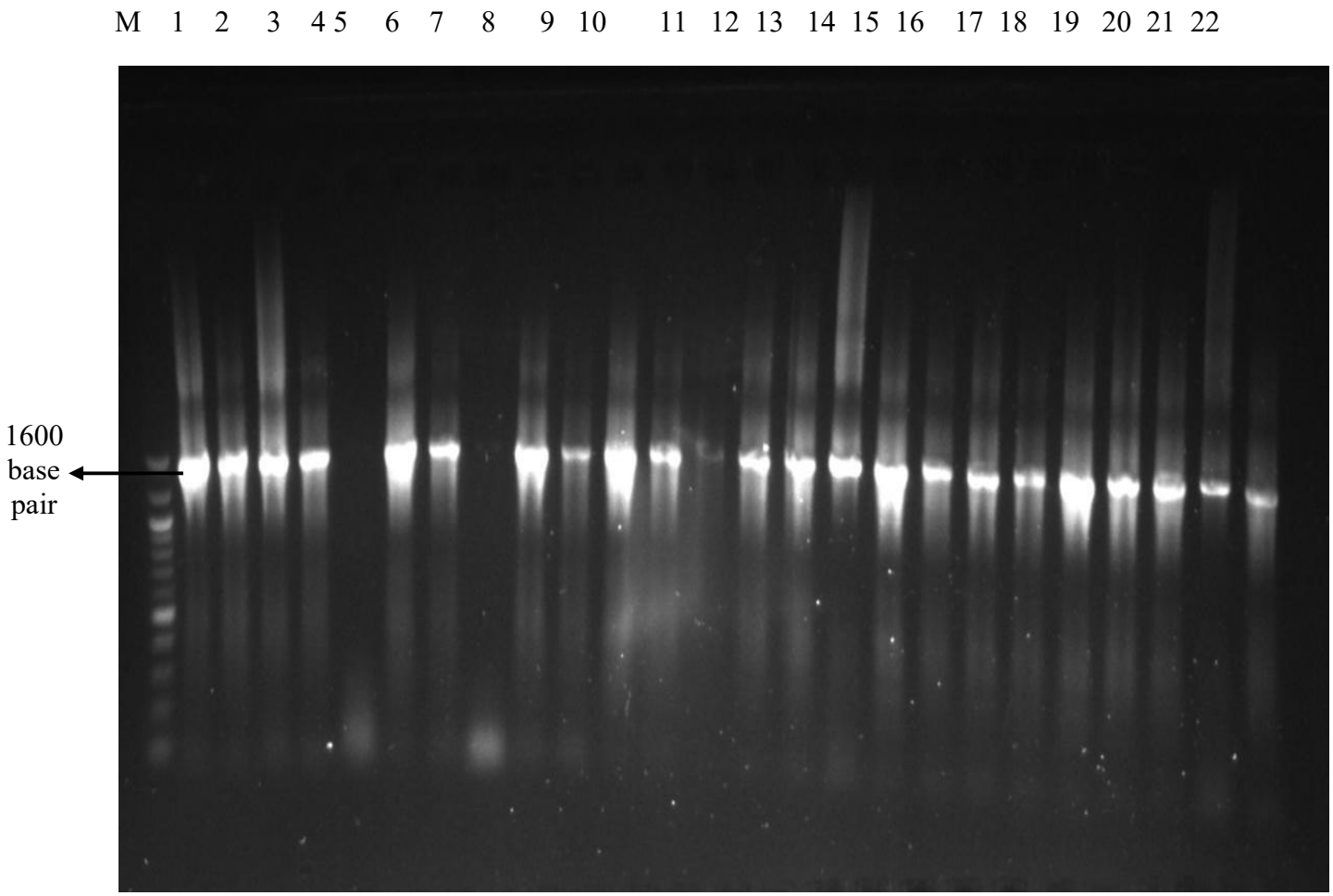


Plate 4.1: DNA amplification bands for breadfruit and pigeon-pea isolates

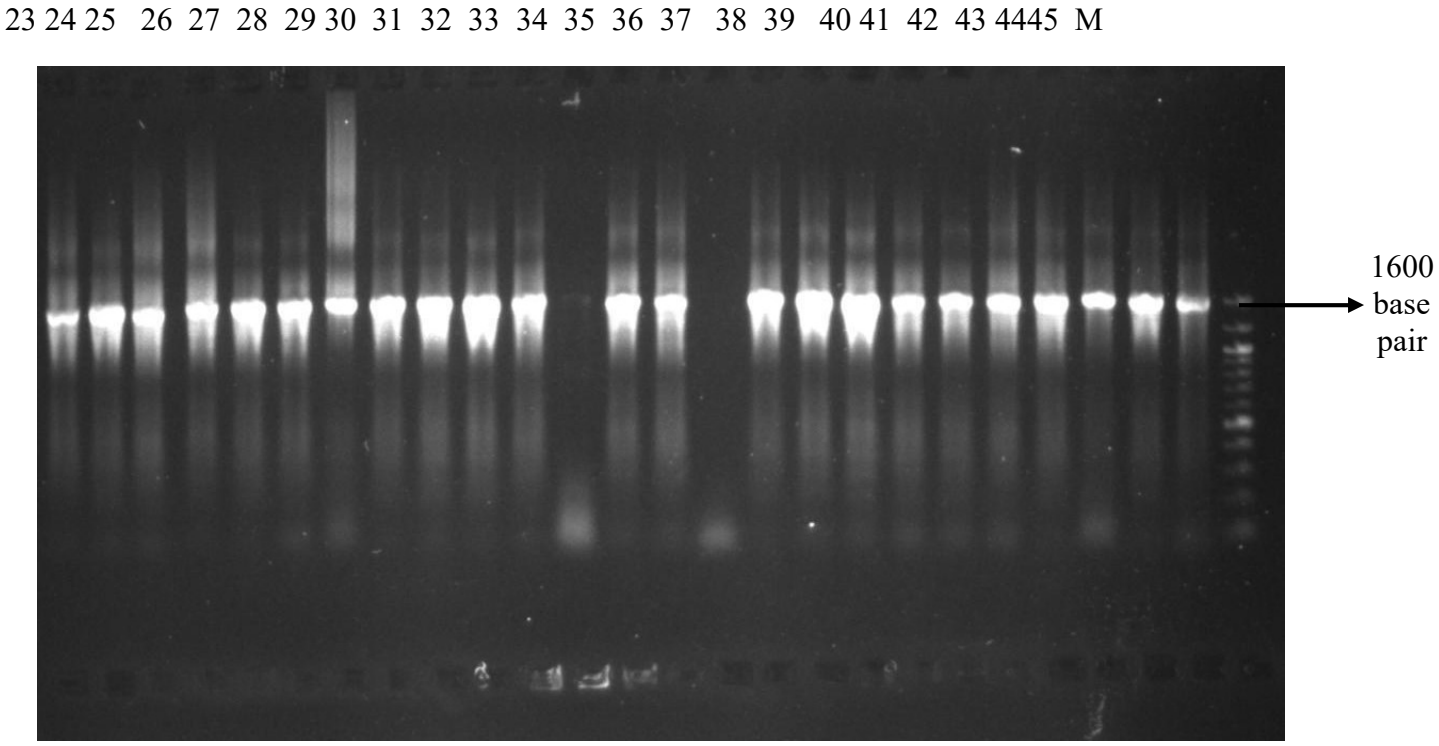


Plate 4.1: DNA amplification bands for breadfruit and pigeon-pea isolates (contd)

46 47 M

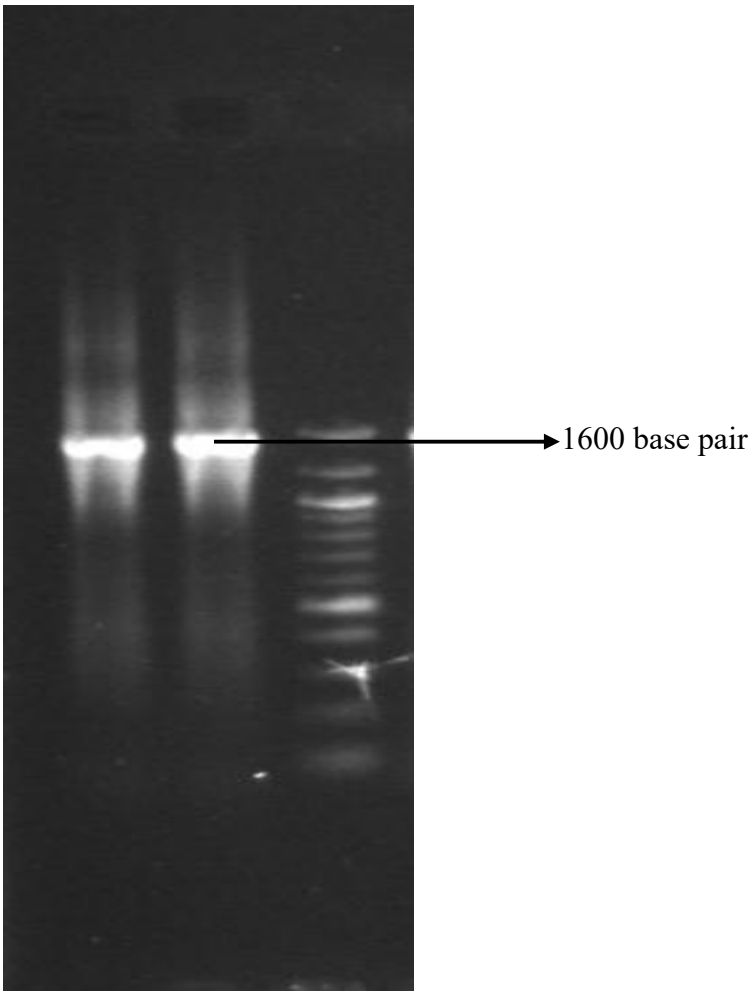


Plate 4.1: DNA amplification bands for breadfruit and pigeon-pea isolates (contd)

Key:

M: Marker

Lane 25: *B. thuringiensis*

Lane 1: *L. fermentum*

Lane 2:*L. fermentum 2*

Lane 3:*L. fermentum 2*

Lane 4:*L. fermentum 3*

Lane 5: *B. cereus*

Lane 6: *B. anthracis*

Lane 7: *B. cereus 2*

Lane 8: *B. anthracis 2*

Lane 9:*B. cereus*

Lane 10: *B. thuringiensis*

Lane 11: *L. plantarum*

Lane 12:*L. plantarum 1*

Lane 13: *L. plantarum 2*

Lane14: *B. anthracis*

Lane 15: *B. cereus*

Lane 16: *B. thuringiensis*

Lane 17: *L. fermentum 3*

Lane 18: *L. fermentum 3*

Lane 19: *L. fermentum*

Lane20:*L. plantarum*

Lane 21:*L. plantarum 1*

Lane 22:*L. plantarum 2*

Lane 23: *B. anthracis*

Lane 24: *B. cereus*

Lane 26: *L. fermentum 2- 1*

Lane 27: *L. fermentum*

Lane 28: *L. fermentum*

Lane 29: *L. plantarum*

Lane 30: *L. plantarum 2*

Lane 31: *L. plantarum 3*

Lane 32: *L. plantarum 1*

Lane 33: *L. plantarum 4*

Lane 34: *B. cereus*

Lane 35: *B. cereus 1*

Lane 36: *B. cereus*

Lane 37: *L. fermentum*

Lane 38: *L. plantarum*

Lane 39: *L. plantarum*

Lane 40: *B. thuringiensis*

Lane 41: *B. anthracis*

Lane 42: *L. fermentum 3*

Lane 43: *L. fermentum 1*

Lane 44: *L. fermentum 2*

Lane 45: *Alcaligenes*

Lane 46: *Bacillus pumillus*

Lane 47: *Paenibacillus thuringiensis*

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

Fig. 4.1 (a)

*Lactobacillus fermentum*CP011536.1

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG

GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

Fig. 4.1(b)

*Lactobacillus fermentum*CP002033.1

AAAAACATACAAATAGACGAGGAGTGCTTAATTATGTTATCAGTACCTGATTATGAG
TTTTGGTTTGTTACCGGTTCACAACACCTTTATGGTGAAGAACAATTGAAGTCTGTTG

CTAAGGATGCGCAAGATATTGCGGATAAATTGAATGCAAGCGGCAAGTTACCTTAT
AAAGTAGTCTTCAAAGATGTTATGACGACGGCTGAAAGT

ATCACCAACTTTATGAAAGAAGTTAATTACAATGATAAGGTAGCCGGTGTTATTACT
TGGATGCACACATTCTCACCAGCCAAGAACTGGATTCGTGGAACTGAACTGTTACAA
AAACCATTATTACACTTAGCAACGCAATATTTGAATAATATTCCATATGCAGACATT
GATTTTGATTACATGAACCTTAACCAAAGTGCGCATGGC

GACCGTGAATATGCCTACATTAACGCCCGGTTGCAGAAACATAATAAGGTTGTCTAC
GGCTATTGGGGCGATGAAGATGTGCAAGAACAGATTGCGCGTTGGGAAGACGTCGC
AGTAGCGTACAATGAGAGCTTTAAAGTTAAGGTTGCTCGTTTTGGCGACACGATGCG
TAATGTGGCCGTTACTGAAGGTGACAAGGTTGAAGCTCAA

ATTAAGATGGGCTGGACAGTTGACTATTATGGTATCGGTGACTTAGTTGAAGAGATC
AATAAGGTTTCGGATGTTGATATTGATAAGGAATACGCTGACTTGGAGTCTCGGTAT
GAAATGGTCCAGGGCGATAACGATGC

Fig.4.1 (c)

*Lactobacillusplantarum*CP012122.1

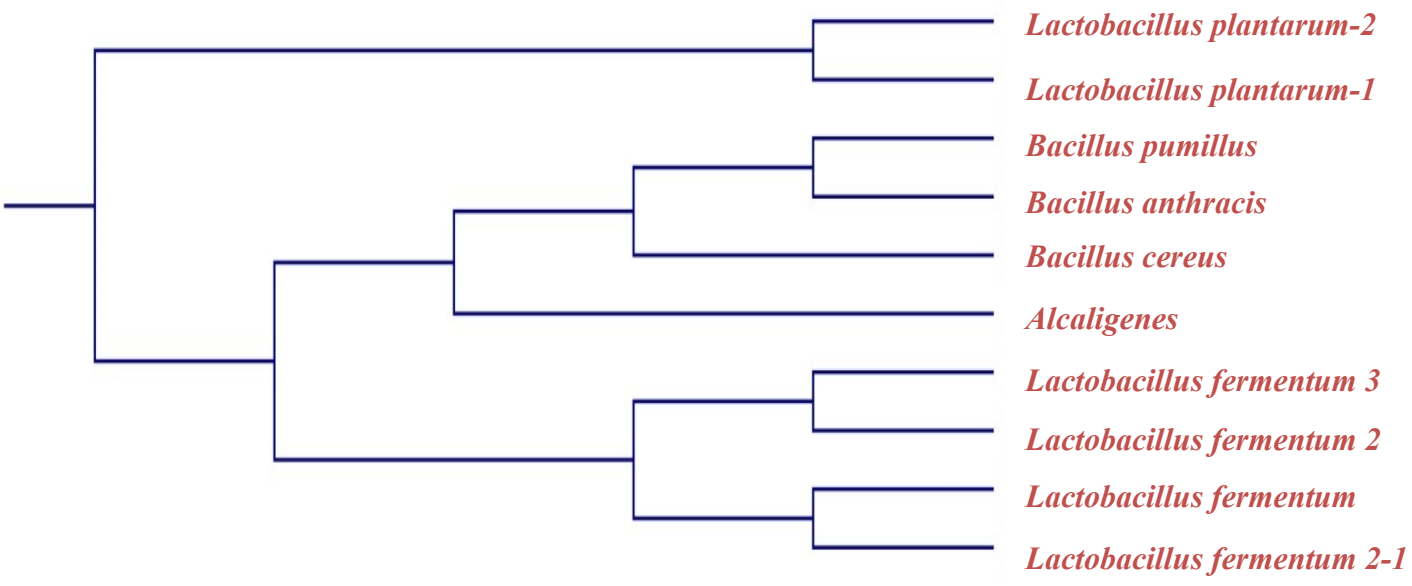


Fig. 4.1(d): Phylogenetic Tree of Breadfruit Isolates at 28±2^{o}C

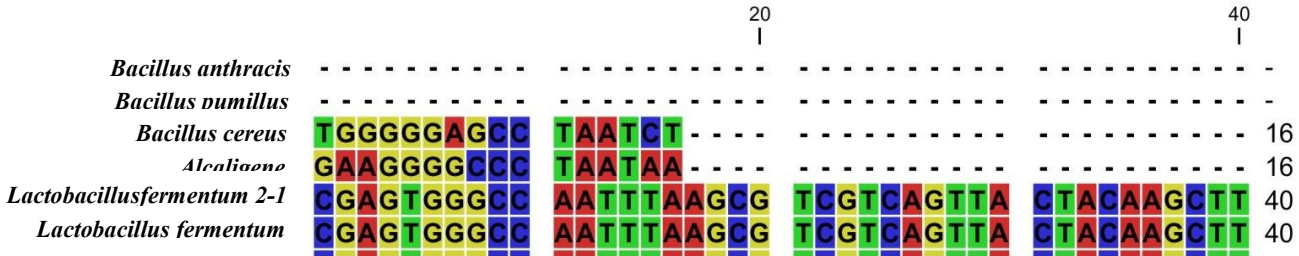


Fig. 4.1 (e) Breadfruit Isolates Alignment at 28±2^{o}C

4.15 Sensory Properties of Pigeon-pea- EnrichedBreadfruitProducts

Sensory assessment is countenance of individual`s like or dislike for product due to biological

difference in human and what is observed as suitable during evaluation. It is distinctive foundation of product evidence not simply acquired anyhow. This evaluatespeople responses to food samples based onquality attributes,lackinglabelling help, valuing and additional descriptions (Iwe, 2003).Table 4.16 and 4.17 show the mean sensory scores of pigeon-pea-fortified breadfruit products (breakfast mealand pizelle cookies). The fermented breadfruit-pigeon-pea breakfast mealat 28±2^{o}C for 24hassessedonaroma appearance, colour,taste and generalsuitability viahedonic scale(9-point).Organoleptic attributes of fermented blends shown (Table 4.16). Sample 553(breakfast meal produced from commercial flour blends) preferred to others based on appearance, colour, aroma, taste including overall acceptability (8.16) while sample 554 (7.68) which is the 10% pigeon-pea not statistically different at $p \leq 0.05$ after commercial.Trial558 (50:50 breadfruit: pigeon-pea) was least acceptable. Little differences were observedvia appearance, colour, aroma, taste and overall acceptability from other samples at 5% significant level. However, the samples were rated above average and scores higher than(Adebayo-Oyetoro *et al*., 2012) findings.Fermented Breadfruit flour enriched with pigeon-pea flour to make breakfast meal agreed with observations of Muoki *et al*. (2012); Monayajo and Nupo (2011) and Osho (2003)for cassava-based products improved with soybean.Olatidoye *et al*., (2010) also reported nutritional enhancement on a product enriched with soybean flour. Improvement in protein status of pigeon-pea enriched-breadfruit meal will havenutritivesignificance for developing countries such as Nigeria, where cost of protein-rich foods is higher. Badmus *et al*. (2006) corroborates the present findings that though breadfruit flour samples were improved nutritionally by enriching with pigeon-pea flour, this does not translate to consumer acceptance as shown. There is need for public enlightenment and sensitization on the nutritional quality and importance of new products in order to stimulate higher consumer's acceptance as suggested byOlaoye *et al.* (2006).

Table 4.17shows sensory evaluation of pigeon pea-enriched breadfruit breakfast meal at 37±1^{o}C for 24h.The observation shown substantial difference in the commercial meal sample and breadfruit meal samples in terms of general acceptability. Commercial meal rated highest (8.08) followed by breadfruit meal samples. This might be ascribed to consumer's familiarity with the commercial sample, which is processed from corn starch.The 10% pigeon-pea inclusion (6.44) rated next to commercial meal, but no significant difference among samples at 10%, 20% and 30% pigeon-pea flour supplementation in overall

acceptability.Overallsuitabilitygivespanelistotalopiniontomea1. Breakfast meal at50% fermented breadfruit: 50% fermented pigeon-pea hadthe lowest rating (Table 4.17).Hence, the observations suggest thatpigeon-pea-enriched breadfruit-based flour can be useful in food preparationsand desirable for making thinner gruels (Alake *et al*., 2016).Although, samples at 28±2^{o}C were rated better than this, but the ratings show that the new products can be acceptable commercially based on awareness.

The sensory scores of pigeon-pea-enriched breadfruit cookiesat 28±2^{o}C for 24h are shown in Table 4.18 and it was found that thecommercial cookie was rated higher (6.02) while the least was 50:50 of breadfruit and pigeon-pea (4.90). Chocolate pizzelle cookies produced from 10% and 50% pigeon-pea flour rated (5.80 - 4.90) where 5.80 is for 10% pigeon-pea flour supplementationand other values have no much difference on appearance, aroma, taste and texture (Table 4.18). Some of the mean scores above average through9-point hedonic scale show sample`s reasonably acceptability. Appearance as well as other sensory properties were not that bad.Appearancecan be definedasoneofthemost vitalfeaturesaffecting products acceptability bythe consumers(Suknark*etal*., 1998). Food acceptance hang on responds to consumer requests and satisfaction provided (Heldman, 2004). FAO (2006) suggested indigenousflour ingredientscan be includedto product with no negative impart toflavour,particle size,primitive and envisioned colourof product.

Breadfruit blends might be suitable in confectionery products and can replace starchy staples as well as imported foods of lower nutritive values.Sensory properties of pigeon-pea-enriched breadfruit pizzelle cookies at 37±1^{o}C for 24h showed (Table 4.19). Cookie from commercial sample had highest ratingbasedon overall acceptability (6.86). This was followed byfermented and unfermented breadfruit (100%). The 10% and 20% pigeon-pea inclusion not meaningfully different at 5% level (4.96, 4.80).The obtained results showeddecreasedin general acceptability as fermented pigeon-peainclusion increased. Adebayo-Oyetoro *et al*., 2017obtained similar findings for cookies processed from soybean and sorghum blends.Sample 377 (50% pigeon-pea) rated least considering aroma,appearance, taste, texture with overall acceptability.

Pigeon-pea inclusion influencedsensory qualities including cookies generalsuitability.Adebayo-

Oyetoro *et al*. (2017) had similar report on cookies processingusingsoybean and sorghum whileOkpala and Chinyelu (2011) reported the same trend on cookies evaluationin pigeon-pea and cocoyam.However, substitution of pigeon-pea flours up to 20% in cookies production in order to enhance nutritive value is feasible.

Table 4.16: Sensory Evaluation Scores of Breakfast Meal Processed from Pigeon-pea-

Enriched Fermented Breadfruit at28±2°C for 24h

Samples	Appearance	Taste	Colour	Aroma	Overall Acceptability
551	5.72± 1.10^{d}	5.84± 1.03^{d}	6.08± 1.35^{d}	5.80± 1.00^{c}	6.08± 0.81^{e}
552	6.68± 1.41^{b}	6.68± 1.14^{b}	6.68± 1.18^{b}	6.48± 1.63^{bc}	7.12± 0.93^{bc}
553	7.52± 1.16^{a}	7.88± 0.73^{a}	7.40± 1.08^{a}	7.24± 1.27^{a}	8.16± 0.62^{a}
554	6.88± 1.20^{b}	7.12± 1.09^{ab}	6.88± 1.05^{b}	6.92± 1.35^{ab}	7.68± 0.69^{b}
555	6.40± 1.53^{c}	6.48± 1.22^{c}	6.76± 1.13^{bc}	6.16± 1.03^{c}	7.04± 1.01^{c}
556	6.28± 1.46^{c}	6.80± 0.58^{b}	6.52± 1.16^{c}	6.64± 1.22^{b}	6.96± 0.84^{c}
557	5.60± 1.22^{d}	6.04± 1.51^{cd}	5.44± 1.35^{e}	5.64± 1.58^{cd}	6.60± 1.41^{d}
558	5.44± 1.04^{d}	5.92± 1.18^{d}	5.16± 1.11^{e}	5.40± 1.41^{d}	6.04± 0.97^{e}

Values within same columns with differentalphabet(s)were statistically differentat 5%

Key:

551 – Unfermented Breadfruit flour

552 – 100% Fermented Breadfruit Flour at 28±2°C for 24h.

553 – Commercial Flour

554 – 90% Fermented Breadfruit Flour: 10% Pigeon-pea at 28±2°C for 24h.

555 – 80% Fermented Breadfruit Flour: 20% Pigeon-pea at 28±2°C for 24h.

556 - 70% Fermented Breadfruit Flour: 30% Pigeon-pea at 28±2°C for 24h.

557 – 60% Fermented Breadfruit Flour: 40% Pigeon-pea at 28±2°C for 24h.

558- 50% Fermented Breadfruit Flour: 50% Pigeon-pea at 28±2°C for 24h

Table 4.17: Sensory Evaluation Scores of Breakfast Meal Processed from Pigeon-pea

Enriched Fermented Breadfruit at 37±1°C for 24h

Samples	Appearance	Taste	Colour	Aroma	Overall Acceptability
661	5.84± 0.94^{d}	6.24± 1.13^{b}	5.76± 1.01^{bc}	5.88± 1.20^{b}	6.12± 0.97^{b}
662	6.00± 0.64^{c}	5.40± 0.76^{c}	5.00± 0.91^{d}	4.84± 0.94^{d}	5.36± 0.64^{d}
663	8.12± 0.83^{a}	7.92± 0.64^{a}	8.12± 0.73^{a}	7.76± 0.66^{a}	8.08± 0.70^{a}
664	5.60± 1.44^{d}	6.12± 1.33^{b}	5.92± 1.18^{b}	5.48± 1.56^{c}	6.44± 1.33^{b}
665	5.84± 0.94^{d}	6.24± 1.13^{b}	5.76± 1.01^{bc}	5.88± 1.20^{b}	6.12± 0.97^{b}
666	6.52± 1.16^{b}	6.12± 1.05^{b}	6.00± 1.19^{b}	5.68± 1.25^{b}	6.08± 0.75^{b}
667	6.12± 1.13^{c}	5.88± 1.09^{bc}	5.92± 0.76^{b}	5.64± 0.64^{b}	5.72± 0.73^{c}
668	5.96± 1.10^{cd}	5.08± 1.26^{d}	5.48± 0.71^{c}	5.32± 1.42^{c}	5.56± 0.96^{c}

Values within same columns with different alphabet(s) were statistically different at 5%

Key:

661 – Unfermented Breadfruit flour.

662 – 100% Fermented Breadfruit Flour at 37±1°C for 24h.

663 – Commercial Flour

664 – 90% Fermented Breadfruit Flour: 10% Pigeon-pea at 37±1°C for 24h.

665 – 80% Fermented Breadfruit Flour: 20% Pigeon-pea at 37±1°C for 24h.

666 – 70% Fermented Breadfruit Flour: 30% Pigeon-pea at 37±1°C for 24h.

667 – 60% Fermented Breadfruit Flour: 40% Pigeon-pea at 37±1°C for 24h.

668 - 50% Fermented Breadfruit Flour: 50% Pigeon-pea at 37±1°C for 24h

Table 4.18: Sensory Scores of FermentedPigeon-pea-EnrichedBreadfruitPizzelle Cookieat 28±2°C for 24h

Samples	Appearance	Taste	Crispiness	Aroma	Overall Acceptability
240	6.44± 1.88[a]	5.64± 2.30[b]	5.42± 1.89[bc]	5.46± 1.85[bc]	5.42± 2.32[b]
241	5.30± 2.10 [ab]	5.10± 2.34[d]	5.10± 1.85[cd]	4.90± 1.92 [d]	4.98± 1.85[c]
242	6.54± 3.07[a]	6.52± 3.32[a]	6.48± 3.10[a]	6.22± 3.06[a]	6.02± 1.85[a]
243	5.94± 1.71[a]	5.48± 2.41[bc]	5.70± 2.10[b]	5.92± 1.60[a]	5.80± 2.32[a]
244	5.94± 1.75[a]	5.26± 2.44[c]	5.40± 1.78[c]	5.40± 2.08 [c]	5.52± 2.53[b]
245	5.46± 1.98[b]	5.32± 2.38[c]	5.46± 2.04[bc]	5.62± 1.85[b]	5.12± 2.43[c]
246	5.46± 1.98[b]	5.32± 2.38[c]	5.46± 2.04[bc]	5.62± 1.85[b]	5.12± 2.43[c]
247	5.04± 2.10[c]	4.96± 2.33[d]	5.02± 2.31[d]	5.36± 2.05 [c]	4.90± 2.53[c]

Values within same columns with diverse alphabet(s) were statistically different at 5%

Key:

240 – Unfermented Breadfruit flours

241 – 100% Fermented Breadfruit Flour at 28±2^{o}C for 24h.

242 – Commercial cookie

243 – 90% Fermented Breadfruit Flour: 10% Pigeon-pea at 28±2^{o}C for 24h.

244 – 80% Fermented Breadfruit Flour: 20% Pigeon-pea at 28±2^{o}C for 24h.

245 – 70% Fermented Breadfruit Flour: 30% Pigeon-pea at 28±2^{o}C for 24h.

246 – 60% Fermented Breadfruit Flour: 40% Pigeon-pea at 28±2^{o}C for 24h.

247- 50% Fermented Breadfruit Flour: 50% Pigeon-pea at 28±2^{o}C for 24 h

Table 4.19: Sensory Scores of Pigeon-pea Enriched Fermented BreadfruitPizzelle Cookie at 37±1^{o}C for 24h

Samples	Appearance	Taste	Crispiness	Aroma	Overall

					Acceptability
370	6.42± 1.86[b]	5.61± 1.28[b]	5.42± 1.87[b]	5.43± 1.83[b]	5.40± 1.31[b]
371	5.62± 1.47[c]	5.16± 0.10[c]	4.98± 1.30[c]	5.02± 1.97[d]	5.24± 1.85[b]
372	7.16± 1.32[a]	6.90± 1.54[a]	7.04± 1.36[a]	6.66± 0.79[a]	6.86± 0.85[a]
373	5.22± 1.08[c]	4.68± 1.38[d]	4.68± 1.28[cd]	5.14± 0.14[c]	4.96± 1.85[bc]
374	5.28± 1.03[c]	4.78± 1.41[d]	4.94± 1.31[c]	5.14± 2.15[c]	4.80± 1.85[c]
375	5.06± 1.14[cd]	4.86± 1.32[cd]	4.46± 1.31[d]	4.76± 1.87[e]	4.62± 1.85[c]
376	4.76± 0.18[d]	4.14± 1.24[d]	4.24± 1.16[e]	4.38± 0.22[e]	4.56± 1.85[c]
377	4.84± 0.32[d]	4.14± 1.04[d]	4.56± 1.08[d]	4.34± 0.16[e]	4.26± 1.85[d]

Means within same columns with dissimilar alphabet(s) are statistically different at 5%

Key:

370 – Unfermented Breadfruit flour.

371 – 100% Fermented Breadfruit Flour at 37±1^{o}C for 24h.

372 – Commercial cookie.

373 – 90% Fermented Breadfruit Flour: 10% Pigeon-pea at 37±1^{o}C for 24h.

374 – 80% Fermented Breadfruit Flour: 20% Pigeon-pea at 37±1^{o}C for 24h.

375 – 70% Fermented Breadfruit Flour: 30% Pigeon-pea at 37±1^{o}C for 24h.

376 – 60% Fermented Breadfruit Flour: 40% Pigeon-pea at 37±1^{o}C for 24h.

377- 50% Fermented Breadfruit Flour: 50% Pigeon-pea at 37±1^{o}C for24h.

Plate 4.2: Breadfruit – Pigeon-pea Breakfast Meal

Plate 4.3:Breadfruit-Pigeon-pea Pizzelle Cookie

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 General Conclusion

Generally,fermentationshownimprovements on nutrients and reduction ofanti-nutrients in breadfruit-pigeon-pea using different temperatures and durations. Reduction of antinutritional substances during fermentation at 28±2^{o}C and 37±1^{o}Cwere within permissible safe level as recommended by Codex Alimentarius Commission. There was establishment of *Lactobacillus plantarum and fermentum* which can serveas starter culture inproducts development at 28±2^{o}C and 37±1^{o}C.Some nutrients were negatively affected during the fermentation while some were not, because of long periods of fermentation. The data presented in this study in terms of chemical, functional, pasting properties, anti-nutrients including molecular characteristicsestablished potentials of using fermented breadfruit and pigeon-pea in producing varieties of convenience foods, formulations development and commercial starter culture.

Study also showed enrichment withfermented pigeon-pea at different percentage (10%, 20%, 30%, 40% and 50%)led to increase in nutrients of breadfruit. Precisely, enrichment improved the protein content which is insufficient in breadfruit and carbohydrate content decrease with increase pigeon- pea. Appreciable rise in protein level was perceived in breadfruit flour at 50% level of substitution. Sensory scoresfrom breakfast meal and pizzelle cookies revealed that the productsmight not be acceptable beyond 10-20% pigeon-pea substitution, but in presence of nutritional awareness percentage level may increase.

Worldwide, changes in consumer styles geared towards convenient foods andfoods with extra worth in form of health benefits.Also, presently in Nigeria, research has been focused on adding value to locally available crops as well as developing alternative ways of producing flour for confectionery products and complementary foods. Hence, enriched fermented breadfruit flour for producing breakfast meal and cookies could mitigate the level of wastages in breadfruitand reduce the protein insufficiency of this crop, then reducing problem ofmalnutrition among populace.In conclusion,more support for production of culturally familiar formsat numerous food shortage countries where fermented productsaccepted as foodnecessitate. Also, other processing techniques might help put this time- honoured staple crops back on the menu.

5.2 Recommendations

i. With the national policy of 10% cassava flour supplement for wheat flour in Nigeria, substitution of breadfruit and pigeon-peaup to 10% in complementary and convenience foods is recommended. This will alleviate hunger, improving and stimulating breadfruit-pigeon pea demand in largeand small scale industries.
ii. Based on observed nutritional improvement in enriched breadfruit flour, usage of breadfruit and pigeon pea in food formulations will be of advantage in reducingundernourishment.
iii. Future research should focus on value-additions that are breadfruit-pigeon-pea based for local and export markets.
iv. Additionalstudies should be carried out on breadfruit-pigeon-pea in molecular aspect.
v. Efforts should be made to convert breadfruit to storable form and commercializationshould proceed toimprove food and nutrition in Nigeria.

5.3 Contributions to knowledge

i. Proximate, physico-chemical, then samples functional propertiesas influencedby fermentation periods and temperature established.
ii. Study provided information on changes in nutrients and anti-nutrients fromfermented breadfruit and pigeon-pea.
iii. The fermentation conditions, periods for nutrients retention and reduction of anti-nutrients in breadfruit and pigeon-peadocumented.
iv. Dominant lactic acid bacteria (*Lactobacillus plantarum and fermentum*) detected might be useful as starter culture for other food production.
v. The research established thatpigeon-pea-enriched fermented breadfruit flours are highly nutritious and can be useful for food formulations.
vi. Breakfast meal and pizzelle cookies can be produced from fermented breadfruit-pigeon-pea blends.
vii. Processing methods for value-added products from underutilised crops like breadfruit and pigeon-peawas established.

REFERENCES

AACC. 1995. Approved methods of AACC (9th ed., Vols. 1 and 3). St. Paul, MN: *American Association of Cereal Chemists*.

Abbey, B.W. and Ibeh, G.O. 1988. Functional properties of raw and heat processed cowpea (*Vigna unguiculata* Walp) flour. *Advance Journal of Food Science and Technology.* 2.1: 41–44.

Achi, O. K. 2005. Traditional fermented protein condiments in Nigeria. *African Journal of Biotechnology.* 4.13: 1612-1621.

Adebayo, S.F. and Ogunsola, E.M. 2005. The proximate analysis and functional properties of fortified instant pounded yam flour. In: Proceed.29th Annual Conference of the Nigerian Institute of Food Science and Tech. (NIFST). 244.

Adebayo-Oyetoro,A. O., Adeyeye, S. A. O., Olatidoye,O. P., Ogundipe, O.O and Adenekan, O.E. 2017. Effect of Co-fermentation on the quality attributes of weaning food produced from sorghum (Sorghum bicolor) and pigeon pea *(Cajanus cajan*). *Journal of Culinary Science and Technology.* 17.2:118-135.

Adebayo - Oyetoro, A. O., Ogundipe, O. O., Ntuen, I. G. and Akinboyetan, B. E. 2012. Proximate composition, functional and sensory properties of breakfast meal from composite flours of African breadfruit(*T. africana*) and pigeon pea (Cajanus cajan).*Journal of Applied Agricultural Research.* 4.1: 173 – 178.

Adebowale, A.A., Sanni, S.A. and Oladapo, F.O. 2008.Chemical, functional and sensory properties of instant yam-breadfruit flour. *Nigerian Food Journal*. 26.1:2-12.

Adebowale, O.K. and Lawal, O. S. 2003. Functional properties and retrogradation behaviourof native chemically modified starch of Mucuna bean (*Mucuna pruriens*). *Journal ofFood Science and Agriculture.* 83: 1511–1516.

Adebowale, O. J. and Maliki, K. 2011. Effect of fermentation period on the chemical composition and functional properties of pigeon pea (*Cajanus cajan*) seed flour.*InternationalJournal of Food Research.* 18.4: 1329-1333.

Adebowale, Y.A., Adeyemi, I.A. and Oshodi, A.A. 2005. Variability in the physicochemical, nutritional and antinutritional attributes of six mucuna species. *Food Chemistry*. 89:37-48.

Adegbehingbe, K. T., Adetuyi, A.C. and Akinyosoye, F.A. 2014. Effect of fermentation on nutrient and anti-nutrient contents of ground-cooked lima bean (*Phaseolus lunatus*) seeds using *Bacillus subtilis* and *Bacillus pumilus*. *British Microbiology Research Journal. 4.11*: 1285-1298.

Adegbehingbe, K. T., Fakoya, S. and Adeleke, B. S. 2017. Effect of fermentation on nutrient and anti-nutrient contents of fermented whole and ground African breadfruit (*Treculia africana*) Seeds. *Microbiology Research Journal International.*20.5: 1-11.

Adegoke, G.O. 2004. Understanding Food Microbiology. 2nd ed. Alleluia Ventures, Ibadan.79-86.

Adegoke, G.O. and Babalola, A.K. 1988. Characteristics of micro-organisms of importance in the fermentation of *fufu* and *ogi* - two Nigerian foods. *Journal of Applied Bacteriology*. 65.6:449- 453.

Adekanmi, A.H., Gbadamosi, S.O. and Omobuwajo, T.O. 2012. Microbiological and physico-chemical characterictics of fufu analogue from breadfruit (Altocarpus altilis F). *Int.J. Food Sci. Technol*. 47.2:332-340.

Adeniran, H. A., Farinde, E. O. and Obatolu, V. A. 2013. Effect of Heat Treatment and Fermentation on Anti-Nutrients Content of Lima Bean (*Phaseolus Lunatus*) during production of daddawa Analogue. *Annual Review and Research in Biology.* 3.3: 256-266.

Adeniran, H.A. and Ajifolokun, O.M. 2015. Microbiological studies and sensory evaluation of breadfruit and cassava co-fermented into gari analogue. *Nigerian Food Journal*.33:39-47.

Adepeju, A. B, Gbadamosi, S. O, Adeniran, A. H and Omobuwajo, T. O. 2011. Functional and pasting characteristics of breadfruit (*Artocarpus altilis*) flours. *African Journal of Food Science.* 5.9: 529-535.

Adepeju, A. B., Gbadamosi S.O., Omobuwajo, T.O. and Abiodun, O.A. 2014. Functional and physico-chemical properties of complementary diets produced from breadfruit (*Artocarpus altilis*). *Afr. J. Food Sci. Technol*. 5.4:106-115.

Adepeju, A.B., Abiodun, O.A., Otutu, O.L. and Pele, I.G. 2015.Development and quality evaluation of wheat / breadfruit cookies. *International Journal of Technical Research and Applications.* 3: 8-12.

Adesokan, I. A., Fawole, A.O., Ekanola, Y.A., Odejayi, D. O. and Olanipekun, O.K. 2011. Nutritional and sensory properties of soybean fortified composite ogi- a Nigerian fermented cereal gruel.*African Journal of Microbiology Research.*5.20: 3144-3149.

Adewusi, S. R. A., Udio, J. and Osuntogun, B.A. 1995. Studies on the carbohydrate content of breadfruit (*Artocarpus commumis Forst*)from south-western, Nigeria.*Starch/Starke*. 47.8:289 – 294.

Adu-Gyamfi, J.J., Myaka, F.A., Sakala, W.D., Odgaard, R., Vesterager, J.M. and Hogh-Jensen, H. 2007. Biological nitrogen fixation and nitrogen and phosphorus budgets in farmer-managed intercrops of maize-pigeon pea in semi-arid southern and eastern Africa. *Plant and Soil*. 295:127-136.

Afoakwa, E. O., Adjonu, R. and Asomaning, J. 2010. Viscoelastic properties and pasting characteristics of fermented maize: influence of the addition of malted cereals. *International Journal of Food Science and Technology.* 45.2: 380-386.

Afoakwa, E.O., Paterson, A., Fowler, M. and Ryan, A. 2008. Flavour formation and character in cocoa and chocolate: a critical review. *Crit.Rev*. *Food Sci. Nutr*. 48.9: 840- 857.

Agbede, J.O. and Aletor, V.A. 2003. Evaluation of fishmeal replacedwith leaf protein concentrate in diets of broiler chicks. Effectson performance, muscle growth, haematology and serum metabolism. *InternationalJournal of Poultry Science.* 2:14–19.

Aguirre, M. and Collins, M.D. 2008. Lactic acid bacteria and human clinical infection. *Journal of Applied Bacteriology.*75.2: 95-107.

Aiyesanmi, A. F. and Oguntokun, M. O. 1996. Nutrient composition of *Dioclearеflexa* seed: an underutilized edible legume. *Rivista Italiana Delle Sostanze Grasse.* 73.11:521–523.

Ajanaku, K. O., Ajani, O., Siyanbola, T. O., Akinsiku, A. A, Ajanaku, C. O. and Oluwole, O. 2013. Dietary fortification of sorghum-ogi using crayfish (*Paranephrops planifrons*) as supplements in infancy. *Food Science and Quality Management*. 15: 1–10.

Ajani, A. O., Alade, O.A., Olagbaju, A.R., Fasoyiro, S.B., Arowora, K. A. and Oyelakin, M. O. 2016. Chemical and sensory qualities of stored gari fortified with soybean and groundnut. *Applied Tropical Agriculture*. 21.2: 79-83.

Ajani, A.O., Osundahunsi, O.F., Akinoso, R., Arowora, K.A., Abiodun, A. A. and Pessu, P. O. 2012. Proximate composition and sensory qualities of snacks produced from breadfruit flour. *Global J. of Science Frontier Research.* 12.7:10-18.

Akanbi, T.O. Nazamid, S. and Adebowale, A.A. 2009. Functional and pasting properties of a tropical breadfruit (*Artocarpus altilis*) starch from Ile-Ife, Osun State, Nigeria. *International Food Research Journal.* 16: 151-157.

Akande, K.E., Abubakar, M.M., Adegbola, T.A., Bogoro, S.E. and Doma, U.D. 2010. Chemical evaluation of the nutritive quality of pigeon pea (*Cajanus cajan* (L). Millsp.). *Int. J. Poultry Sci.* 9:63-65.

Åkerberg, C. and Zacchi, G. 2000. An economic evaluation of the fermentative production of lactic acid from wheat flour. *Bio-resource Technology*. 75.2: 119-126.

Akinjayeju, O. 2010. Humanand Applied Nutrition. Concept Publications Ltd, Lagos. 249.

Akintayo, E.T., Oshodi, A.A. and Esuoso, K.O. 1999. Effects of NaCl, ionic strength and pH on the foaming and gelation of pigeon pea (*Cajanus cajan*) protein concentrates. *Food Chemistry*. 66:51-56.

Akpata, M.I. and Akubor, P.I. 1999. Chemical composition and selected functional properties of sweet orange (*Citrus sinesis*) seed flour. *Plant Foods Hum. Nutri.* 54.4:353–362.

Akubor, P. I., Yusuf, D. and Obiegunam, J. E. 2013.Proximate composition and some functional properties of flour from the kernel of African star apple (*Chrysophyllual albidum). International Journal of Agricultural Policy and Research*. 1.3: 62- 66.

Akusu, O.M. and Wordu, G.O. 2016. Effect of soaking/fermentation periods on phytic acid polyphenolic content and invitrop and "moin-moin" prepared from cowpea. *International Journal of Research Studies in Biosciences.* 4.1: 21-27.

Alake, O.O., Babajide, J.M., Adebowale, A.A. and Adebisi, M.A. 2016. Evaluation of physico-chemical properties and sensory attributes of cassava enriched custard powder. *Cogent Food and Agriculture*. 2: 1246116.

Aletor, V.A. and Adeogun, O.A. 1995. Nutrients and antinutrient components of some tropical leafy vegetables. *Food Chemistry*. 54.4:375-379.

Alfaro, M.J, Alvaraz, I, Khor, E.L. and Padika, F.C. 2004. Functional properties of protein products from Barinus nut. *Am. J. Nutr*. 54:223-228.

Alonso, R., Orue, E. and Marzo, F. 1998. Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chemistry.* 63.4: 505–512.

Amoa-Awua, W.K.A. and Jakobsen, M. 1995. The role of Bacillus species in the fermentation of cassava. *Journal of Applied Bacteriology*. 79:250-256.

Amusa, N.A., Kehinde, I.A. and Ashaye, O.A. 2002. Bio-deterioration of breadfruit *(Artocarpus communis)* in storage and its effects on the nutrient composition. *Afri. J. Biotechnol*. 1.2: 57-60.

Anonymous. 2010. Tropical Africa`s Breadfruit Revolution AD. Accessed on 10 September, 2017 at 19:12.

Anteneh, T., Tetemke, M. and Mogessie, A. 2011. Antagonism of lactic acid bacteria against foodborne pathogens during fermentation and storage of borde and shamita traditional Ethiopian fermented beverages. *Int.Food Res. J*. 18.3:1189-1194.

AOAC. 2012. Official methods of analysis, Association of official analytical chemist. 19th edition, WashingtonD.C., USA.

AOAC. 2006. Official methods of analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD.

AOAC. 2005. Official methods of analysis of AOAC International. 18thed. Association of Official Analytical Chemists.Gaithersburgs, MD, USA.

AOAC. 2002. Official Methods of Analysis of AOAC International. 17th ed., Association of Official Analytical Chemists.Gaithersburgs, MD, USA.

AOAC. 2000. Official Methods of Analysis. 17thEdition. The Association of Official Analytical Chemists. Gaithersburgs, MD, USA.

Appiah, F., Oduro, I. and Ellis, W.O. 2016. Nutritional composition of breadfruits (*Artocarpus spp*. and *Treculia africana*) in Ghana. *Acta horticulturae, Proc. Int. Symp. on Horticulture in Developing Countries.*1-8.

Appiah, F. Oduro, I.and Ellis, W.O. 2011. Pasting properties of *Treculia africana* seed flour in Ghana and the production of a breakfast meal. *Agriculture and Biology Journal of North America.* 2.2: 325-329.

Appiah, F., Oduro, I. and Ellis, W.O. 2011a. Proximate and mineral composition of *Artocarpus altilis* pulp flour as affected by fermentation. *Pak. J.Nutr.* 10.7: 653-657.

Arawande, J. O. and Borokini, F. B. 2010. Comparative Study on Chemical Composition and Functional Properties of Three Nigerian Legumes (Jack Beans, Pigeon Pea and Cowpea). *Journal of Emerging Trends in Engineering and Applied Science*. 1: 89-95.

Aremu, M.O, Olaofe, O. and Akintayo, E.T. 2007. Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. *Journal of Food Technology.* 5.2: 109-115.

Arimah, B.D., Ogunlowo, O.P., Adebayo, M.A. and Jesumirhewe, C. 2014. Identification of lactic acid bacteria isolated from selected Nigerian foods and comparison oftheir bacteriocins activities. *International Journal of Pharmaceutical and Clinical Research*. 6.1: 20-26.

Asfaw, Z. and Nigatu, A. 1995. Home-gardens in Ethiopia: characteristics and plant diversity. *SINET*. 18.2:235-266.

Asif-Ul-Alam, S.M., Islam, Z.M., Hoque, M.M. and Monalisa, K. 2014. Effect of drying onthe Physicochemical and functional properties of greenbanana (*Musa Sapientum*) flour and development of baked product. *American Journal of Food Science and Technology.* 2.4:128– 133.

Atobatele, O.B. and Afolabi, M.O. 2016. Chemical composition and sensory evaluation of cookies Baked from the blends of soya beans and maize flours. *Applied Tropical Agricultural* (special issue). 21.2: 8-13.

Awolu, O.O. 2017. Optimization of the functional characteristics, pasting and rheological properties of pearl millet-based composite flour. *Heliyon*. 3.2:240.

Aworh, O.C. 2008. The Role of Traditional Food Processing Technologies in National Development: The West African experience. In: Using Food Science and Technology to Improve Nutrition and Promote National Development. Chapter 3 (Robertson, G.L. & Lupien, J.R. Eds.), *International Union of Food Science and Technology* (IUFoST). Chapt.3

Awoyemi, T.O. 2012. A Study of Properties of Composite Meal from Breadfruit and Cassava. Unpublished M.Sc.Thesis, Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Ayad, E.H.E., Nashat, S., El-Sadek, N., Metwaly, H. and El-soda, M. 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. *Food microbiology*. 21.6: 715-725.

Ayo-Omogie, H. N.and Ogunsakin, R. 2013. Assessment of Chemical, Rheological and Sensory Properties of Fermented Maize-Cardaba Banana Complementary Food. *Food and Nutrition Sciences*. 4: 844-850.

Babu, K.R. and Satyanarayana, T. 1996. Production of Bacterial Enzymes by Solid-State Fermentation. *Journal of Scientific and Industrial Research*. 55(5-6): 464-467.

Bakare, H. A., Osundahunsi, O. F. and Adegunwa, M. O. 2012. Composition andPasting Properties of Breadfruit (*Artocarpus communis* Forst) from South-West States of Nigeria. *Nigerian Food Journal.* 30.1:11–17.

Balagopalan, C., Padmaja, G.,Nanda, S.K. and Moorthy, S.N. 1988. Cassava in food, feed and industry. C.R.C. Press, Boca Raton, FL., USA. 189-196.

Balogun, M.A., Kolawole, F. L., Karim, O. R. and Fasakin, T. B. 2016. Proximate composition, microbial quality and consumer acceptability of gruel from fermented maize and soybean. *Applied Tropical Agriculture* (Special Issue). 21. 2:53-57.

Batt, C.A. 2000. *Bacillus cereus*. In: Encyclopedia of Food Microbiology. 119-124. (R. K. Robinson*et al*., eds.). Academic Press, London.

Beaumont, M. 2002. Flavouring composition prepared by fermentation with *Bacillusspp*.*Int. J. Food Microbiol.* 75.3:189-196.

Bodner, G., Loiskandl, W. and Kaul, H.P. 2007. Cover crop evapotranspiration under semi-arid conditions using FAO dual crop coefficient method with water stress compensation. *Agricultural Water Management*. 93:85-98.

Boonmee, M., Leksawasdi, N., Bridge, W. and Rogers, P.L. 2003.Batch and continuous culture of *Lactococcus lactic* NZ133:experimental data and model development. *Biochem. Eng. J.* 14: 127-135.

Camille, G., David, E., Hank, G. and George E. 2011. How understanding of local challenges and opportunities influenced the design of post-harvesting technology in Haiti. *Open Catalyzing Innovation*. Washington DC. 1-10.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K. 2001. Quality control *Lactobacillus* strains for use with the API50 CH and APIZYM systems at 37^{o}C. *J. Basic Microbiol*. 41.5: 241–251.

Chen, Y., Wang, Z., Chen, X., Liu, Y., Zhang, H. and Sun, T. 2010. Identification of angiotensin I-converting enzyme inhibitory peptides from koumiss, a traditional fermented mare's milk. *Journal of Dairy Science*. 93.3:884–892.

Chikwendu, N. J. 2005. Production and Availability of Ground bean (*Kerstingiella geocarpa*) in a Tropical Rural Nigerian Community: Implication for Nutrition Education and National Development. *J. Home Economics Research.* 6.1: 135-141

Chinma, C.E., Adewuyi, O. and Abu, J.O. 2009. Effect of germination on the chemical, functional and pasting properties of flour from brown and yellow varieties of tigernut (*Cyperus esculentus*). *Food Research International.* 42.8: 1004-1009.

Chung, Y., Wang, J., and Yu, Y. 1998. Effect of Gamma-Ray Irradiation on the Physicochemical Properties of Flour and Starch Granule Structure for Wheat. *International Journal of Food Science and Technology*. 44:674–680.

C.I.A.T. 2001. Information systems on postharvest management and processing of cassava: cassava and its by-products. Centro internacional de Agriculture Tropical. Cali, Colombia.

Clarridge, J.E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Review*.17.4:840-862.

CodexAlimentarius International Food Standards 1995.General standard for contaminants and Toxins in Food and Feed (CODEX STAN 193-1995) Adopted in 1995. Revised in 1997, 2006, 2008, 2009.Amendment 2010, 2012, 2013, 2014. 1-20.

Coffman, C. W. and Garcia, V. V. 1977. Functional properties and amino acid content of protein isolate from mungbean flour. *J. Food Tech*. 12:473-484.

Daniel, J.N. and Ong, C.K. 1990. Perennial pigeon-pea: a multi-purpose species for agroforestry systems. *Agroforestry Systems*. 10: 113 – 129.

De martinis, E. C. P., Alves, V. F. and Franco, B. D. G. M. 2002. Fundamentals and perspectives for the use of bacteriocins produced by Lactic Acid Bacteria in meat products. *Food Reviews International*. 18.2-3: 191-208.

Deivanai, S. and Subhash, J.B. 2010. Breadfruit (Artocarpus altilis Fosb.). An underutilized and neglected fruit plant species. *Middle-East Journal of scientific Research*. 6.5:1-13.

Dengate, H.N. 1984. Swelling, pasting and gelling of wheat starch. In: Pomeranz,

Y(Ed.).*Advances in Cereal Science and Technology*. America Association of Cereal Chemist (AACC) St. Paul, MN, 49-71.

Dragsted, L.O., Strube, M. and Larsen, J.C. 1993. Cancer-protective factors in fruits andvegetables: biochemical and biological background. *Pharmacol. Toxicol.* Suppl. 1:116–135.

Dresbach, R. H. 1980. Handbook of poisoning, prevention, diagnosis and treatment. Lange Medical Publication, LA California, 488-504.

Echendu, C.A., Onimawo, I.A. and Somtochi, A. 2004. Production and evaluation of doughnuts and biscuits from maize-pigeon pea flour blends. *Nigerian Food Journal.*22: 147-153.

Edema, M.O.,Sanni, L.O. and Sanni, A.I. 2005. Evaluation of maize – soybean flour blends for sour maize bread production in Nigeria. *African Journal of Biotechnology*. 4.9:911-918.

Ehsan, K., Ehsan, O., Rudi, H. and Jaafar, H.Z.E. 2010. Solid state fermentation effects on Pistachio Hulls antioxidant activities. Presented in the 3rd International Conference for Value Added Agricultural Products (3rd FerVAAP Conference). *KKU Research Journal.*15.5: 360 – 366.

Ejigui, J., Savoie, L., Marin, J. and Desrosiers, T. 2005. Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and anti-nutritional factors of yellow maize (Zea mays). *Journal of Biological Sciences*. 5.5: 590-596.

Eke, O. S. and Akobundu, E. N. T. 1993. Functional properties of African yam bean (*Sphenostylis stenocarpa*) seed flour as affected by processing. *FoodChemistry*. 48.4:337-340.

Ekpenyong, T.E. 1985. Chemical composition and amino acid content of African breadfruit (*Treculia africana Decne*). *Food Chem*. 17:59 – 64.

Elangovan, V., Sekar, N., and Govindasamy, S. 1994. Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene-induced tumorigenesis. *Cancer Lett*. 87:107-113.

Elegbede, J.A. 1998.Legumes. In:Osagie, A.L and Eka, O.U (Eds.), Nutritional Quality of Plant Food. Post-Harvest Research Unit, Department of Biochemistry, University of Benin, Benin City, Nigeria. 53-93.

Elkhalifa, A.O., Schiffler, B and Bernhardt, R. 2005. Effect of fermentation on the functional properties of sorghum flour. *Food Chem.*92.1:1–5.

Elliason, D.J. and Tatini, S.R. 1999. Enhanced inactivation of Salmonella typhimurium and verotoxigenic Escherichia coli by nisin at 65^{o}C. *Food Microbiol*. 16.3: 257-67.

Eneche, E.H. 2009. Biscuit-making potential of millet/pigeon pea flour blends. *Plant Foods Hum. Nutr.*, 54.1: 21-27.

Ene-Obong, H.N. and Obizoba, I. C. 1996. Effect of domestic processing on the cooking time, nutrients, anti-nutrients and invitro protein digestibility of the Africa yam bean (*Sphenostylisstenocarpa*). *Plant Food Hum.Nutr*. 49.1:43-52.

Esuoso, K.O. and Bamiro, F.O. 1995. Studies on baking properties of non-wheat flours--1. Breadfruit (*Artocarpus artilis*). *International Jour. of Food Sci. and Nutrition.* 46.3: 267 – 273.

Etok, C.A., Ado, S.A. and Eja, M.G. 2005. General Microbiology practical manual university of Coloban.Published by the Society for General Microbiology, Marlborough House, Basingstoke Road,Spencers Wood, ReadingRG7 1AG, UK. 44 – 49.

Fagbemi, T.N., Oshodi, A.A. and Ipinmoroti, K.O. 2005. Processing effects on some antinutritional factors and In vitro multienzyme protein digestibility (IVPD) of three tropical seeds: breadnut (*Artocarpus altilis*), cashewnut (*Anacardiumoccidentale*) and fluted pumpkin (*Telfairia occidentalis*). *Pakistan Journal of Nutrition*. 4.4:250-256.

Fagbemi, T.N. 1999. Effect of blanching and ripening on functional properties of plantain (*Musaaab*) flour. *PlantFoods Hum. Nutr.* 54.3: 261- 269.

Falade, K.O and Ogunwolu, O.S. 2014. Modelling of drying patterns of fresh and osmotically pretreated cooking banana and plantain slices.*Journal of Food Processing and Preservation.* 38.1:373-388.

FAO. 2006. Starch Market adds value to cassava: spotlight/2006. www.fao.org/ag/magazine/ Accessed 25 October 2014.

FAO/WHO. 1998. Carbohydrates in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation, 14–18 April 1997, Rome. FAO Food and Nutrition Paper No. 66. Rome.

FAO. 1992. Small-scale processing of microbial pesticides.*Agricultural services bulletin No.96.*

FAOSTAT. 2011. FAO Statistical Yearbook. World food and Agriculture, Rome, Italiy. Accessed 21/07/2014.

Farhat, I.A., Oguntona, T and Roger, N. J. 1999. Characterization of starches from West African Yams. *Journalof the Science of Food and Agriculture.* 79.15: 2105-2112**.**

Fasasi, O.S, Adeyemi, I. A. and Fagbenro, O.A. 2007. Functional and Pasting Characteristics of Fermented Maize and Nile Tilapia (*Oreochromisniloticus*) Flour Diet. *Pak. J. Nutri*., 6.4: 304-309.

Fasoyiro, S. B., Farinde, E.O, Adiat, W.T., Zaka, K.O. and Ajani, A.O. 2013. Proximate Composition and Organoleptic Properties of Cassava Snacks fortified with pigeon pea flour.*Science Focus.* 18.1:1- 9.

Fasoyiro, S.B., Akande, S.R., Arowora, K.A., Sodeko, O.O., Sulaiman, P. O. Olapade, C.O. and Odiri, C.E. 2010. Physico-chemical and sensory properties of pigeon pea (*Cajanus caja*n) flours. *Afr. J. Food Sci.* 4.3:120 – 126.

Fasoyiro, S.B., Obatolu, V. A., Ashaye, O. A., Farinde, E.O. and Arowora, K. A. 2009b. Chemical composition of different varieties of pigeon pea (*Cajanus cajans*) growing in South -West Nigeria. Proceeding of Food Data Conference. S3-P5, 1-3rd October, 2009. Mahidol University. Bangkok, Thailand.

Fasoyiro, S.B., Obatolu, V.A., Asaye, O.A., Adeojo, E.A. and Ogunleti, D.O. 2009. Chemical and Sensory qualities of Pigeon Pea (Cajanus cajan) developed into a local spice dawadawa'- Niger. *Food J.* 27.2: 150-158.

Fereidoon, S. 2014. Beneficial health effects and drawbacks of antinutrients and phytochemicals in foods. *Appl Microbiol Biotechnol.*97:45-55

Flemming, H.P. and McFeters, R.F. 1981. Use of microbial cultures: vegetable products. *Food Technology*. 35.1: 84.

Foodnet 2002.R-Directory-of-commodities: Pigeon-pea. http://www.foodnet.cgiar.org/market/Tropcomm/part2R/htm. Accessed 27 March, 2007.

Francis, G., Makkar, H.P.S., and Becker, K. 2001. Antinutritional factors present in plant-derivedalternate fish feeding ingredients and their effects in fish. *Aquaculture.* 199.3-4:197-227.

Fry, N. K., Warwick, S. Saunders, N. A. and Embley, T. M. 1991. The use of 16S ribosomal RNA analyses to investigate the phylogeny of the family Legionellaceae. *J. Gen. Appl.Microbiol*. 137.5: 1215–1222.

Gemede, H.F. and Ratta, N. 2014. Anti-nutritional factors in plant foods: Potential health benefits and adverse effects.*International Journal of Nutrition and Food Sciences.*3.4:284-289.

Ghadge, P.N., Shewalkar, S.V. and Wankhede, D.B. 2008. Effect ofprocessing methods on qualities of instant whole legume: Pigeon pea(*Cajanus cajan L.*). *Agricultural Engineering International. The CIGRe-journal manuscript.* 08004.

Giami. S.Y. Amasisi, T and Ekiyor, T. 2004. Comparison of bread making properties of composite flour from kernels of roasted and boiled African breadfruit *(Treculia africana decne*) seeds. *Journal of Material Research.* 1.1: 16-25.

Goering, K.J. and Dehass, B. 1990. Properties of the small granule starch from *Colocasiaesculenta*. *Starke.* 21:772-791.

Golden, K.D. and Williams, O.J. 2001. Amino acid, fatty acid and carbohydrate content of *Artocarpus altilis* (breadfruit). *J. Chromatogr. Sci*. 39.6:243–250.

Gomes, A.M.P., Malcata, F.X., Klaver, F.A.M. and Grande, H.G. 1995. Incorporation survival of *bifidobacterium sp*. strain Bo and *lactobacillus acidophilus* strain Ki in a cheese product. *Netherlands Milk Dairy Journal.* 49.2-3:71-95.

Gomez-Gil, B., Thompson, F.L., Thompson, C.C., Garcia-Gasca, A., Roque, A., and Swings, J. 2004. Vibrio hispanicus sp. nov., isolated from Artemia sp. and sea water in Spain.*Int. J. Syst. Evol*. *Microbiology.*54.1:261-265.

Graham, H.D. and Negron de Bravo,E. 1981. Composition of the Breadfruit. *J. Food Science*. 46.2: 535 – 539.

Granito, M., Frias, J.,Doblado, R., Guerra, M., Champ, M., and Vidal-valverde, C. 2002. Nutritional improvement of beans (*Phaseolus vulgaris*) by natural fermentation. *Eur. Food Res. Technol.*214.3: 226-231.

Griffiths, D. W. 1979. The inhibition of enzymes by extract of field beans, *Viviz feba. Journal of Agric. Food Chem*.28:459-461

Guasch-Jane. M.R., Andrés-Lacueva, C., Jáuregui, O. and Lamuela- Raventos, R.M. 2006. First evidence of white wine in ancient Egypt from Tutankhamun's tomb. *J. Archaeol. Sci*. 33.8: 1075-1080.

Halliwell, B. 1997. Antioxidants and human disease: a general introduction. *Nutr. Rev.* 55.1:S49–52.

Hammes, W.P., Weiss, N and Holzaptel, W.P. 1992. Lactobacillus and carnobacterium. In: A. Balows, H.G. Truper, M. Dworkin, W. Harder and K-H. Schleifer (Eds.). The prokaryotes, 2nd ed Springer-Verlag, New York, USA.1535-1594.

Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis, Chapman and Hall New York. 185-286.

Harinder, K., Kaur, B. and Sharma, S. 1999**.** Studies on the bakingproperties of wheat: Pigeon pea flour blends. *Plant Foods for Human Nutrition*. 54.3: 217 – 226.

Harland, B. F. and Oberleas, D. 1977. A modified method for phytate analysis using an ion-exchange procedure: Application to textured vegetables proteins. *Cereal Chem*. 54:827-832.

Harrigan, W.F. and McCance, M. E. 1976. Laboratory Methods in Food and Dairy Microbiology, Second Ed. Academic Press, London.

Hedberg, M., Hasslöf, P., Sjöström, I., Twetman, S. and Stecksén-Blicks, C. 2008. Sugar fermentation in probiotic bacteria-an in vitro study. *Oral Microbiology Immunology.* 23.6: 482-485.

Heldman, D.R. 2004. Identifying food science and technology research needs. *Food Technology*. 58.12:32-34.

Hithamani, G. and Srinivasan, K. 2014. Effect of domestic processing on the polyphenol content and bioaccessibility in finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum). Food Chem. 164: 55-62.

Holzapfel, W.H., Haberer, P., Geisen, R., Björkroth, J. and Schillinger, U. 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. *American Journal of Clinical Nutrition.*73: 365-373.

Hu, F.B. 2003. Plant-based foods and prevention of cardiovascular disease: an overview.*Am. J. Clin. Nutr* .78.3: 544S-551S.

Hugenholtz, J, and Smid, E.J. 2001. Improving nutrition - Researchers believe fermentation of micro-organisms could be the key to enhancing nutritional content in dairy foods. *Dairy Ind. Int.*66: 32-33.

Hugo, L. F., Rooney, L. W and Taylor, J. R. N. 2000. Malted Sorghum as a functional ingredient in composite bread. *Cereal Chem.*77.4:428–432.

Igbabul, B.D., Bello, F. A. and Ani, E. C. 2014. Effect of fermentation on the proximate composition and functional properties of defatted coconut (*Cocosnucifera* L flour.*Sky Journal of Food Science*. 3.5: 34 – 40.

Ige, M. M.2017**.** Physicochemical, pasting and sensory characteristicsof complementary foods formulated from plantain,pigeon pea and maize flours. *Donnish Journal of Food Science and Technology.* 3. 2: 7-15.

Igene, F.U., Oboh, S.O. and Aletor, V.A. 2005. Effects of some processing techniques on the functional properties of winged bean seed flours. *J. Food Agric*. *Environ*. 3.2: 28-31.

Ihekoronye, A. I. and Ngoddy, P. O. 1985. In Integrated Food Science and Technology forthe Tropics. London: Macmillan Education Ltd. 224-229.

Ijarotimi, S. O. and Aroge, F. 2005. Evaluation of the nutritional composition, sensory and physical properties of a potential weaning food from locally available food materials – breadfruit (*artocarpus altilis*) and soybean (*glycine max*).*Pol. J. Food Nutr. Sci.*14/55.4: 411–415.

Ikegwu, O.J., Nwobasi, V.N., Odoh, M.O. and Oledinma, N.U. 2009. Evaluation of the pasting and some functional properties of starch isolated from some improved cassava varieties in Nigeria. *Afr. J. Biotechnol*. 8.10: 2310-2315.

Ikemefuna, C., Obizoba, J., and Atii, J.V. 1991. Effects of soaking, sprouting, fermentation and cooking on nutrient composition and some antinutritional factors of sorghum (*Guinesia*) seeds. *PlantFoods for Hum. Nutr*. 41: 203-212.

Ikujenlola, V.A. and Fashakin, J.B. 2005. The physico-chemical properties of acomplementary diet prepared from vegetable protein. *Journal of Food Agriculture and Environment.* 3.4:23-26.

Ilori, M.O. and Irefin, L.A. 1997. Development of non-alcoholic beverages from some tropical crops. Master Brewers Association of the Americans (MBA).Technical Quarterly. 34.4: 229– 234.

Ishaya, F.A. and Oshodi, A.A. 2013. The Proximate Composition and Sensory Evaluation of the flours of breadfruit (*Artocarpus altilis*), benth seed (*Adenopus breviflorus*) and their composite bread. *Chemistry and Materials Research*. 3.9: 79-83.

Ishola, R.O. and Adebayo-Tayo, B. C. 2012. Screening of Lactic Acid Bacteria Isolated from Fermented Food for Bio-molecules Production.*AU J.T.* 15. 4: 205-217.

Iwe, M. O. 2003. The Science of Soybean Chemistry Processes and Utilization. In:*The Science and Technology of Soybean Chemistry, Nutrition, Processing and Utilization.*1st Ed. Rojoint Communication Services Ltd, Uwani, Enugu.

James, C. J. 1995. The Analytical Chemistry of Foods. Chapman and Hall, New York. 86.

James, S and Nwabueze, T. U. 2014. Influence of Extrusion Condition and Defatted Soybean Inclusion on the Functional and Pasting Characteristics of African Breadfruit (Treculia Africana) Flour Blend.*Food Science and Quality Management.* 34:1-33.

Jeevaratnam, K., Jamuna, M. and Bawa, A.S. 2005. Biological preservation of foods –

Bacteriocin of lactic acid bacteria. *Indian J. Microbiol.* 4:446-454.

Jideani,I.A. andJideani,V.A.2006. Laboratory manual of food bacteriology. Amana printing and Advertising Ltd, Kaduna. 90-112.

Jimoh, F.O. and Oladiji, A.T. 2005. Preliminary Studies on Piliostigma thonningii seeds: Proximate analysis, mineral composition and phytochemical screening. *African Journal of Biotechnology.* 4.12:1439-1442.

Jimoh, K. O. and Olatidoye, O. P. 2009. Evaluation of physicochemical andrheological characteristics of soybean fortified yam flour. *Journal of AppliedBiosciences.*13: 703 – 706.

Kesmen, Z., Yetiman, A.E., Gulluce, A., Kacmaz, N., Sagdic, O., Cetin, B. and Yetim, H. 2012. Combination of culture-dependent and culture-independent molecular methods for the determination of lactic microbiota in Sucuk. *International Journal of Food Microbiology.* 153.3: 428-435.

Kerr, B. 2009. Breadfruit. [http://globalgardensfoundation.org/wordpress/?page_id=127 Breadfruit].Accessed on 2nd January, 2010.

Khandelwal, S., Udipi, S.A. and Ghurge, P. 2010. Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. *Food Research International.* 43.2:526-530.

Kim, Y. S., Wiesenborn, D. P., Orr, P. H. and Grant, L. A. 1995. Screening potato starch for novel properties using differential scanning calorimetry. *Journal of Food Science.*60.5: 1060–1065.

Kleerebezemab, M., Hols, P. and Hugenholtz, J. 2000. Lactic acid bacteria as a cell factory: rerouting of carbon metabolism in *Lactococcus lactis* by metabolic engineering. *Enzyme Microb. Technol.* 26. 9-10: 840-848.

Kobawila, S.C, Louembe, D., Keleke, S., Hounhouigan, J. and Gamba, C. 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *AfricanJournal of Biotechnology.* 4.7: 689-696.

Konings, W. N., Kok, J., Kuipers, O. P. and Poolman, B. 2000. Lactic acid bacteria: the bugs of the new millennium. *Current Opinion in Microbiology*. 3.3: 276-282.

Lasekan, O.O. and Shabnam, M. 2013. Effect of traditional fermentation as a pretreatment to decrease the antinutritional properties of rambutan seed *(Nephelium lappaceum L.). International Conference on Food and Agricultural Sciences.* 55.13:67–72.

Laurentiu, B., Benten, W.P.M., Engelhardt, E., Ko¨hrer, K., Gougoula, C. and Sager, M. 2014. 16S ribosomal DNA sequence-based identification of bacteria in laboratory rodents: a practical approach in laboratory animal bacteriology diagnostics. *Lab. Anim*. 48: 305–312.

Lawal, O.S. 2004. Composition, physicochemical properties and retrogradation characteristics of native, oxidised, acetylated and acid-thinned new cocoyam (*Xanthosoma sagittifolium*) starch. Food Chemistry. 87:205-218.

Leszek, M. 2011. Extrusion-cooking techniques applications, theory and sustainability. WILEY-VCH Verlag & Co. KGaA, Weinheim, Germany; 2011.

MacFarland, L. 2000. A review of the evidence of health claims for biotherapeutic agents. *Microbial Ecology in Health and Disease*. 12.2: 65-76.

Macrae, R., Robinson, R.K. and Sadler, M.J. 1993. Encyclopedia of Food Science,*Food Technology and Nutrition.* Academic Press, London. 3:2120-2123.

Makinde, M.A., Elemo, B.O., Arukwe, U. and Pellett, P. 1985. Ukwa seed (*Treculia Africana Decne*) protein I: Chemical evaluation of the protein quality. *J. Agric. Food Chem*. 33:70-72.

Mayaki, O. M., Akingbala, O. J., Baccus – Taylor, G. S. H. and Thomas, S.C. 2003. Evaluation of breadfruit (*A. communis*) in traditional stiff porridge foods. *Food, Agriculture and Environment.* 1.2:54-59.

Maziya-Dixon, B.,Sanni, L.O., Adebowale, A. A., Onabanjo, O. O. and Dixon, A.G. O. 2005. Effect of variety and drying methods on proximate composition and pasting properties of highquality cassava flour from yellow cassava roots. *In Proceedings ofAfrican Crop Science Conference Proceedings*. Enttebe, Uganda. 5-9

Mbata, T. I., Ikenebomeh, M. J. and Alaneme, J. C. 2009b. Studies on the microbiologial,

nutrient composition and anti-nutritional contents of fermented maize flour fortified with bambara groundnut (*Vigna subterranean* L.). *African Journal of Food science.* 3.6:165–171.

Mbata, T. I., Ikenebomeh, M. J. and Ezeibe, S. 2009. Evaluation of mineral content and functional properties of fermented maize (*Zea mays*) flour blended with bambara groundnut (*Vigna subterranean L*).*Afri. J. Food Sci*. 3.4:107-112.

Medlicott, A.P. 2002. Post harvest handling of breadfruit. http://wwwfintrac.com/gain/guides/ph/postbrdf.html.

Mensah, P. and Tomkins, A. 2003. Household-level technologies to improve the availability and preparation of adequate and safe complementary foods. *Food Nutr. Bull*. 24.1: 104-125.

Mensah, P.P., Tomkins, A.M., Drasar, B.S. and Harrison, T. J. 1990. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. *Lancet*.336: 140-143.

Modu, S., Ibrahim Z., Hajjagana L., Falmata, A. S., Babagana, M. and Bintu, B. P. 2013. Production and evaluation of weaning meal from fermented red maize fortified with cowpea. *Academia Journal of Food Research*. 1.3:50–58.

Monayajo, S. A. and Nupo, S. S.2011. Nutrient composition and acceptability of "Pupuru" fortified with soy flour. *Journal of Agriculture and Veterinary Sciences*.3: 346–423.

Moneim, A., El khalifa, O. and El Tinay, A.H. 1995. Effect of Fermentation and Germination on the invitro protein digestibility of low and high tannin cultivars of sorghum. *Food Chem.* 54.2: 147-150.

Moorthy, S. N. 2002. Physicochemical and functional properties of tropical tuberstarches: A review. *Starch*. 54.12: 559-592.

Morton, J.F. 1987. Breadfruit: Fruits of warm climates. In proceedings of the sixth National Symposium on creating markets for economic development of new crops and new uses, Purdue University, Miami, USA, 50-58.

Mountney, G. J. and Gould, W.A. 1988. Practical Food Microbiology and Technology. AVI

Books, Van Nostrand Reinhold Company, New York, USA.

Mugula, J.K., Nnko, S.A., Narvhus, J.A. and Sorhaug, T. 2003. Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. *International Journal of FoodMicrobiology*. 80.3: 187-199.

Mukesh, S.S., Boey, J. H., Kumutha, S., Bavani, D.V., Ling Kar, Y. and Kaveti, B. 2014. A Review on *Artocarpus altilis* (Parkinson) Fosberg (breadfruit). *Journal of Applied Pharmaceutical Science*. 4.8: 91-97.

Muoki, R.C., Paul, A., Kumari, A., Singh, K. and Kumar, S. 2012. An improved protocol for the isolation of RNA from roots of tea (*Camellia sinensis* (L.) O. Kuntze). *Molecular Biotechnology.* 5.21:82-88.

Narayana, K. and Narasinga Rao, M. S. 1982. Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour.*J. Food Sci.* 47.5: 1534-1538.

Ngaba, P. R. and Lee, J. S. 1979. Fermentation of cassava (Manihot esculenta Crantz). *J. Food Sci.* 44.5: 1570-1571.

Nguyen, Q. and Eun, J. 2011. Antioxidant activity of solvent extracts from Vietnamese medicinal plants. *Journal of Medicinal Plants Research*. 5.13: 2798-2811.

Noonan, S.C. and Savage, G.P. 1999. Oxalate content of foods and its effects on humans. *AsiaPac.J.Clin. Nutr*. 8.1:64-74.

Nout, M.J.R. and Motarjemi Y. 1997. Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control*. 8.5-6: 221- 226.

Nwaigwe, J. O. and Adejumo, B. A. 2015.Qualities of African breadfruit (*Treculia Africana*) seed flour as influenced by thermal processing methods and duration. *International Journal of Technology Enhancements and Emerging Engineering Research*. 3.4:102-108.

Nwokolo, E. 1996. The need to increase consumption of pulses in the developing world. In: Nwokolo, E.A., Smart, J. Food and Feed from legumes and oil seeds.Chapman and Hall, London.3-11

Obasi, N. E. and Wogu, C. O. 2008. Effect of soaking time on proximate, mineral compositions and anti-nutritional factors of yellow maize (*Zea mays*). *Nigerian Food Journal*, 26.2: 70-77.

Obizoba, I.C. and Atii, J.V. 1991. Effect of soaking, sprouting, fermentation and cooking on nutrient composition and some anti-nutritional factors of sorghum (*Guinesia*) seeds. *Plant Foods for HumanNutrition.* 41.3:203-212.

Odeny, D.A. 2007. The potential of pigeonpea (*Cajanus cajan* (*L*.) *Millsp*.) in Africa. *Natural Resources Forum.* 31.4:297–305.

Odoemelam, S.A. 2003. Chemical composition and functional properties of conophor nut (*Tetracarpidium conophorum*) flour. *Int. J. Food Sci. Technol*. 38.6: 729-734.

Ogungbenle, H.N. 2009. Chemical and amino acid composition of cooked walnut (*Juglans regia*) flour. *Pakistan Journal of Science and Industrial Research.* 52.3:130 – 133.

Ojinnaka, M.C, Anyanwu F. A. and Ihemeje, A. 2013**.** Nutritional evaluation of cookiesproduced from African breadfruit (*Treculia africana)* starch and wheat flour.*International Journal of Agriculture and Food Science*. 3.3: 95-99.

Ojokoh, A. O., Fayemi, O. E., Ocloo, F.C.K. and Alakija, O. 2014. Proximate composition, antinutritional contents and physicochemical properties of breadfruit (*Treculia africana*) and cowpea (*Vigna unguiculata*) flour blends fermented with *Lactobacillus plantarum*. *African Journal of Microbiology Research*. 8.12: 1352-1359.

Ojokoh, A. O., Daramola, M. K. and Oluoti, O.J. 2013. Effect of fermentation on nutrients and antinutrients composition of breadfruit (*Treculia africana*) and cowpea (*Vigna unguiculata*) blend flours. *African Journal of Agricultural Research*. 8.27: 3566-3570.

Okaka J. C. 2005.Processing of starchy roots, tubers and fruits. In: Handling, Storage and Processing of Plants Foods Academic Publishers, Enugu. 237-255.

Oke, O.L. 1969. Oxalic acid in plants and nutrition. *World Rev. Nutr. Dietetics*.10:262-302.

Okigbo, B.N. 1980. Nutritional implications of projects giving high priority to the production of staples of low nutritive quality: The case for cassava (*Manihot esculenta crantz*) in the humid tropics of West Africa. *Food and Nutrition Bulletin*. 2.4:1-10.

Okoye, J.I. and Obi, C.D. 2017. Nutrient composition and sensory properties of wheat-African breadfruit composite flour cookies.*Sky Journal of Food Science.* 6.3:27 – 32.

Okpala, L. C. and Chinyelu,V. A. 2011. Physicochemical, nutritional and organolepticevaluation of cookies from pigeon pea (Cajanus Cajan) and cocoyam (Xanthosoma Sp) flour blends.*African Journal of Food, Agriculture, Nutrition and Development.* 11.6:1–13.

Okpala, L., Okoli, E. and Udensi, E. 2013. Physico-chemical and sensory properties of cookies made from blends of germinated pigeon pea, fermented sorghum and cocoyam flours. *Food Sci. Nutr.,* 1.1: 8 –14.

Okpala, L.C., and Okoli, E.C. 2011. Nutritional evaluation of cookies produced from pigeon pea, cocoyam and sorghum flour blends. *African Journal of Biotechnology.* 10.3:433-438.

Oladele, A.K. and Aina, J.O. 2007. Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). *African Journal of Biotechnology*. 6.21: 2473-2476.

Oladunjoye, I.O., Ologhobo, A.D. and Olaniyi, C.O. 2010. Nutrient composition, energy value and residual anti-nutritional factors in differently processed breadfruit (*Artocapus altilis*) meal. *Afr. J. Biotechnol*. 9.27:4259 – 4263.

Olaoye, O. A., Onilude, A.A and Oladoye, C.O. 2007. Breadfruit flour in biscuit making: effects on product quality. *African Journal of Food Science*. 1: 20-23.

Olaoye, O.A, and Onilude, A.A. 2008. Microbiological, proximate analysis and sensory evaluation of baked products from blends of wheat-breadfruit flours. *Afr. J. Food Agric.Nutr. Dev*., 8.2: 192-203.

Olatunji, O. and Akinrele, I.A. 1978. Comparative rheological properties and bread qualities of wheat flour diluted with tropical tuber and breadfruit flours. *Cereal*

Chemistry.55:1 – 6.

Oliveira, R.B.P., Oliveira, A. de L. and Glória, M.B.A. 2008. Screening of lactic acidbacteria from vacuum packaged beef forantimicrobial activity. *Braz. J. Microbiol*. 39.2: 368-374.

Oloyede**,** A. R., Oyelakin, O. O,Akinyemi, A. A.,Ekelemu, J. K.,Onireti, O.B. and Quadri, A. 2016.Genetic Diversity Studies of Bacteria Associated with *Brycinus longipinnis* from Eggua on Yewa River using random amplified polymorphic DNA (RAPD) markers. *Scholars. J. Agric. Vet. Sci.* 3.3:167-171.

Oloyo, R.A. 2004. Chemical and nutritional quality changes in germinating seeds of *Cajanus cajanL.Food Chemistry* 85.4: 497-502.

Oluwamukomi, M. O. and Jolayemi, O. S. 2012.Physico-thermal and pasting properties of soy melon-enriched "gari" semolina from cassava.*Agric Eng Int: CIGR Journal.* 14.3:105-116.

Omobuwajo, T.O. 2003. Compositional characteristics and sensory quality of biscuit, prawn crackers and fried chips produced from breadfruit. *J. Innovative Food Science and Emerging Technologies*. 4.2: 219-225.

Omobuwajo, T.O. 2007. Overview of the status of breadfruit in Africa. Acta Hort. 757:61-64.

Omole, A., Adewusi, S.A., Adeyemo, A. and Oke, O.L. 1978. The nutritive value of tropical fruits and root crops. *Nutr. Rep. Int*. 17:575–580.

Omoniyi, S.A., Awonorin, S.O., Idowu, M. A. and Adeola, A.A. 2016. Physicochemical and functional properties of sweet potato-soybean flour blends. *Applied Tropical Agriculture* (special issue). 21.2:84-88.

Onilude, A.A., Olaoye, O, Fadahunsi, I.F., Owoseni, A, Garuba, E.O. and Atoyebi, T. 2014. Effects of cultural conditions on dextran production by *Leuconostoc spp*. *International Food Research Journal.* 20.4:1645-1651.

Onimawo, A.I. and Akubo, P.I. 2005. Food Chemistry(Integrated approach with biochemical background) Published by Ambik Press limited, Benin City, Edo State Nigeria. 222 –228.

Onimawo, I.A. and Akubor, P.I. 2012. Food Chemistry (Integrated Approach with Biochemical

background) 2nd edn. Joytal printing press, Agbowo, Ibadan, Nigeria.

Onweluzo, J.C. and Nwabugwu, C.C. 2009. Fermentation of millet (*Pennisetumamericanum*) and pigeon pea (*Cajanus cajan*) seeds for flour production: Effectson composition and selected functional properties. *Pakistan Journal of Nutrition.* 8.6: 737-744.

Onwuka, G.I. 2005. FoodAnalysis and instrumentation: theory and practice. Naphathali Prints, Lagos, Nigeria. 95-96

Onwuka, G.I. 2006. Soaking, boiling and antinutritional factors in pigeon peas (*Cajanuscajan*) and cowpeas (*Vigna unguiculata*). *Journal Food Processing and Preservation*. 30.5: 616-630.

Oppong, D., Eric, A., Kwadwo, S.O., Eric, B. and Patrick, S. 2015. Proximate composition and some functional properties of soft wheat flour. *Int.J. Innovative. Res. Sci. Eng. Tech.*4.2:753 –758.

Oral jenson Axelsson, L., Salminen, S. and Wright, A. 1998. Lactic Acid Bacteria: microbiology and functional aspects, 2nd Edition, Marcel Dekker Inc., New York. 617

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. 2009. Agroforestree Database: A tree reference and selectionguide. version 4.0. International Center for Research in Agroforestry.

Osabor, V. N., Ogar, D. A., Okafor, P. C and Egbung, G. E. 2009. Profile of the African breadfruit (*Treculiaafricana*). *Pakistan Journal of Nutrition*. 8.7:1005-1008.

Osho, S. M. 2003. The processing and acceptability of a fortified cassava-based product (gari) with soybean. *Nutrition and Food Science*. *33*.6:278-283.

Otegbayo, B., Aina, J., Asiedu, R. and Bokanga, M. 2006. Pasting characteristics offresh yams (*Dioscoreaspp*.) as indicators of textural quality in a major food product – pounded*yam*.*Food Chemistry.* 99.4: 663-669.

Otegbayo, B.O., Ogunniyan, D. and Akinwumi, B. 2014. Physicochemical and functional characterization of yam starch for potential industrial applications. *Starch/Starke* 66: 235 –250.

Otunola, E. T., Adelakun, E. O. and Olaniyan, T. O. 2006. Evaluation of 'ogi' and pawpaw

mixes. *Journal of Engineering Science and Technology*. 13.3:6983–6993

Otunola, E. T., Sunny-Roberts, E.O and Solademi, A.O. 2007. Influence of the addition of okra seed flour on the properties of 'ogi' a Nigerian fermented maize food. A paper from the conference on International Agricultural Research at the University of Kassel-Witzenhausen and University of Gottingen.

Oulaï, S.F., Ahi, A.P., Kouassi-Koffi, J.D., Gonnety, J.T. Faulet, B.M. Djè, K.M. and Kouamé, P.L. 2014. Treatments effects on functional properties of breadfruit (Artocarpus altilis) pulp flour harvested in Côte D'ivoire. *International Journal of Recent Biotechnology*. 2.4:1-12.

Oyarekua, M.A. 2013. Effect of co-fermentation on nutritive quality and pasting properties of maize/cowpea/sweet potato as complementary food. *African Journal of food, Agriculture, Nutrition and development*. 13.1:7171-7191.

Oyarekua, M.A. 2011. Biochemical and Microbiological changes during the production of fermented pigeon pea (*Cajanus cajan*) flour.*African Journal of Food Science and Technology.* 2.10:223-231.

Oyewole, O.A. and Isah, P. 2012. Locally Fermented Foods in Nigeria and their Significance to National Economy: Areview. *Journal of Recent Advances in Agriculture*. 1.4: 92-102.

Pandey, A., Selvakumar, P., Soccol, C. and Nigam P. 1999.Solid state fermentation for the productionof industrial enzymes. *Current Science*, 77. 1: 149-162.

Pang, H., Tan, Z., Qin, G., Wang, Y., Li, Z., and Cai, Y. 2012. Phenotypic and phylogenetic analysis of lactic acid bacteria isolated from forage crops and grasses in the Tibetan Plateau. *J Microbiol.* 50.1: 63–71.

Pang, H., Zhang, M., Qin, G., Tan, Z., Li, Z, Wang, Y. and Cai, Y. 2011. Identification of lactic acid bacteria isolated from corn stovers. *Anim Sci. J.* 82.5: 642–653.

Paull, R.E. and Chen, N.J. 2004. Tropical Fruit Postharvest: The Impact of Biotechnology. *Acta. Hort.* 632: 303-308.

Pele, G.I., Oladiti, E.O., Bamidele, P.O. and Fadipe, E.A. 2016. Influence of Processing Techniques on the Nutritional and Anti-Nutritional Properties of Pigeon Pea

(*Cajanus cajan*). Int.J.Eng.App.Sci.3:92-94.

Petti, C.A., Polage, C.R. and Schreckenberger, P. 2005. The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. J. Clin. Microbiol. 43.12: 6123-6125.

Plahar, W. A. and Hoyle, N. T. 1991. Estimated protein quality of weaning blends from local cereals and legumes. In Sefa-Dedeh (Ed.): The development of high protein-energy foods from grains legumes. Proceedings of the AAU/UNU International Seminar in Accra (75– 87) Ghana.

Potter, N.N. and Hotchkiss, J.H. 1998. Food Science. 5th Edition. New York: Springer. 402.

Prescott, Harley and Klein. 2008. Microbiology. 7th Edition.Published by McGraw-Hill Companies, Inc.,1221 Avenue of the Americas, New Yolk, NY 10020.

Raboy, V. 2001. Seeds for a better future: ` low phytate` grains help to overcome malnutrition and reduce pollution. *Trends Plant Science*. 6.10:458-462.

Ragaee, S., Abdel-Aal, E.M. and Noaman, M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*.98.1:32-38.

Ragone, D. 1997. Breadfruit, *Artocarpus altilis*(Parkinson) *Fosberg*. Promoting theconservation and use of underutilized and neglected crops. *International Plant Genetic Resources Institute*, Rome, Italy. 77

Ragone, D.2007. Breadfruit: Diversity, Conservation and Potential. In Proceedings of the 1st International Symposium on Breadfruit Research and Development. *ActaHorticulturae* 757: 19-30.

Ragone, D. 2011. Farm and forestry production and marketing profilefor breadfruit (*Artocarpus altilis*). In: Elevith CR (ed). SpecialtyCrops for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR) Hulualoa. 1–19.

Ragone, D. and Cavaletto, C.G. 2006. Sensory evaluation of fruit quality and nutritional composition of 20 breadfruits (*Artocarpus*, Moraceae) cultivars. *Economic Botany.* 60.4:335-346.

Rahul, G., Kadirvelu, J. and Amrin,F. 2018. Lactic Acid Bacteria: Probiotic Characteristic, Selection Criteria and its Role in Human Health (A Review). *Journal of*

Emerging Technologies and Innovation Research. 5.10:2349-5162.

Randazzo, C.L., Restuccia, C., Romano, A.D. and Caggia, C. 2004. Lactobacillus casei, dominant species in naturally fermented silican green olives. *International Journal of Food Microbiology.* 90.1:9-14.

Ravi, R. and Susheelamma, N. S. 2005. "Simultaneous optimization of a multi-response system by desirability function analysis of `Boondi` making: A case study". *Journal of FoodScience*. 70.8: 539–547.

Relman, D. A. 1999. The search for unrecognized pathogens. *Science.* 284: 1308-1310.

Rita, E.S., Adiza, S. and Sophia, D. 2010. Nutritional and sensory analysis of soya bean and wheat flour composite cake. *Pakistan Journal of Nutrition*. 9.8:794-796.

Robert, S. and William, C.S. 2008. Ecology of fermented foods. *Human Ecology Review* 15.1: 25-31.

Roberts, D. and Greenwood, M. 2003. Practical Food Microbiology. Third edition, Blackwell publishing Ltd. 1-19.

Roberts-Nkrumah, L. B. 2005. Fruit and seed yields in chataigne (*Artocarpuscamansi* Blanco) in Trinidad and Tobago. *Fruits.*60.6:387-393.

Salminen, S., Ouwehanda, A.C. and Isolaurie, E. 1998. Clinical applications of probiotic bacteria. *International Dairy Journal*. 8: 563-572.

Salminen, S. J., Gueimonde, M. and Isolauri, E. 2005. Probiotics that modify diseaserisk. *The Journal of Nutrition*. 135.5.1294-1298.

Sanni, L.O, Ikuomola, D.P. and Sanni, S. A. 2001. Effect of length of fermentation and varieties on the qualities of sweet potato garri. Proc. 8th Triennaial Symposium of the International Society for Tropical Root Crop- Africa Branch (ISTRC-AB), Ed. M.O. Akoroda, IITA, Ibadan, Nigeria. 208-211.

Sanni, L. O., Adebowale, A. A., Awoyale, W. and Fetuga, G. O. 2008. Quality of gari (roasted cassava mash) in Lagos State, Nigeria. *Nigerian food journal.* 26. 2: 125-130.

Saraithong, P., Li, Y., Saenphet, K., Chen, Z. and Chantawannakul, P. 2014. Bacterial community structure in Apis florea larvae analyzed by denaturing gradient gel electrophoresis and 16S rRNAgene sequencing. *Insect Science*. 22.5:606-618.

Sathe, S.K, Desphande, S.S, Salunkhe, D.K. 1982. Functional properties of lupinseed (*Lupinus mutabilis*) proteins and protein concentrates. *J. Food Sci*. 47.2: 491-497.

Saxena, K.B., Faris, D.G. Singh, U. and Kumar, R.V. 1987. Relationship between seed size and protein content in newly developed high protein lines of pigeonpea. *Plant Foods Hum.Nutr.* 36.4: 335-340.

Sefa-Dedeh, S., Cornelius, B., Sakyi-Dawson, E. and Afoakwa, E.O. 2004. Effect of nixtamalization on the chemical and functional properties of maize. *Food Chemistry.* 86.3: 317 – 324.

Sharpe, M.E. 1979. Identification of the lactic acid bacteria. In: Skinner, F.A., Lovelock, D.W. (Eds.), Identification Methods for Microbiologist. Academic Press, London. 233–259.

Shankar, S. and Lanza, E. 1991. Dietary fiber and cancer prevention. *Hematol. Oncol.Clin. North Am*. 5.1: 25-41.

Sheldrake, A. R. 1984. Pigeonpea. In: The Physiology of tropical field crops. Edited by P. R. Goldsworthy and N. M. Fisher. John Wiley & Sons, Chichester, U.K. 385-417.

Shen, P., Fan, S. R. and Li, G. W. 1999. Microbiology Experiment, 3rd Ed. High Education Press, Beijing, China.

Shimelis, A.E., Meaza, M. and Rakshit, S. K. 2006. Physico-chemical properties, pasting behaviour and functional characteristics of flours and starches from improved bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Agricultural Engineering International*. 8:18-36.

Siddhuraju, P., Vijayakumari, K.and Janardhanan, K. 1996. Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens*(*L.*) *DC*). *J. Agric*. *Food Chem.* 44.9: 2636- 2641.

Simon, C., Frati, F., Beckenbach, A, Crespi, B., Liu, H. and Flook, P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the *Entomological Society of America*. 87.6: 651-701.

Singh, H. 2009. Tapping into breadfruit`s bounty. Available online at http://www.universityaffairs.ca/tapping-into-breadfruits_bounty.aspx. Accessed 21 June, 2010.

Singh, U. 1988. Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods for Human Nutrition*. 38.3: 251–261.

Singh, U., Jambunathan, R., Saxena, K.B. and Subramaniam. N. 1990. Nutritional quality evaluation of newly developed high protein genotypes of pigeonpea (*Cajanus cajan* L.). *Journal of the Science of Food and Agriculture*.50.2: 201-209.

Singhania, R.R, Patel, A.K., Soccol, C.R. and Pandey, A. 2009. Recent advances in solid-state fermentation. *Biochem Eng. J.* 44.1:13–18.

Sinha, S.K. 1977. Food Legumes: distribution, adaptability and biology of yield. In: *Plant production and protection paper.* FAO, Rome, Italy. 130.

Smith, W.H. 1972. Wire-cut cookies. In: Smith, W.H. (Ed.). Biscuits, crackers and cookies:Technology, production and management.*Applied Science publishers, London.*737.

Soetjipto, N.N, and Lubis, A.S. 1981. Vegetables: The International Board for Plant Genetic Resources (IBPGR) Secretariat, Rome. 330.

Secretariat of the Pacific Community. 2006. Breadfruit: A publication of the Healthy Pacific Lifestyle Section of the Secretariat of the Pacific Community.Pacific food leaflet N0.3 http://www.spc.int/lifestyle/

Spore. 2007. Breadfruits: Ripe for the new markets. Technical Centre for Agricultural and Rural Cooperation (CTA). 46:131.

Steinkraus, K.H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control. 8.5-6:311-317.

Steve, R. A., Adewusi, A., Akpobome, U. I. and Osuntogun. B. A. 1995. Studies on the carbohydrate content of breadfruit (*Artocarpus communisFors*t) from South-western Nigeria. *J. Starch.* 47:289–294

Subramaniyam, R. and Vimala, R. 2012.Solid state and submerged fermentation for the production of bioactive substances: a comparative study. *International Journal of Science and Nature.*3.3: 480-486.

Suknark, K., Mcwatters, K.H. and Phillips, R.D. 1998. Acceptance by American and Asianconsumers of extruded fish and peanut snack products. *J. Food Sci*. 63:721–725.

Tai Situ, Osiboye, O. and Akah, B. 2009. Production Manual of Food, Fruit Juice, Drinks and Allied. Published in Nigeria by ARCH-ADEBAYO Nig. Ent.Ijebu-Ode Ogun State.12-19.

Taylor, M.B. and Tuia, V.S. 2007. Breadfruit in the Pacific Region. Acta Hortic.757.4:43 – 50.

Temmerman, R., Pot, B., Huys, G. and Swings, J. 2003. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int. J. Food Microbiol*. 81.1: 1–10.

Thottappilly, G., Mignouna, H. D., Onasanya, A., Abang, M., Oyelakin, O. and Singh, N.K. 1999. Identification and differentiation of isolates of *Collectotrichum gloesporiodes* from yam by random amplified polymorphic DNA markers.*Afr.Crop Sci.J.*7.2:195-206.

Ting, L., Peter, G.S. and Daniel, B.O. 2009. Reverse Transcription of 16S rRNAto Monitor Ribosome-Synthesizing Bacterial Populations in the Environment. *Appl. Environ. Microbiol*. 75.13:4589-4598.

Torres, A., Frias, J., Granito, M., Vidal-Valverde, C. 2006. Fermented Pigeon Pea (*Cajanus cajan*). Ingredients in Pasta Products. *J. Agric Food Chem*. 54.18: 6685-6691.

Towo, E., Matuschek, E. and Svanberg, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Food Chem.* 94.3: 369-376.

Tresina, P.S., Kala, S.M.J. and Mohan, V.R. 2012. HPTLC finger print analysis of phytocompounds and *in vitro* antioxidant activity of *Eugenia singampattiana* Bedd. *J. App. Pharma. Sci*. 2.12: 112-124.

Udensi, E. A., Oselebe, H. O. and Iweala, O.O. 2008. The investigation of chemicalcomposition and functional properties of water yam (*Dioscorea alata*): Effect of varietal

differences. *Pakistan Journal of Nutrition.* 7.2: 342-344.

Udensi, O.U., Ntia, M.I. and Obianwa, C.U. 2014. Optimizing induced mutation technique for the improvement of agronomic traits in pigeon pea [Cajanus cajan (L.) Millsp.] landraces, *Comunicata Scientiae*. 5.3:267-278

Ukegbu, P.O. and Anyika, J.U. 2012. Chemical analysis and nutrient adequacy of maize gruel (pap) supplemented with other food sources in Ngor-Okpala LGA, Imo State, Nigeria. *J. Biol. Agric. Healthcare.* 2:13–21.

Usman, M.A., Bolade, M.K. and James, S. 2016.Functional properties of weaning food blends from selected sorghum (*Sorghum bicolor(L.*) *Moench*) varieties and soybean (*Glycine max*). *African Journal of Food Science.* 10.8:112-121.

Uzogara, S. G., Morton, I.D. and Daniel, J.K. 1990. Changes in some antinutrients of cowpeas (*Vigna unguiculata*) processed with 'kanwa' alkaline salt. *Plant Foods for HumanNutrition*. 40:249-258.

Uzo-Peters, P.I., Arisa, N.U., Lawrence, C.O., Osondu, N.S. and Adelaja, A. 2008. Effect of partially defatted soybeans or groundnut cake flours on proximate and sensory characteristics of kokoro. *African Journal of Food Science.* 2: 98-101.

Valenzuela, H. and Smith,J. 2002. Sustainable Agriculture Green Manure Crops.SA-GM-7. Cooperative Extension Service, Collegeof Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

Van Den Beldt, R.J. 1988. *Cajanus cajan*: It's more than just a pulse crop. Forest, Farm and Community Tree Network (FACTNet News). ICRISAT Sahalian Centre. (http://www.winrock.org/forestry/factnet.htm). Accessed 8 November, 1988.

Vani. B. and Zayas. J.F. 1995. Wheat germ protein flour solubilitv and water retention. *J. Food Sci.* 60.4:845-848.

Venkateswaran, K., Dohmoto, N. and Harayama, S. 1998. Cloning and nucleotide sequence of the*gyrB*gene of *Vibrio parahaemolyticus* and its application in detection of this pathogen in shrimp. *Applied and Environmental Microbiology*. 64.2: 681-687.

Venzon, M., Rosado, M.C., Euzebio, D.E., Souza, B. and Schoereder, J.H. 2006. Suitability of leguminous cover crop pollens as food source for the green lacewing chrysoperla

externa (Hagen) (Neuroptera: chrysopidae). *Neotropical Entomology*. 35.3: 371–376.

Vinderola, C.G., Prosello,W., Ghiberto, T.D. and Reinheimer, J.A. 2000. Viability of probiotic (bifidobacterium, lactobacillus acidophilus and lactobacillus casei) and nonprobiotic microflora in Argentinian Fresco cheese. *Journal of Dairy Science.*83. 9: 1905-1911.

Walker, P.M.B. 1988. *Chambers Science and Technology Dictionary*,Chambers, Cambridge Univesrsity Press, UK.

Weinberg, Z. Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D. and Miron, J. 2007. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages.*J. Dairy Sci.* 90.10: 4754-4762.

Welman, A.D. and Maddox, I.S. 2003. Exopolysaccharides from lactic acid bacteria: perspectivesandchallenges. *Trends in Biotechnology*. 21.6:269-74.

Wilhelm, W., Johnson, J.M.F., Hatfield, J.L., Voorhees, W.B. and Linden, D.R. 2004. Crop and soil productivity response to corn residue removal: A literature review. *Agronomy Journal.* 96:1-17.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990.DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.,* 18.22:6531-6535.

Woese, C. R. 1987. Bacterial evolution. *Microbiology Reviews*. 51.2: 221-271.

Yameguchi, M. I. 1983. World Vegetables: Principles,Production and Nutritive values. Ellis Horwood Limited; Chichester, England. 182 – 183.

Yang, J., Cao, Y., Cai, Y. and Terada, F. 2010. Natural populations of lactic acid bacteria isolated from vegetable residues and silage fermentation. *J Dairy Sci*. 93.7: 3136–3145.

Yasumatsu, K., Sawada, L., Moritaka, S., Misaki, M., Toda, J., Wada, T. and Ishii, K. 1972. Whipping and emulsifying properties of soybean products. *Agriculturaland Biological Chemistry*. 36.5:719 – 727.

Zerega, N. J. C., Ragone, D. and Motley, T. J. 2004. Complex origins of breadfruit (*Artocarpus altilis, Moraceae*): implications for human migrations in Oceania. *AmericaJournal of Botany.* 91.5: 760 – 766.

APPENDICES

Appendix 1a

16SrRNA Sequence of Breadfruit Isolates at 28±2^{o}C

TAAGCCAGTATGTTGGGATGGCTCAACGCACGCAGACCCAGTTTGCAATTCGAAATG
ACCGTTTATATCGTTATGAGCGGGAAAGTGCAGCGATTATACTGGCTTCGCTTGGGG
CCATTAATATGCCGACTATTGACATTGTTGAGCGCTTTCCAGAGCCGTTTGTGGAAA
CTACCAGCAATAGAGCTGGTAGTTTTTGGAATTGGTTAT

TTAAGATGAAACACTAGTATTCAATATATACGTTATCAATATTCAATTTATAGAATTT
TAGTCTTGGCATATTGACAGTTAAATCCTTACTTGCTTATGATTAAAGCGAAAGGGA
TTGAGTATTGATCATGGCAAAAGATAAGGTAATAATTGATCCAGACGCGTTTGCACG
TGCGGTAGTCAGCGGTTCTAACTTGGAGGCCAAAGATG

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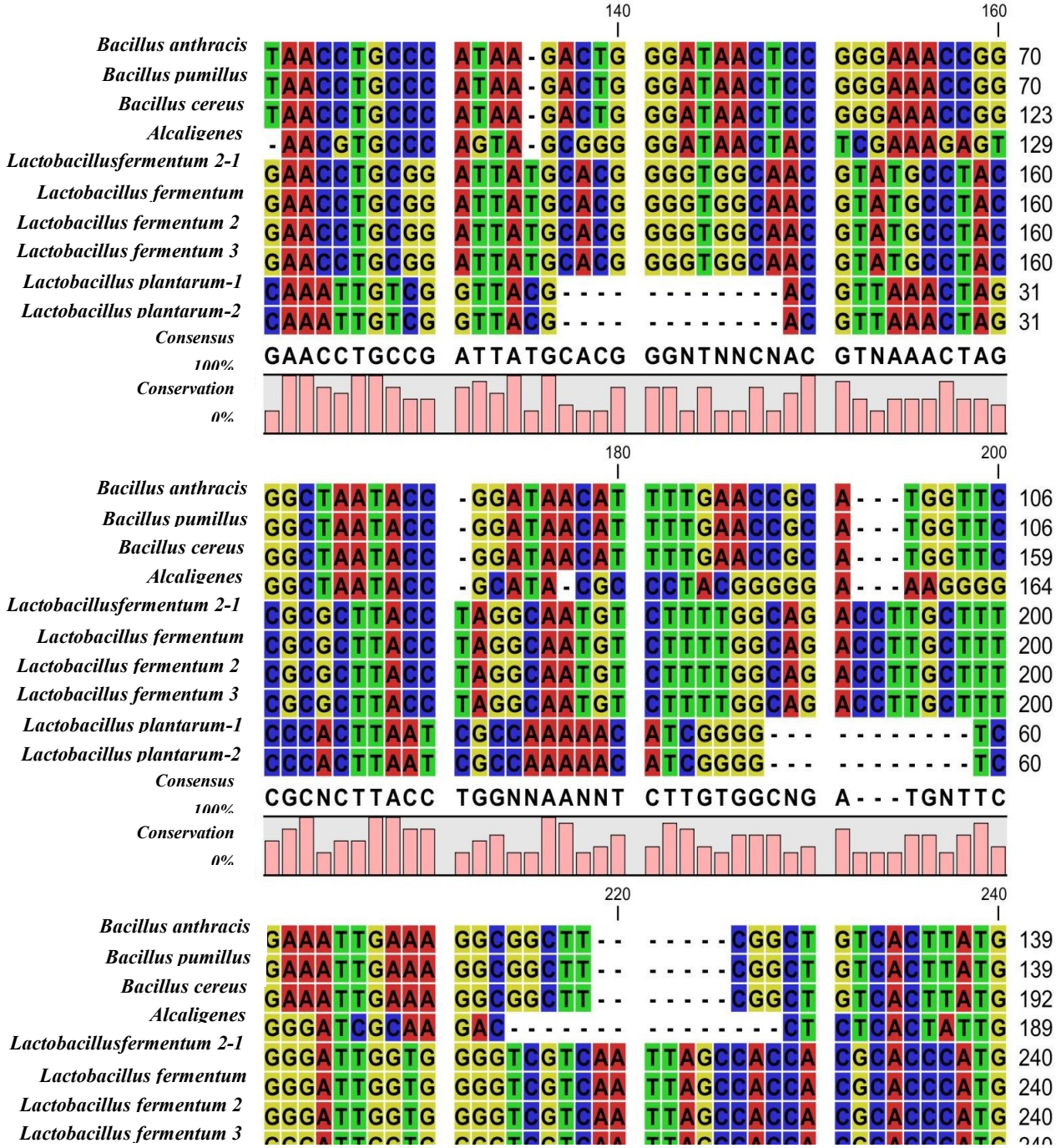
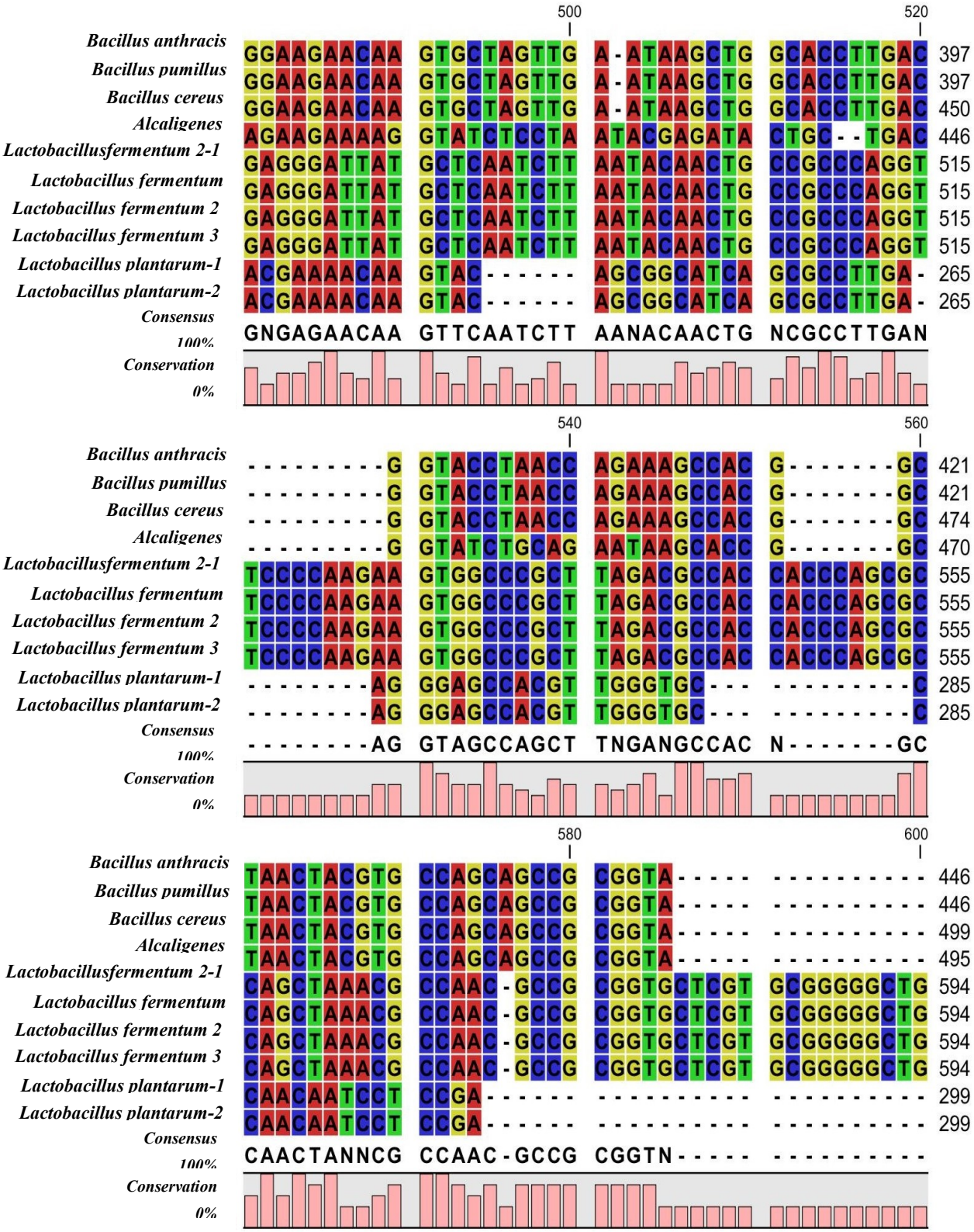
AAAAGTTTAATAA

*Lactobacillusplantarum*CP015308.1

Appendix 1b


140
160
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
GAACCTGCCG ATTATGCACG GGNTNNCNAC GTNAAACTAG
180
200
CGCNCTTACC TGGNNAANNT CTTGTGGCNG A---TGNTTC
220
240
380
400


500
520
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
GGAAGAACAA GTGCTAGTTG A-ATAAGCTG GCACCTTGAC 397
GGAAGAACAA GTGCTAGTTG A-ATAAGCTG GCACCTTGAC 397
GGAAGAACAA GTGCTAGTTG A-ATAAGCTG GCACCTTGAC 450
AGAAGAAAAG GTATCTCCTA ATACGAGATA CTGC--TGAC 446
GAGGGATTAT GCTCAATCTT AATACAACTG CCGCCCAGGT 515
GAGGGATTAT GCTCAATCTT AATACAACTG CCGCCCAGGT 515
GAGGGATTAT GCTCAATCTT AATACAACTG CCGCCCAGGT 515
GAGGGATTAT GCTCAATCTT AATACAACTG CCGCCCAGGT 515
ACGAAAACAA GTAC------ AGCGGCATCA GCGCCTTGA- 265
ACGAAAACAA GTAC------ AGCGGCATCA GCGCCTTGA- 265
GNGAGAACAA GTTCAATCTT AANACAACTG NCGCCTTGAN
540
560
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
---------G GTACCTAACC AGAAAGCCAC G-------GC 421
---------G GTACCTAACC AGAAAGCCAC G-------GC 421
---------G GTACCTAACC AGAAAGCCAC G-------GC 474
---------G GTATCTGCAG AATAAGCACC G-------GC 470
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TCCCCAAGAA GTGGCCCGCT TAGACGCCAC CACCCAGCGC 555
TCCCCAAGAA GTGGCCCGCT TAGACGCCAC CACCCAGCGC 555
TCCCCAAGAA GTGGCCCGCT TAGACGCCAC CACCCAGCGC 555
--------AG GGAGCCACGT TGGGTGC--- ----------C 285
--------AG GGAGCCACGT TGGGTGC--- ----------C 285
--------AG GTAGCCAGCT TNGANGCCAC N-------GC
580
600
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
TAACTACGTG CCAGCAGCCG CGGTA----- ---------- 446
TAACTACGTG CCAGCAGCCG CGGTA----- ---------- 446
TAACTACGTG CCAGCAGCCG CGGTA----- ---------- 499
TAACTACGTG CCAGCAGCCG CGGTA----- ---------- 495
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CAGCTAAACG CCAAC-GCCG CGGTGCTCGT GCGGGGGCTG 594
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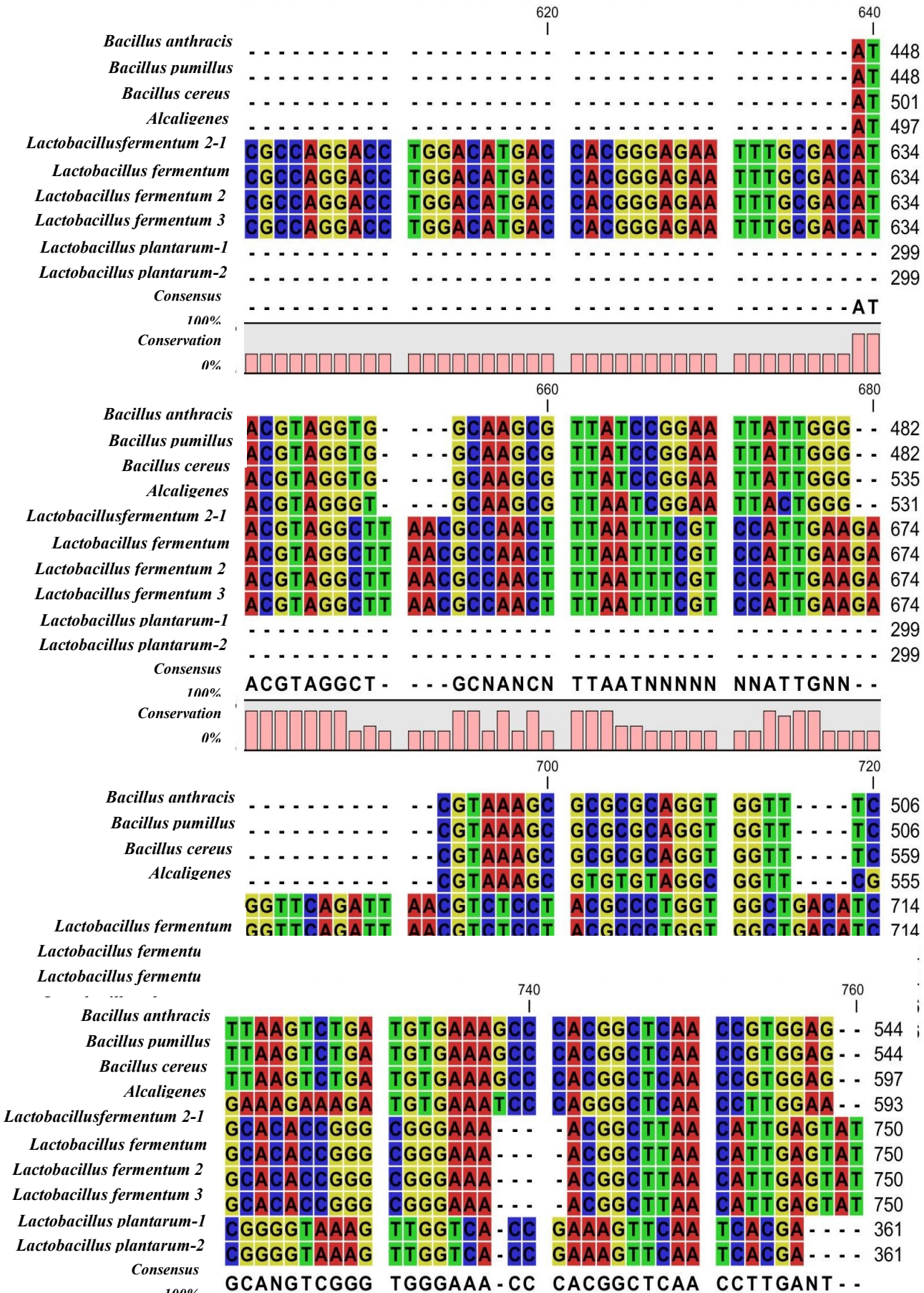
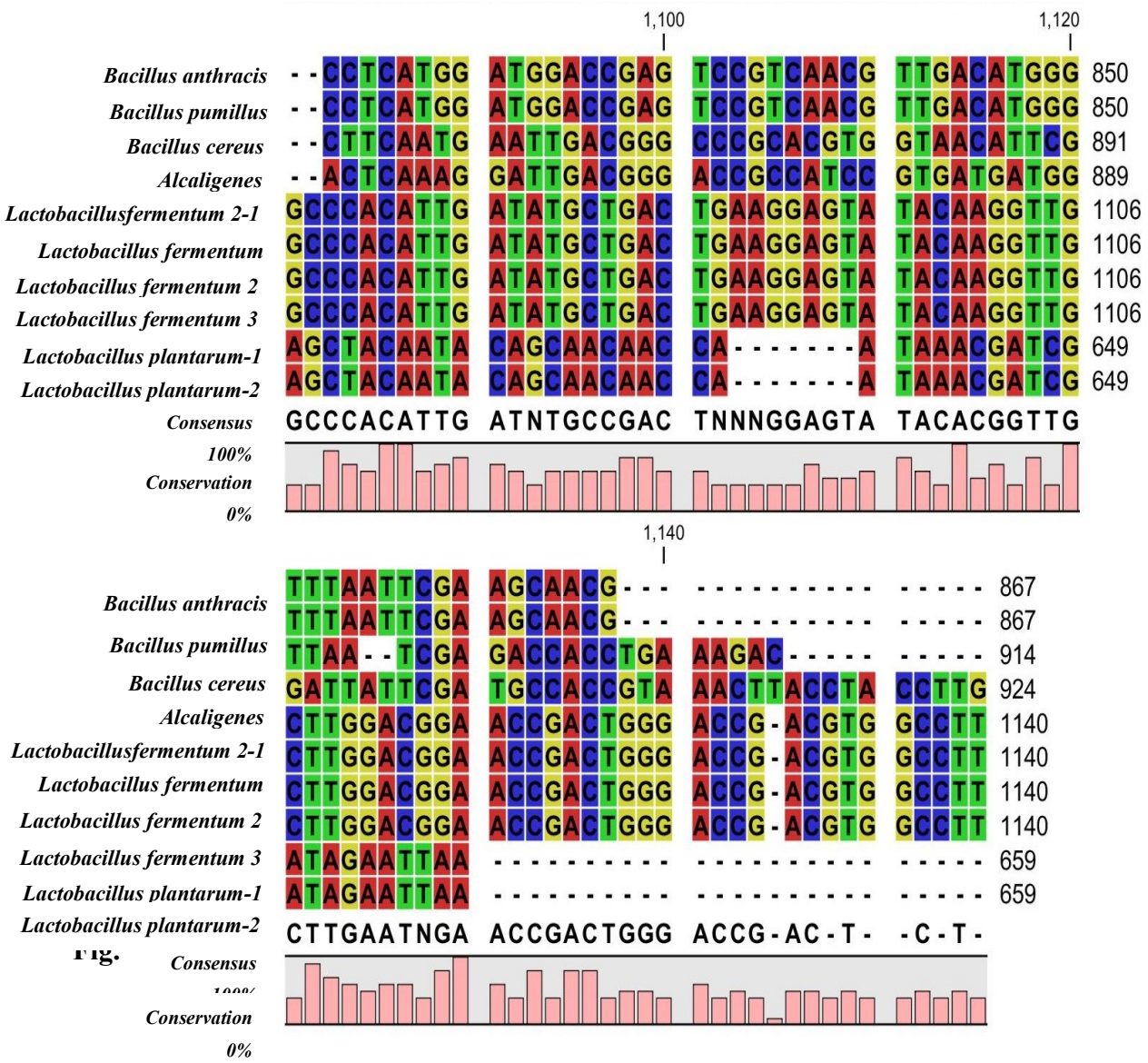
CAACAATCCT CCGA------ ---------- ---------- 299
CAACTANNCG CCAAC-GCCG CGGTN----- ----------


620
640
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
660
680
ACGTAGGCT- ---GCNANCN TTAATNNNNN NNATTGNN--
700
720
Lactobacillus fermentum
Lactobacillus fermentu
Lactobacillus fermentu
740
760


1,100
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Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
100%
Conservation
0%
1,140
Bacillus anthracis
Bacillus pumillus
Bacillus cereus
Alcaligenes
Lactobacillusfermentum 2-1
Lactobacillus fermentum
Lactobacillus fermentum 2
Lactobacillus fermentum 3
Lactobacillus plantarum-1
Lactobacillus plantarum-2
Consensus
Conservation
0%

Fig.

Appendix 2 a

16SrRNA Sequence of Breadfruit Isolates at 37±1^{o}C

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

*Lactobacillus fermentum*CP011536.1

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

Lactobacillus fermentum CP005958.1

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

*Lactobacillus fermentum*P005958.1

TAAGCCAGTATGTTGGGATGGCTCAACGCACGCAGACCCAGTTTGCAATTCGAAATG
ACCGTTTATATCGTTATGAGCGGGAAAGTGCAGCGATTATACTGGCTTCGCTTGGGG
CCATTAATATGCCGACTATTGACATTGTTGAGCGCTTTCCAGAGCCGTTTGTGGAAA
CTACCAGCAATAGAGCTGGTAGTTTTTGGAATTGGTTATTTAAGATGAAACACTAGT
ATTCAATATATACGTTATCAATATTCAATTTATAAATTTTAGTCTTGGCATATTGACA
GTTAAATCCTTACTTGCTTATGATTAAAGCGAAAGGGATTGAGTATTGATCATGGCA
AAAGATAAGGTAATAATTGATCCAGACGCGTTTGCACGTGCGGTAGTCAGCGGTTCT
AACTTGGAGGCCAAAGATGATGTACGTGCCAGCAAAGATGCCTTGAAACGCTATCT
GGCGGCGTATTTCTTGATTGAAAAGTTTAATAA

*Lactobacillusplantarum*CP015308.1

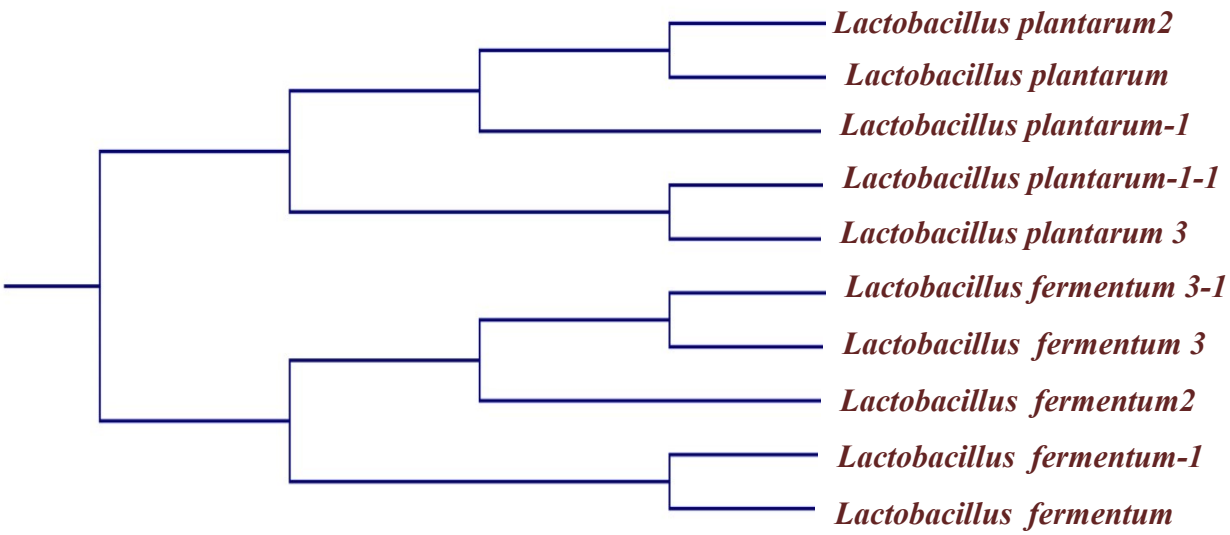
AAAAACATACAAATAGACGAGGAGTGCTTAATTATGTTATCAGTACCTGATTATGAG
TTTTGGTTTGTTACCGGTTCACAACACCTTTATGGTGAAGAACAATTGAAGTCTGTTG
CTAAGGATGCGCAAGATATTGCGGATAAATTGAATGCAAGCGGCAAGTTACCTTAT
AAAGTAGTCTTCAAAGATGTTATGACGACGGCTGAAAGT

ATCACCAACTTTATGAAAGAAGTTAATTACAATGATAAGGTAGCCGGTGTTATTACT
TGGATGCACACATTCTCACCAGCCAAGAACTGGATTCGTGGAACTGAACTGTTACAA
AAACCATTATTACACTTAGCAACGCAATATTTGAATAATATTCCATATGCAGACATT
GATTTTGATTACATGAACCTTAACCAAAGTGCGCATGGC

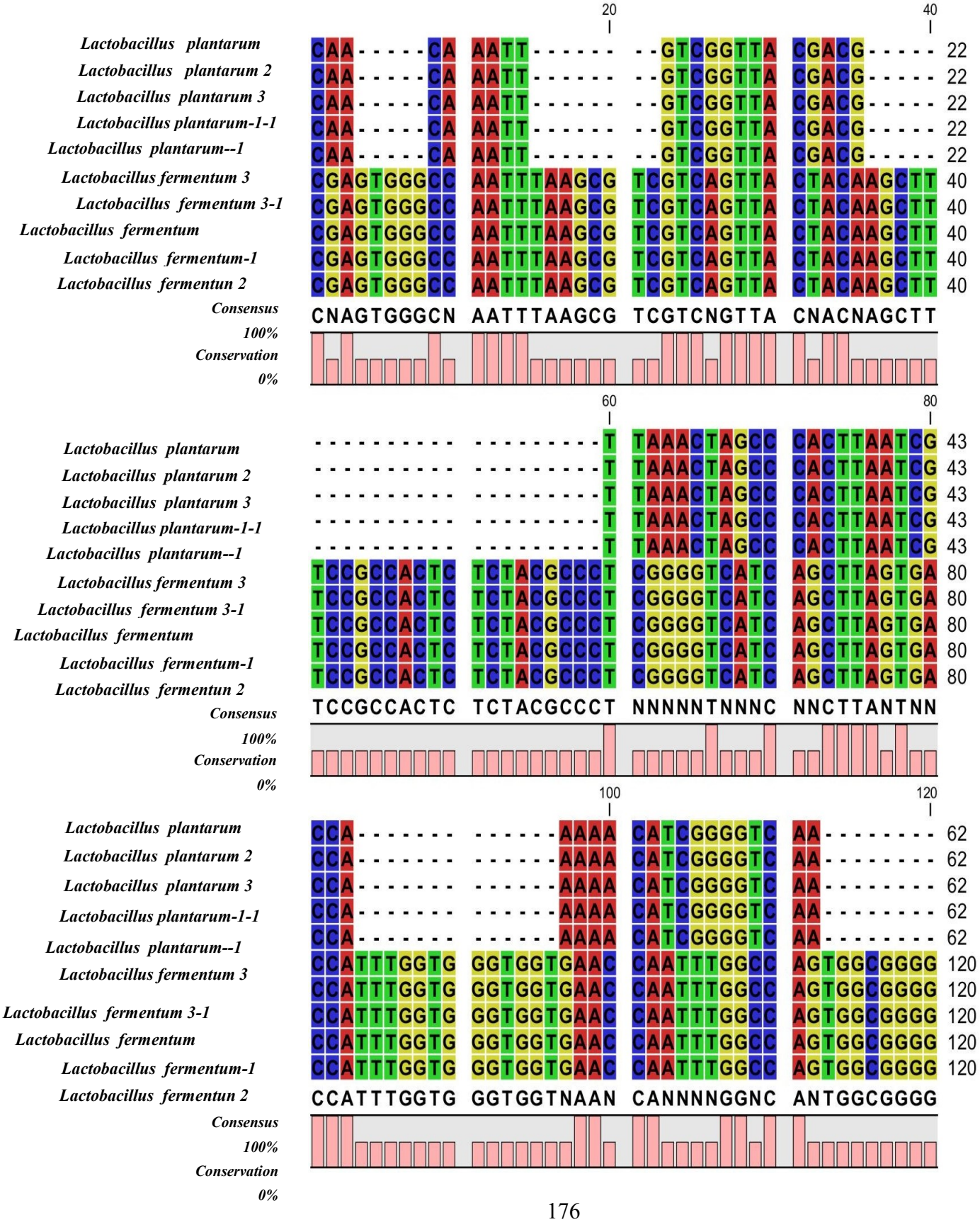
GACCGTGAATATGCCTACATTAACGCCCGGTTGCAGAAACATAATAAGGTTGTCTAC
GGCTATTGGGGCGATGAAGATGTGCAAGAACAGATTGCGCGTTGGGAAGACGTCGC
AGTAGCGTACAATGAGAGCTTTAAAGTTAAGGTTGCTCGTTTTGGCGACACGATGCG
TAATGTGGCCGTTACTGAAGGTGACAAGGTTGAAGCTCAA

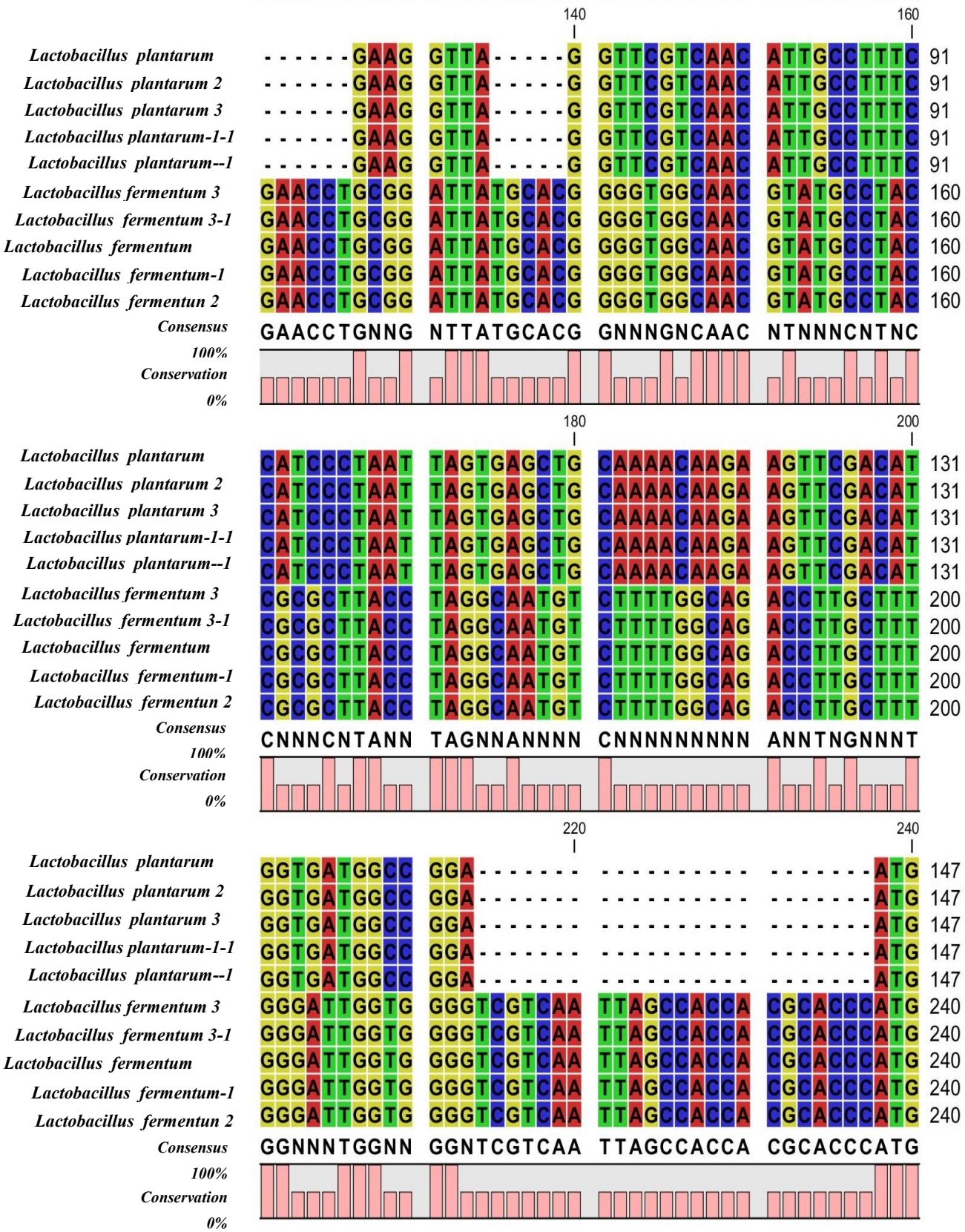
ATTAAGATGGGCTGGACAGTTGACTATTATGGTATCGGTGACTTAGTTGAAGAGATC
AATAAGGTTTCGGATGTTGATATTGATAAGGAATACGCTGACTTGGAGTCTCGGTAT
GAAATGGTCCAGGGCGATAACGATGC

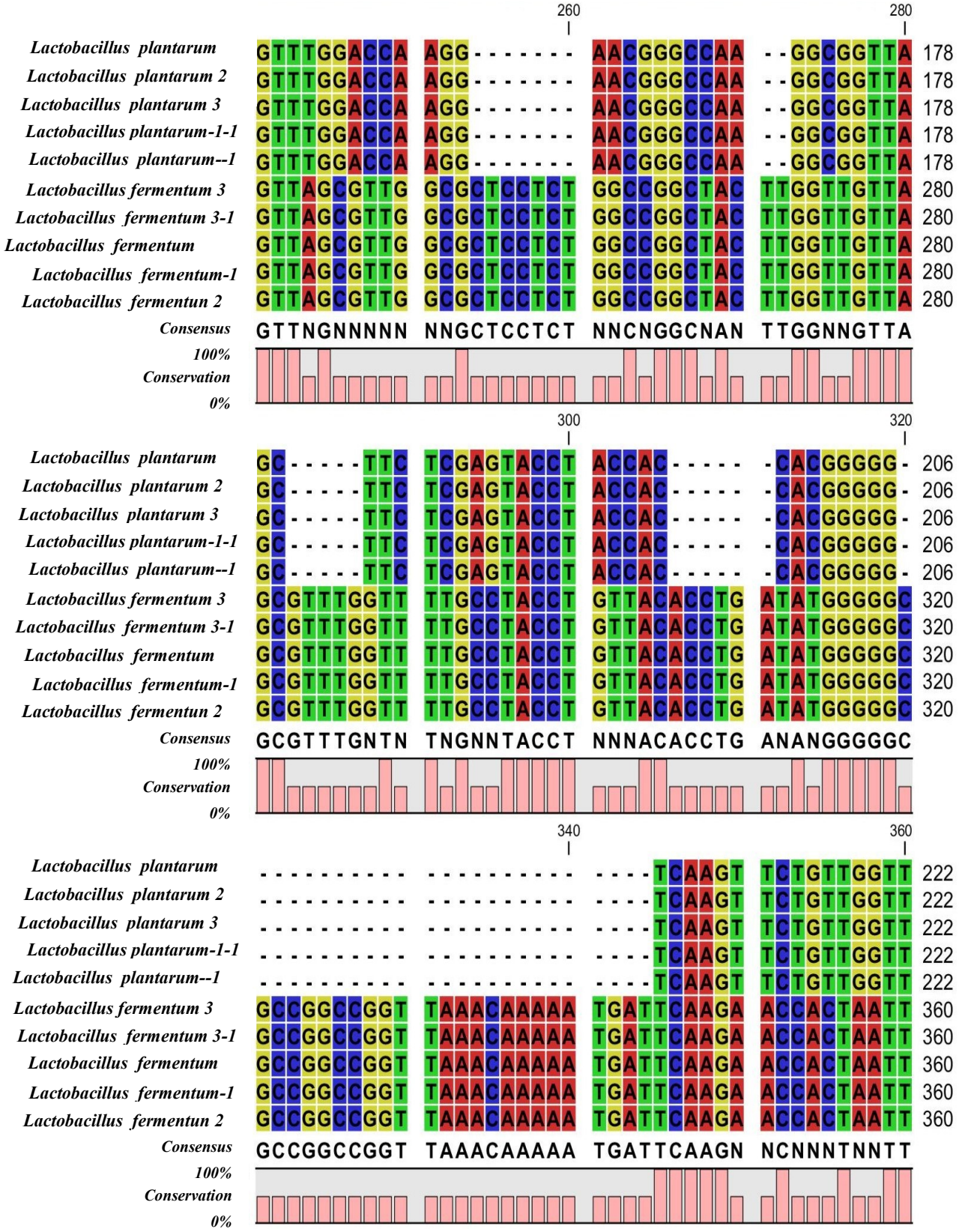
*Lactobacillusplantarum*CP012122.1



Appendix 2 b: Breadfruit Phylogenetic Tree at 37±1^{o}C


20
40
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
CNAGTGGGCN AATTTAAGCG TCGTCNGTTA CNACNAGCTT
100%
Conservation
0%
60
80
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
TCCGCCACTC TCTACGCCCT NNNNNTNNNC NNCTTANTNN
100%
Conservation
0%
100
120
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
CCATTTGGTG GGTGGTNAAN CANNNNGGNC ANTGGCGGGG
100%
Conservation
0%


140
160
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%
180
200
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%
220
240
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%


260
280
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
GTTNGNNNNN NNGCTCCTCT NNCNGGCNAN TTGGNNGTTA
100%
Conservation
0%
300
320
206
320
GCGTTTGNTN TNGNNTACCT NNNACACCTG ANANGGGGGC
340
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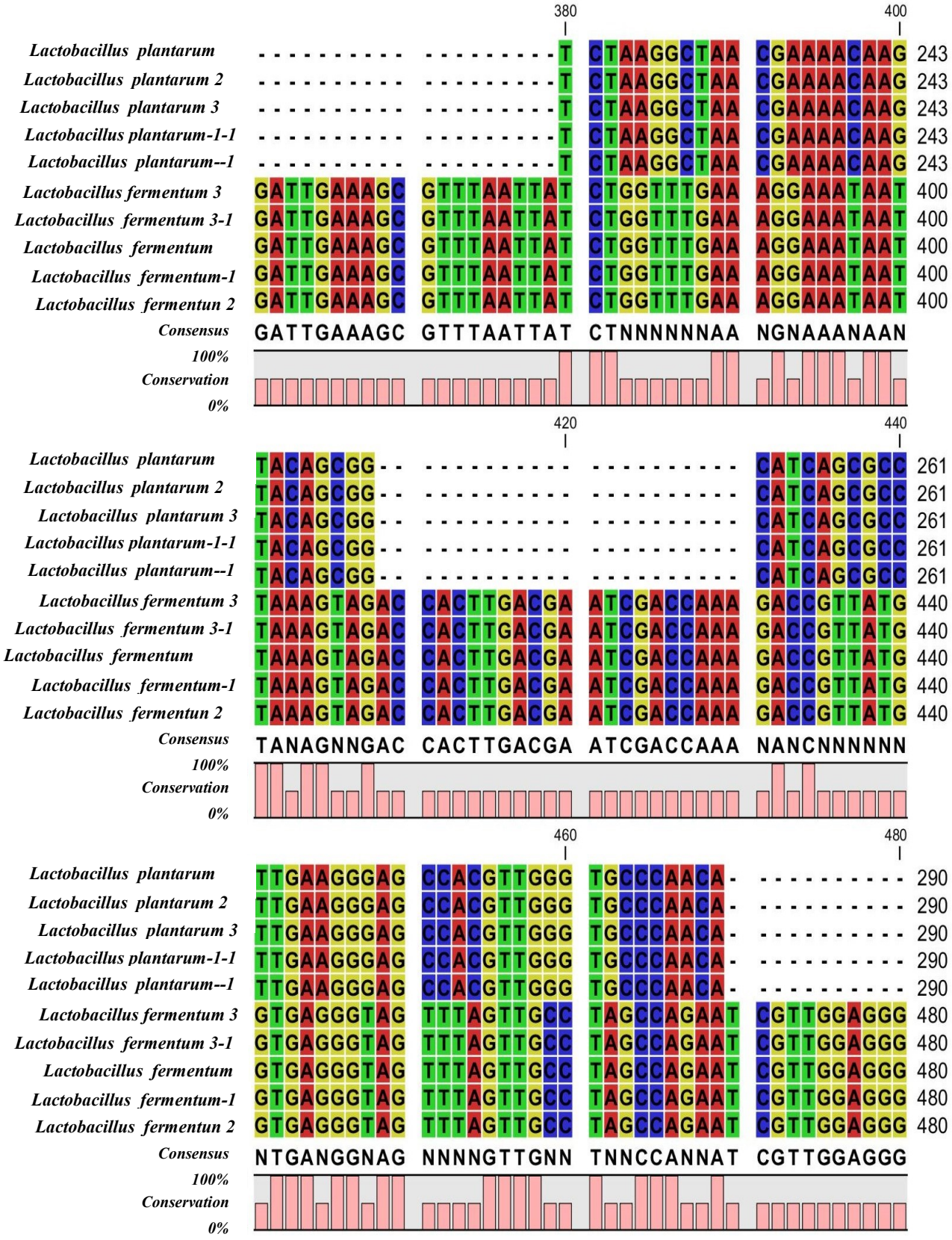
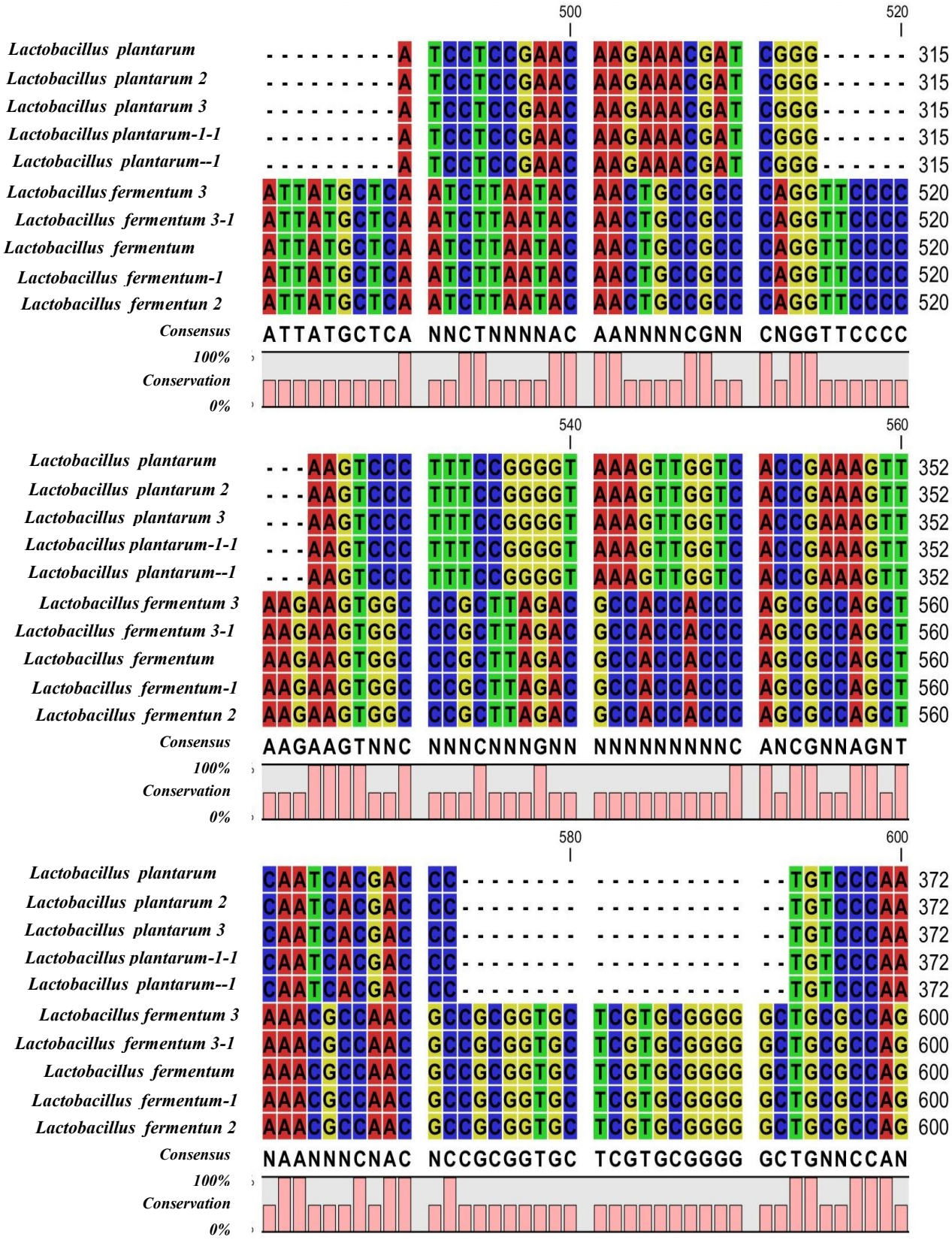
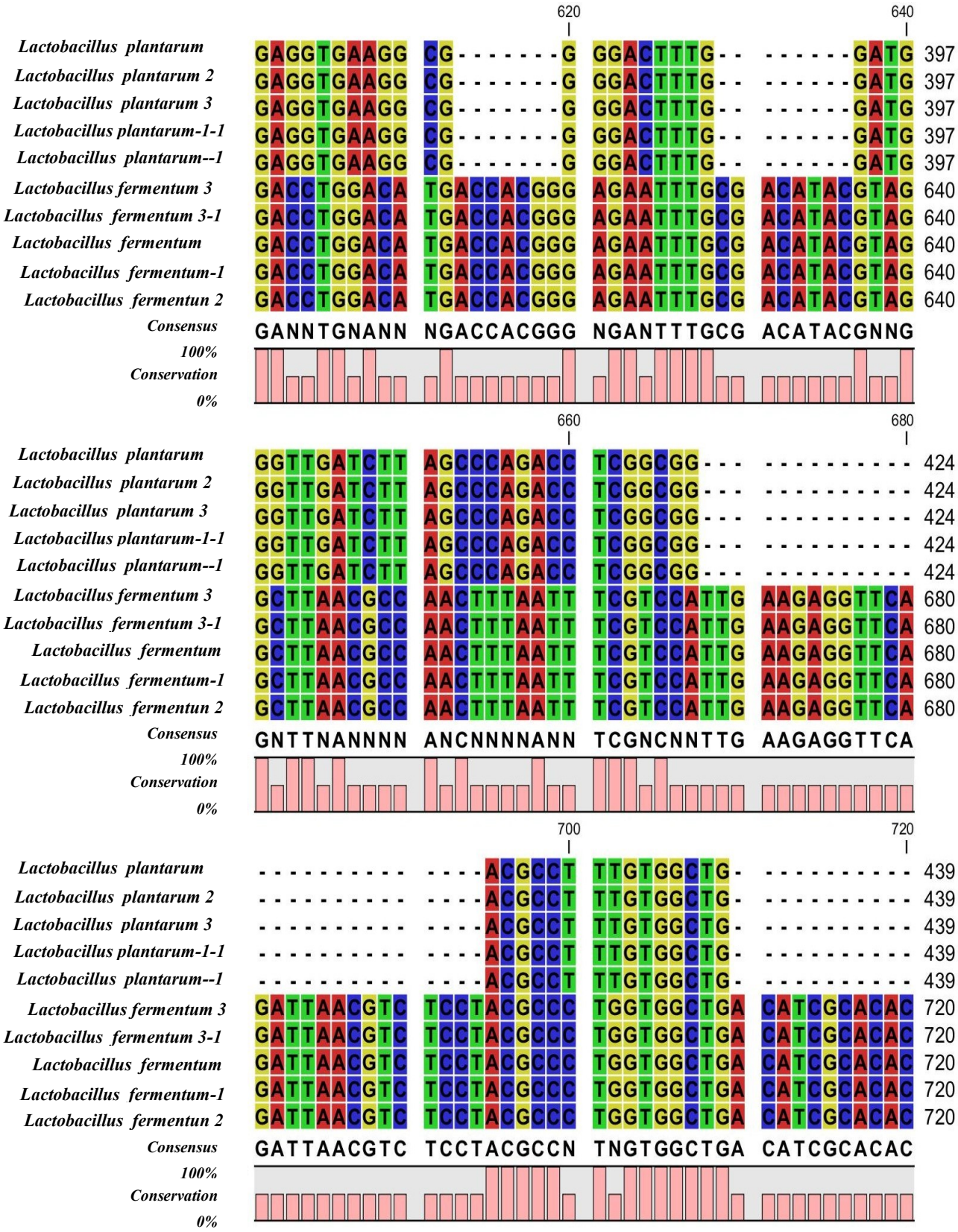
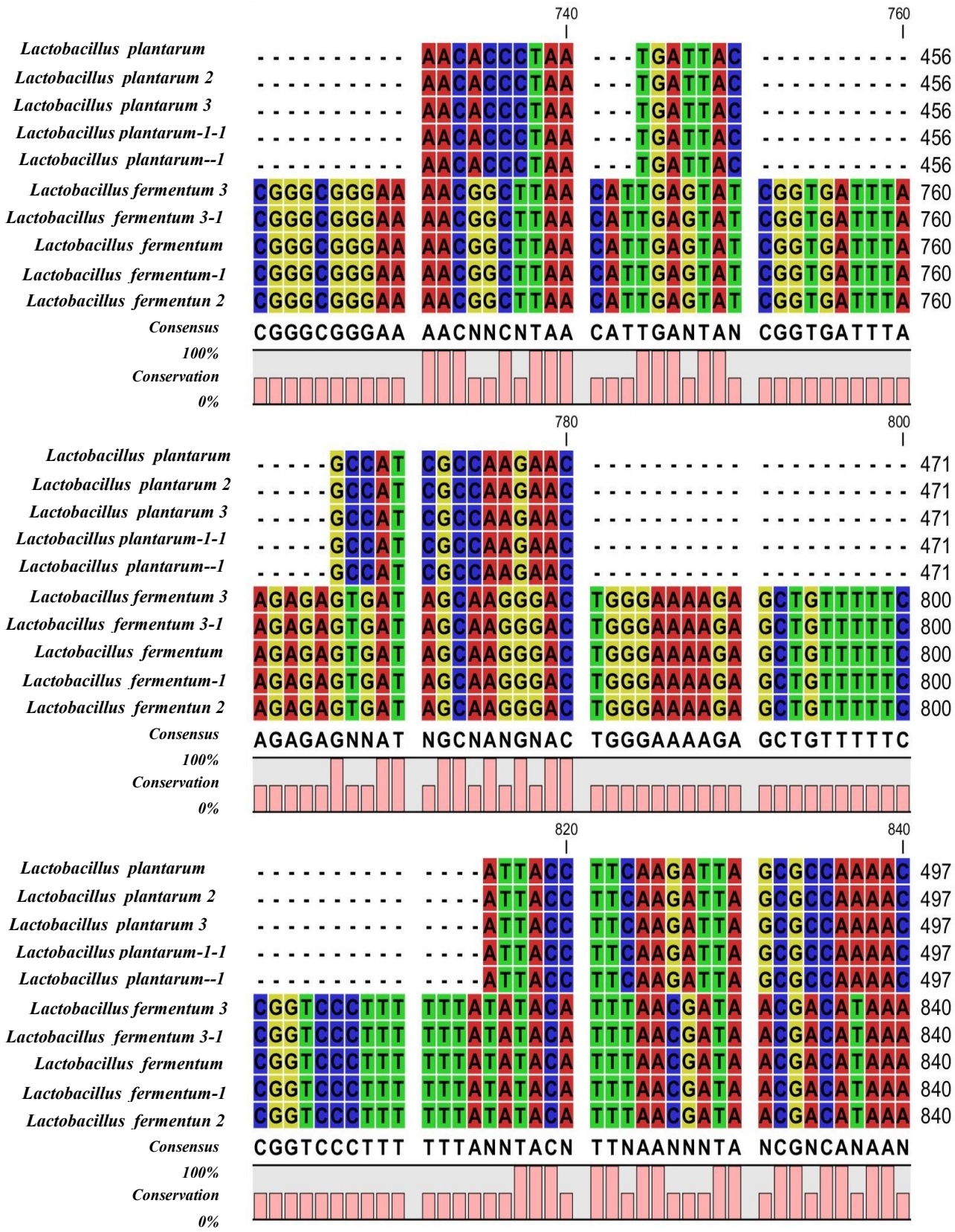
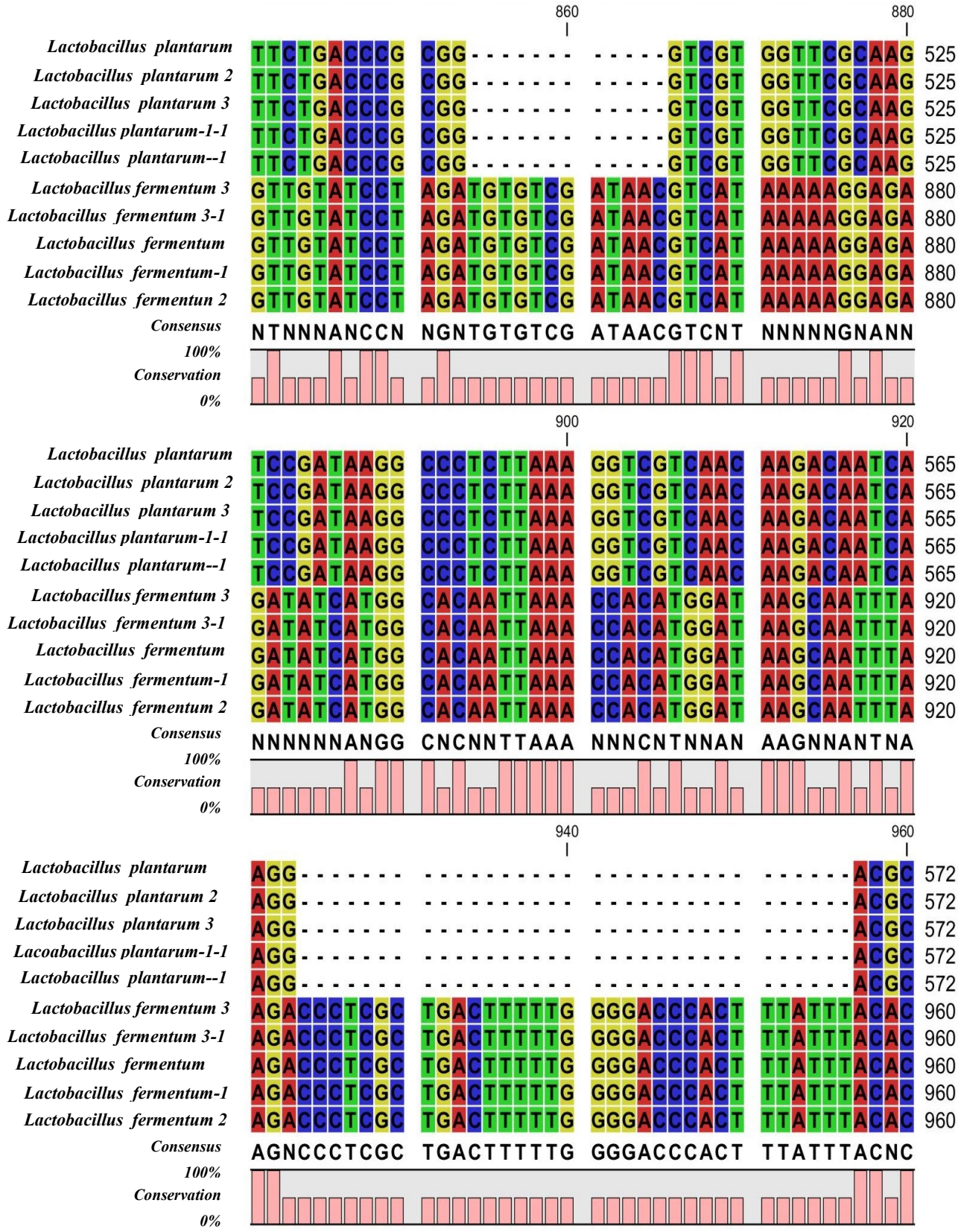
GCCGGCCGGT TAAACAAAAA TGATTCAAGN NCNNNTNNTT

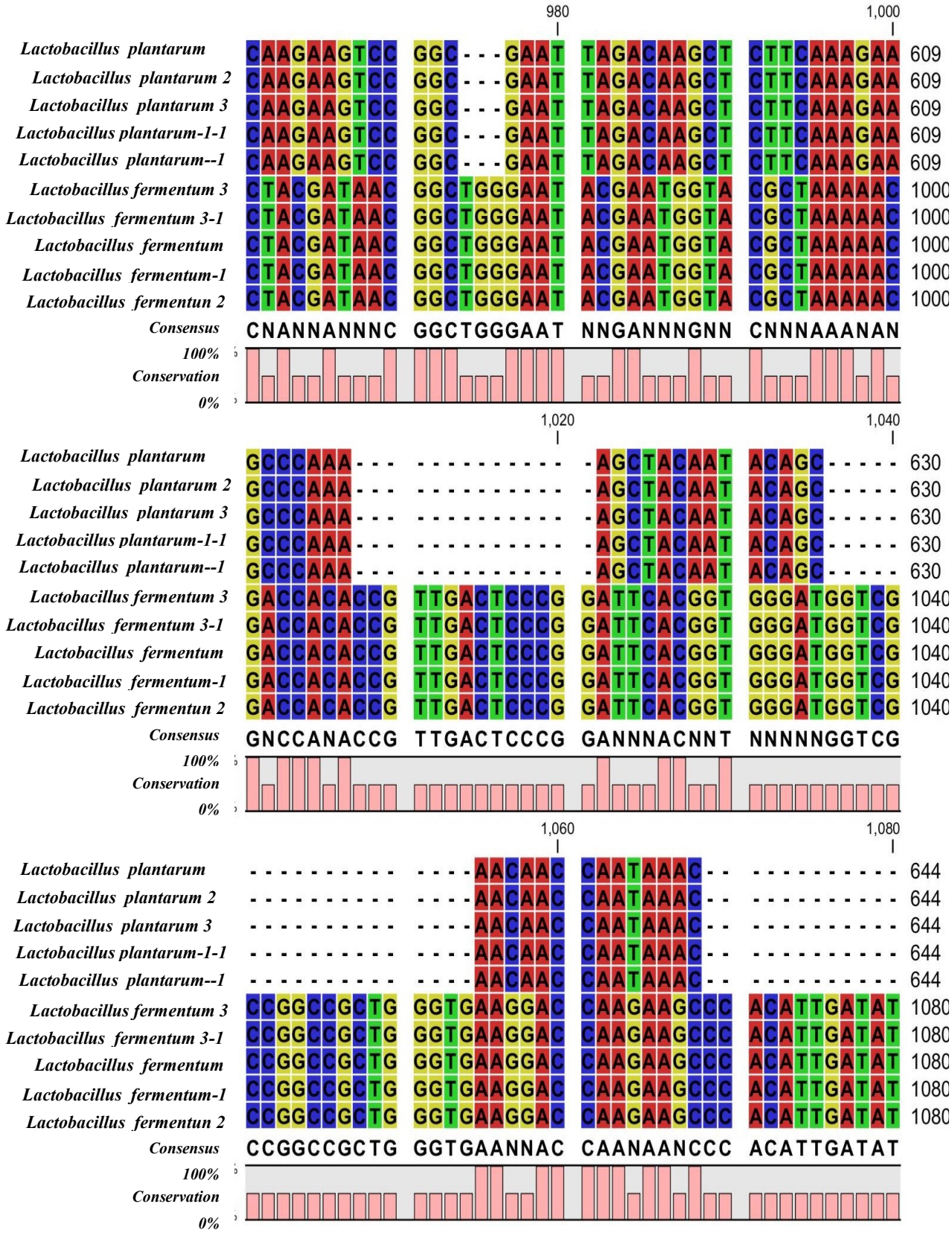

380
400
Lactobacillus plantarum 243
Lactobacillus plantarum 2 243
Lactobacillus plantarum 3 243
Lactobacillus plantarum-1-1 243
Lactobacillus plantarum--1 243
Lactobacillus fermentum 3 400
Lactobacillus fermentum 3-1 400
Lactobacillus fermentum 400
Lactobacillus fermentum-1 400
Lactobacillus fermentun 2 400
Consensus GATTGAAAGC GTTTAATTAT CTNNNNNNAA NGNAAANAAN
100%
Conservation
0%
420
440
Lactobacillus plantarum 261
Lactobacillus plantarum 2 261
Lactobacillus plantarum 3 261
Lactobacillus plantarum-1-1 261
Lactobacillus plantarum--1 261
Lactobacillus fermentum 3 440
Lactobacillus fermentum 3-1 440
Lactobacillus fermentum 440
Lactobacillus fermentum-1 440
Lactobacillus fermentun 2 440
Consensus TANAGNNGAC CACTTGACGA ATCGACCAAA NANCNNNNNN
100%
Conservation
0%
460
480
Lactobacillus plantarum 290
Lactobacillus plantarum 2 290
Lactobacillus plantarum 3 290
Lactobacillus plantarum-1-1 290
Lactobacillus plantarum--1 290
Lactobacillus fermentum 3 480
Lactobacillus fermentum 3-1 480
Lactobacillus fermentum 480
Lactobacillus fermentum-1 480
Lactobacillus fermentun 2 480
Consensus NTGANGGNAG NNNNGTTGNN TNNCCANNAT CGTTGGAGGG
100%
Conservation
0%

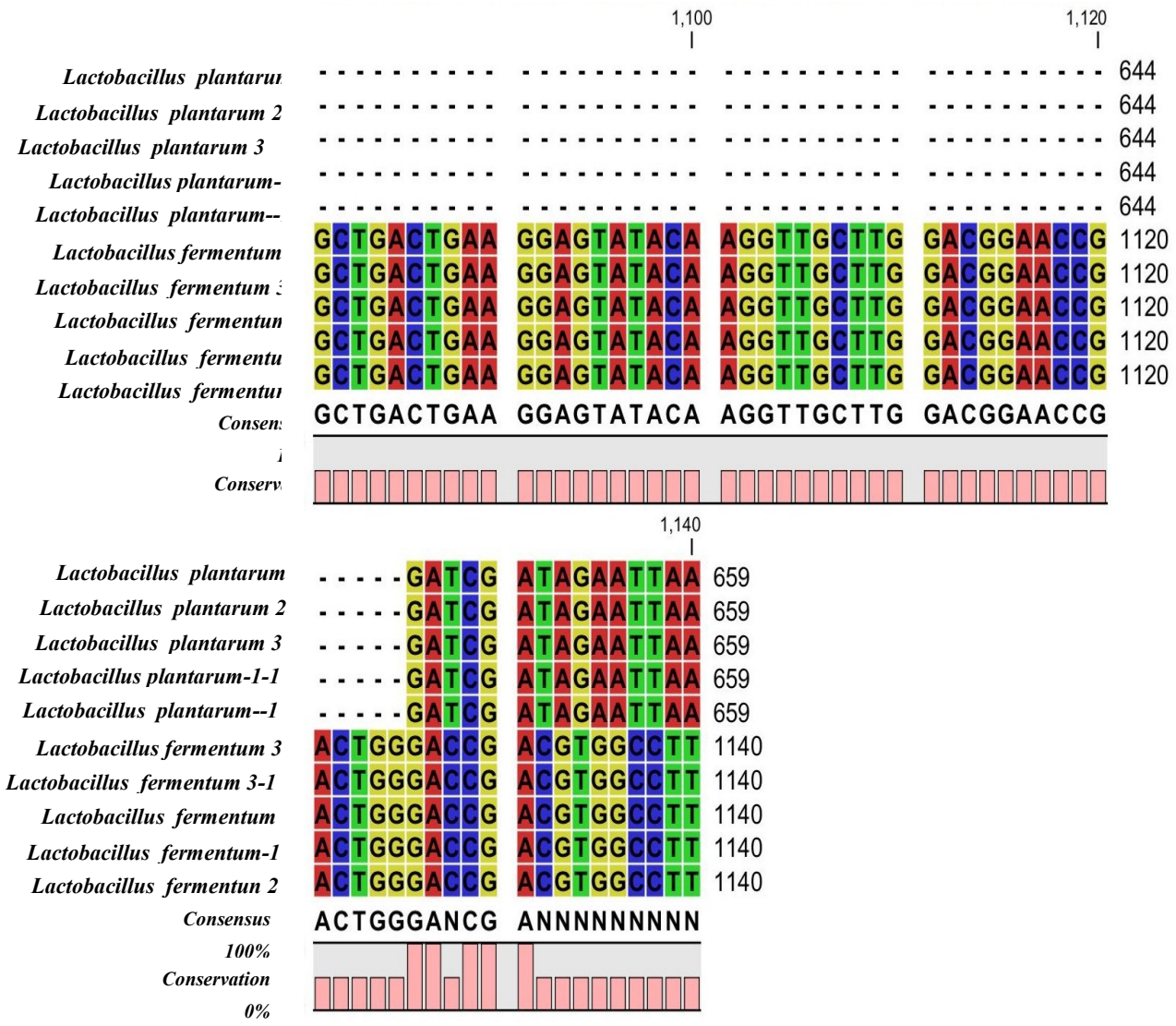

500
520
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%
540
560
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%
580
600
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
100%
Conservation
0%


620
640
Lactobacillus plantarum GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum 2 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum 3 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum-1-1 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum--1 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus fermentum 3 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum 3-1 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum-1 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentun 2 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Consensus GANNTGNANN NGACCACGGG NGANTTTGCG ACATACGNNG
100%
Conservation
0%
660
680
Lactobacillus plantarum GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum 2 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum 3 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum-1-1 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum--1 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus fermentum 3 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum 3-1 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum-1 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentun 2 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Consensus GNTTNANNNN ANCNNNNANN TCGNCNNTTG AAGAGGTTCA
100%
Conservation
0%
700
720
Lactobacillus plantarum ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum 2 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum 3 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum-1-1 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum--1 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus fermentum 3 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentum 3-1 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentum GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentum-1 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentun 2 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Consensus GATTAACGTC TCCTACGCCN TNGTGGCTGA CATCGCACAC
100%
Conservation
0%


740
760
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
CGGGCGGGAA AACNNCNTAA CATTGANTAN CGGTGATTTA
100%
Conservation
0%
780
800
AGAGAGNNAT NGCNANGNAC TGGGAAAAGA GCTGTTTTTC
820
840
CGGTCCCTTT TTTANNTACN TTNAANNNTA NCGNCANAAN


860
880
Lactobacillus plantarum TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum 2 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum 3 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum-1-1 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum--1 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus fermentum 3 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum 3-1 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum-1 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentun 2 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Consensus NTNNNANCCN NGNTGTGTCG ATAACGTCNT NNNNNGNANN
100%
Conservation
0%
900
920
Lactobacillus plantarum TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum 2 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum 3 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum-1-1 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum--1 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus fermentum 3 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum 3-1 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum-1 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum 2 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Consensus NNNNNNANGG CNCNNTTAAA NNNCNTNNAN AAGNNANTNA
100%
Conservation
0%
940
960
Lactobacillus plantarum AGG---------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum 2 AGG---------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum 3 AGG---------- ---------- ---------- ------ACGC 572
Lacoabacillus plantarum-1-1 AGG---------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum--1 AGG---------- ---------- ---------- ------ACGC 572
Lactobacillus fermentum 3 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum 3-1 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum-1 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum 2 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Consensus AGNCCCTCGC TGACTTTTTG GGGACCCACT TTATTTACNC
100%
Conservation
0%


980
1,000
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus plantarum 3
Lactobacillus plantarum-1-1
Lactobacillus plantarum--1
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum
Lactobacillus fermentum-1
Lactobacillus fermentun 2
Consensus
CNANNANNNC GGCTGGGAAT NNGANNNGNN CNNNAAANAN
100%
Conservation
0%
1,020
1,040
GCCCAAA--- ---------- -AGCTACAAT ACAGC----- 630
GACCACACCG TTGACTCCCG GATTCACGGT GGGATGGTCG 1040
Consensus
GNCCANACCG TTGACTCCCG GANNNACNNT NNNNNGGTCG
1,060
1,080
---------- ----AACAAC CAATAAAC-- ---------- 644
CCGGCCGCTG GGTGAAGGAC CAAGAAGCCC ACATTGATAT 1080
Consensus
CCGGCCGCTG GGTGAANNAC CAANAANCCC ACATTGATAT



Appendix 2 c: Breadfruit Isolates Alignment at 37±1^{o}C

Appendix 4 a

16SrRNA Sequenceof Pigeon-pea Isolatesat28±2^{o}C

GTCGGGCCGTACTAAAGATTGCAGTCGAGCGATGGTTTAAAGCTTGTTCTTATGAAG
TTAGCGGCGGACGGGTGAGAAACACGTGGGTAACCTGCCCATAAGACTGGGATAAC
TCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTG
AAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGG
TCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTG
ACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
TAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTT
AAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGA
CTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA
TATGGAGGAACACCAGTGGCGAAGGCGACTTTTCTGGTCTGTAACTGACACTGAGG
CTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGAAGTCTAAGTGTTAAAAGGGTTTCGCCCTTTTAGTTGCTGGAAATTTAACGCA
TTTAAGCAACTCCGCTTGCGGAGTACGGCCGTAAGGTTTGAAAACTTCAATATAAAT
TGAACGGCGGCCTGCCAAGAAGATACGTTGTGGCGCGTACGAACAAGTATATAGTT
GTACTGGTTTTATTTTCTCGATAAGTCTGCATCTGCCGCACACAGAAACCTC.

*Bacillus Thuringiensis*JQ289048.1

TGGTCGGCGTTGTTGTTGCGAGATGGACAATTCTATGAAAGTGTTGTTTATCAGTTG
CACGTTTATGAAGGTGTTGCCAGTGGTGATGCATTTGGTGCTGGACTTGTACATGGC
TTCTTACATGGTTTCAAAGGACAAGAACAAGTTGATTACGCAATTGCTGCTAGTGTT
CTAAAATTGACCGTCAGTGGTGATTTGAACCTGGTCAGT

GAACCTGAGATTCGCAATATCATGCAAGATGGTGGCTCCGCAATGAGTCGCTGATA
AGCTACCCATTAGTTATTCATTAACCTAGCCTTGATCGAATAAGTTAGAGGTGTTATT
TATGGCTGACAATGAGAGCGATTTTGAGCGTAGGTTGCAGAACGCAATTTGGGGGA
TGCCGGTATTACGTCCGGATGAACAGCATCGATGTTTGGG

GACCTTTTATGAGCGCATTGATTTAAGAGTAAGCTTTCAGCAGGCACTACAACGTGA
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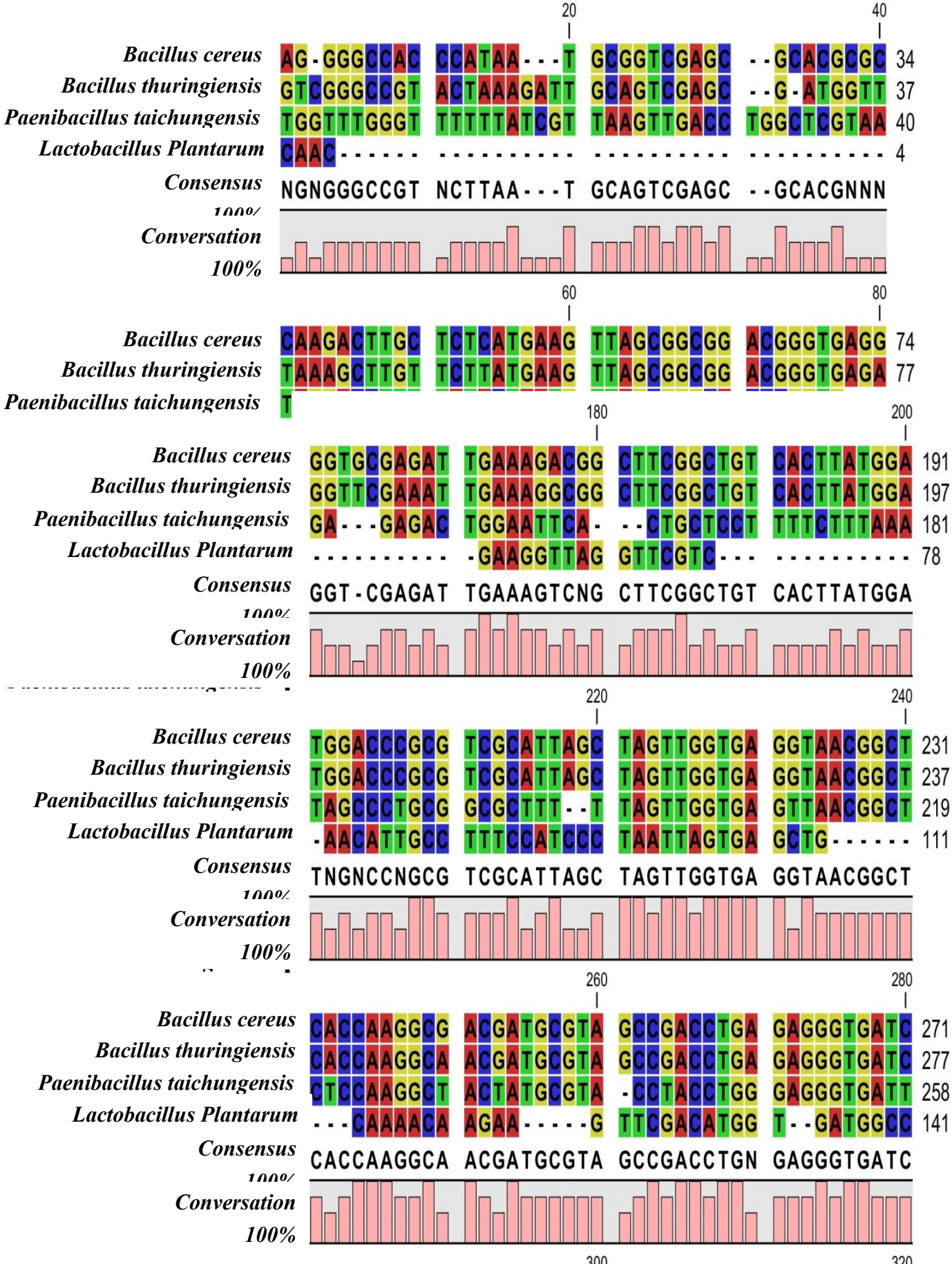
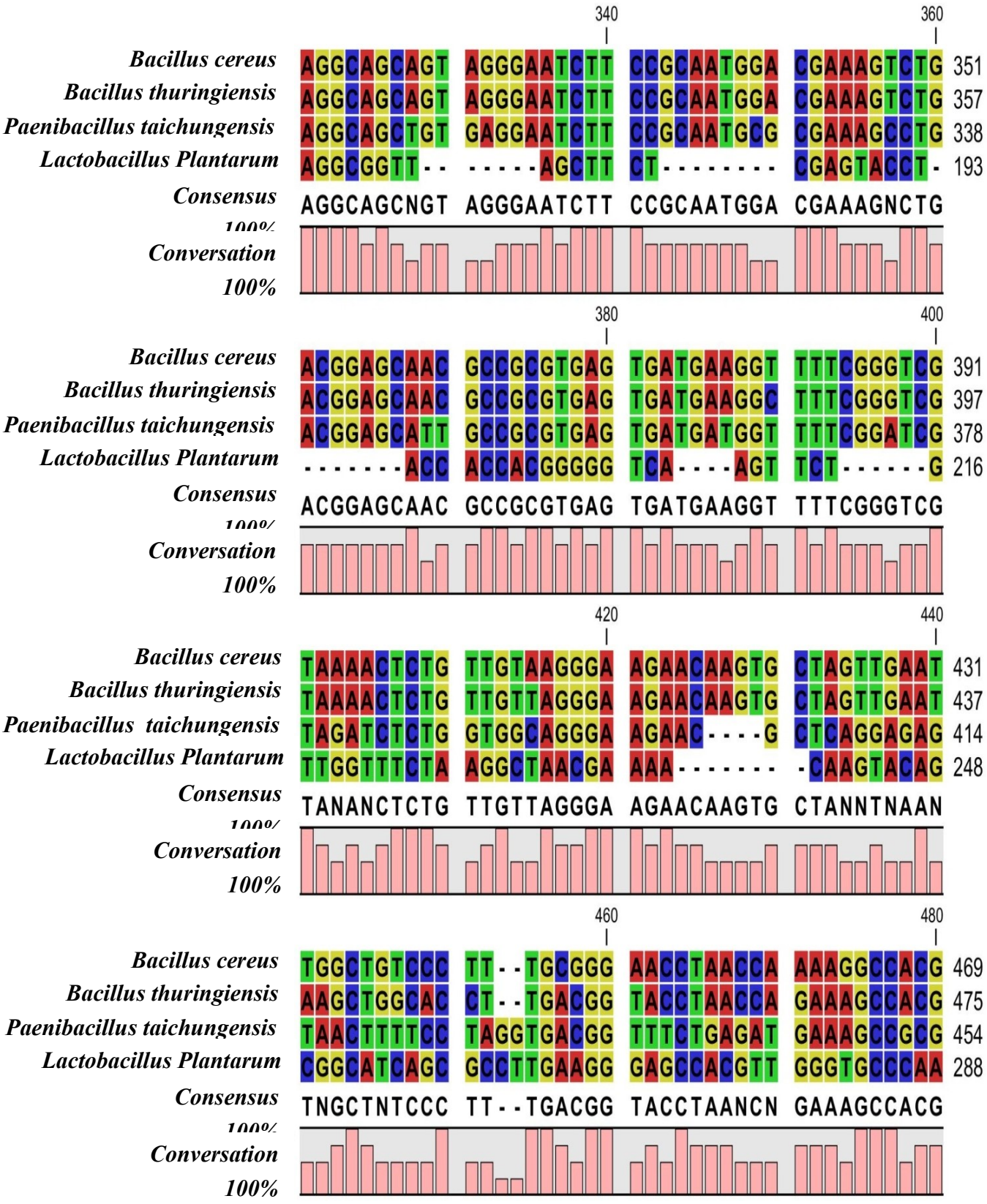
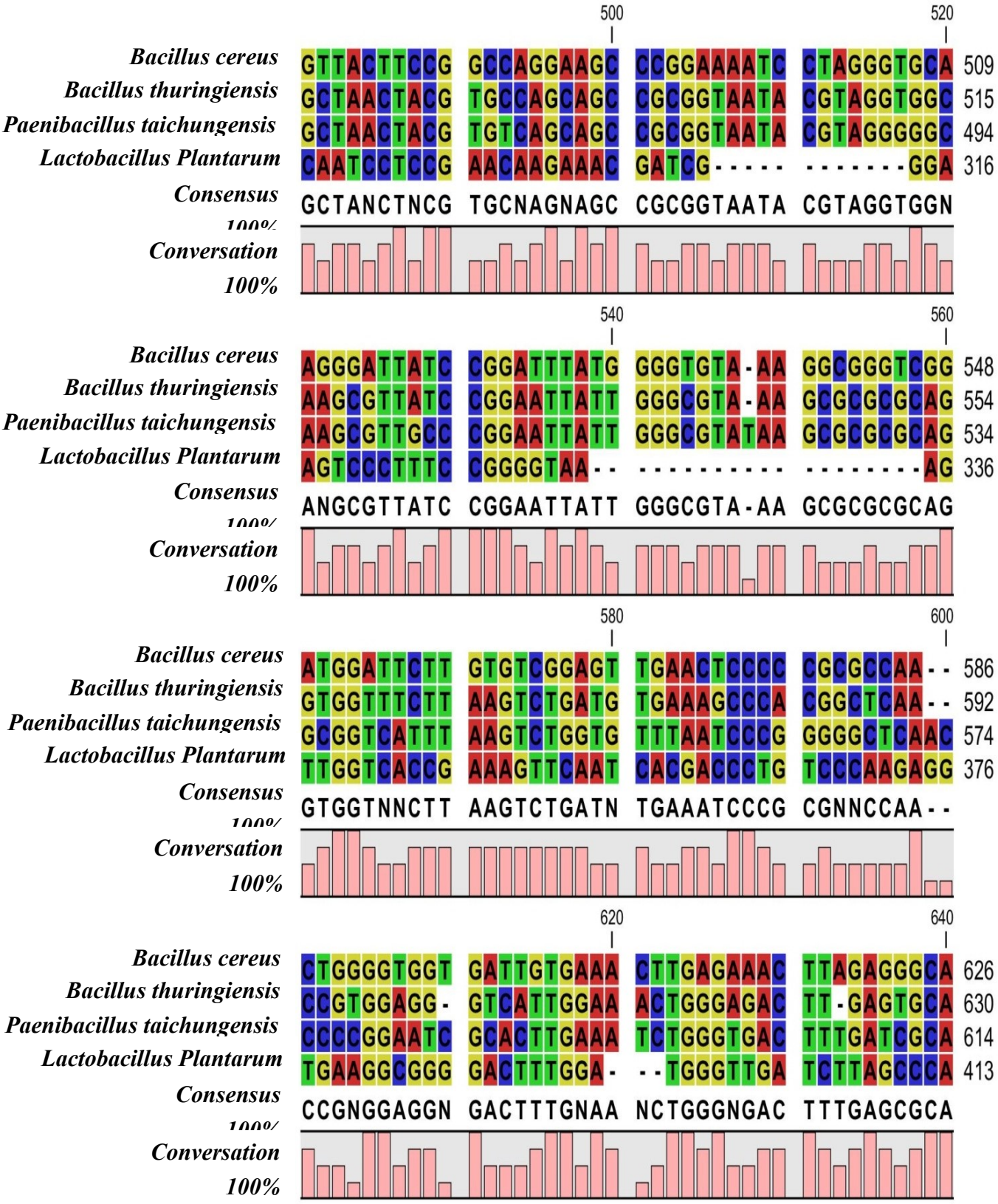
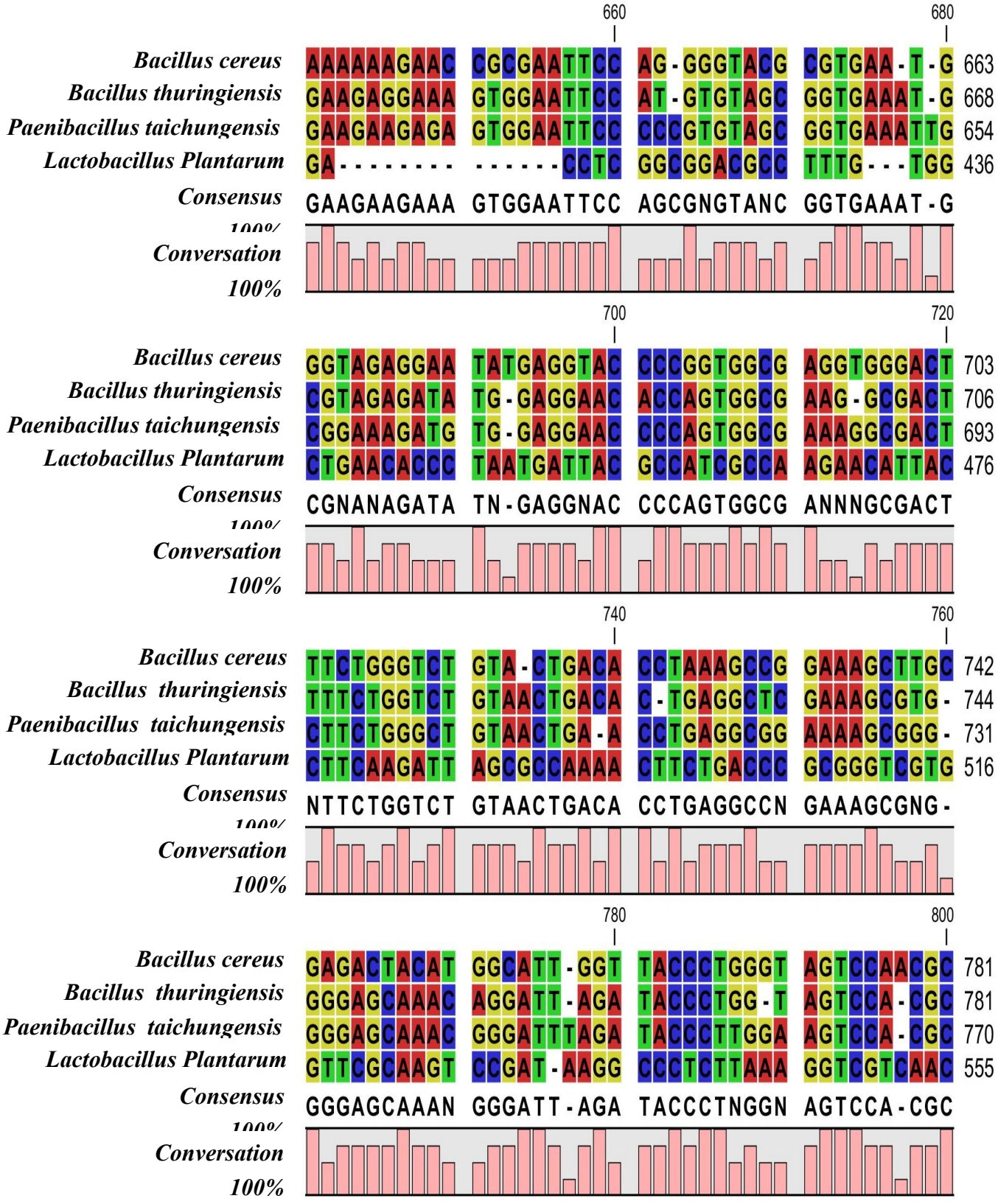
AATGGCAAGCTTGACGATGATATTT

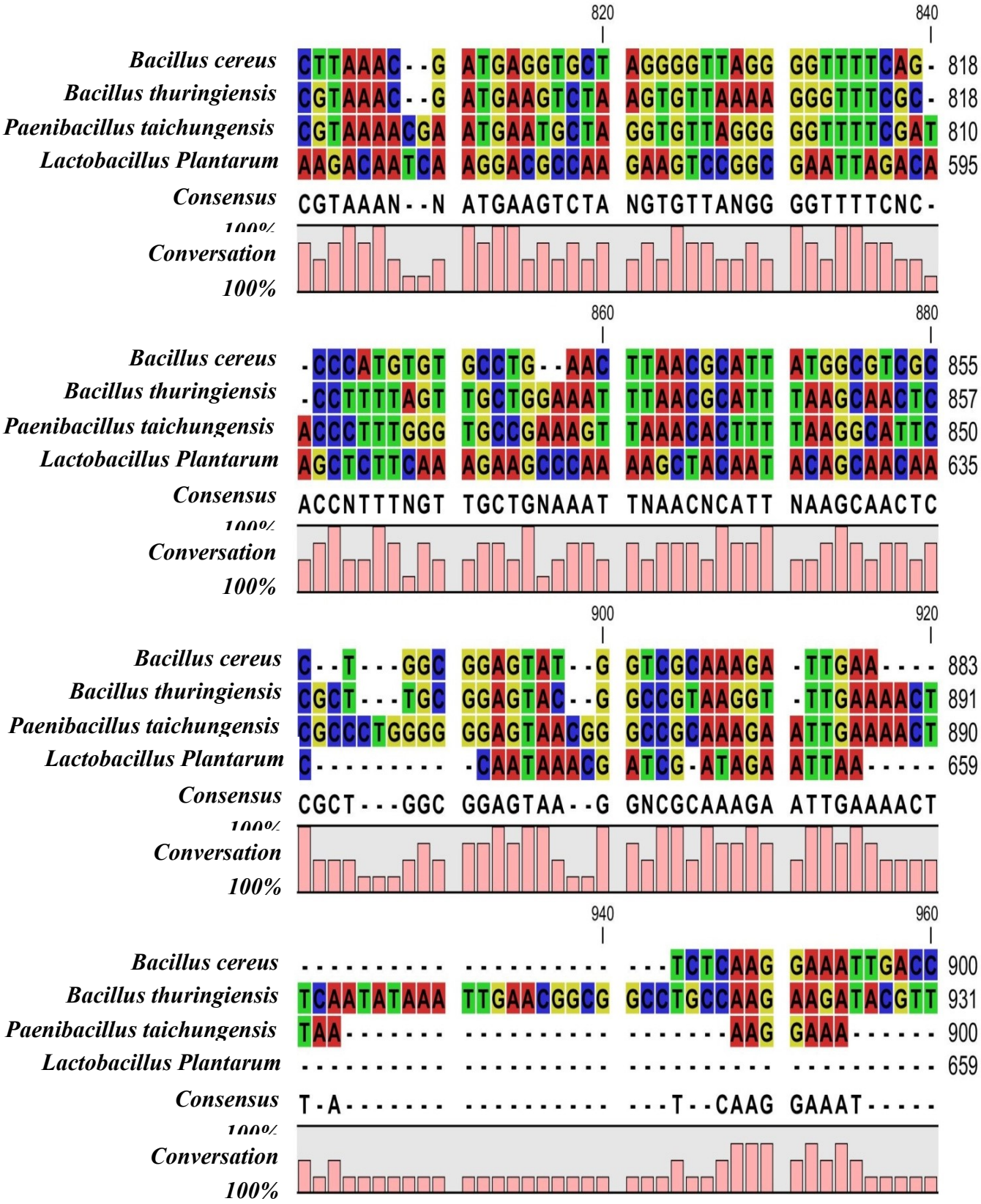
*Lactobacillusplantarum*CP013753.1

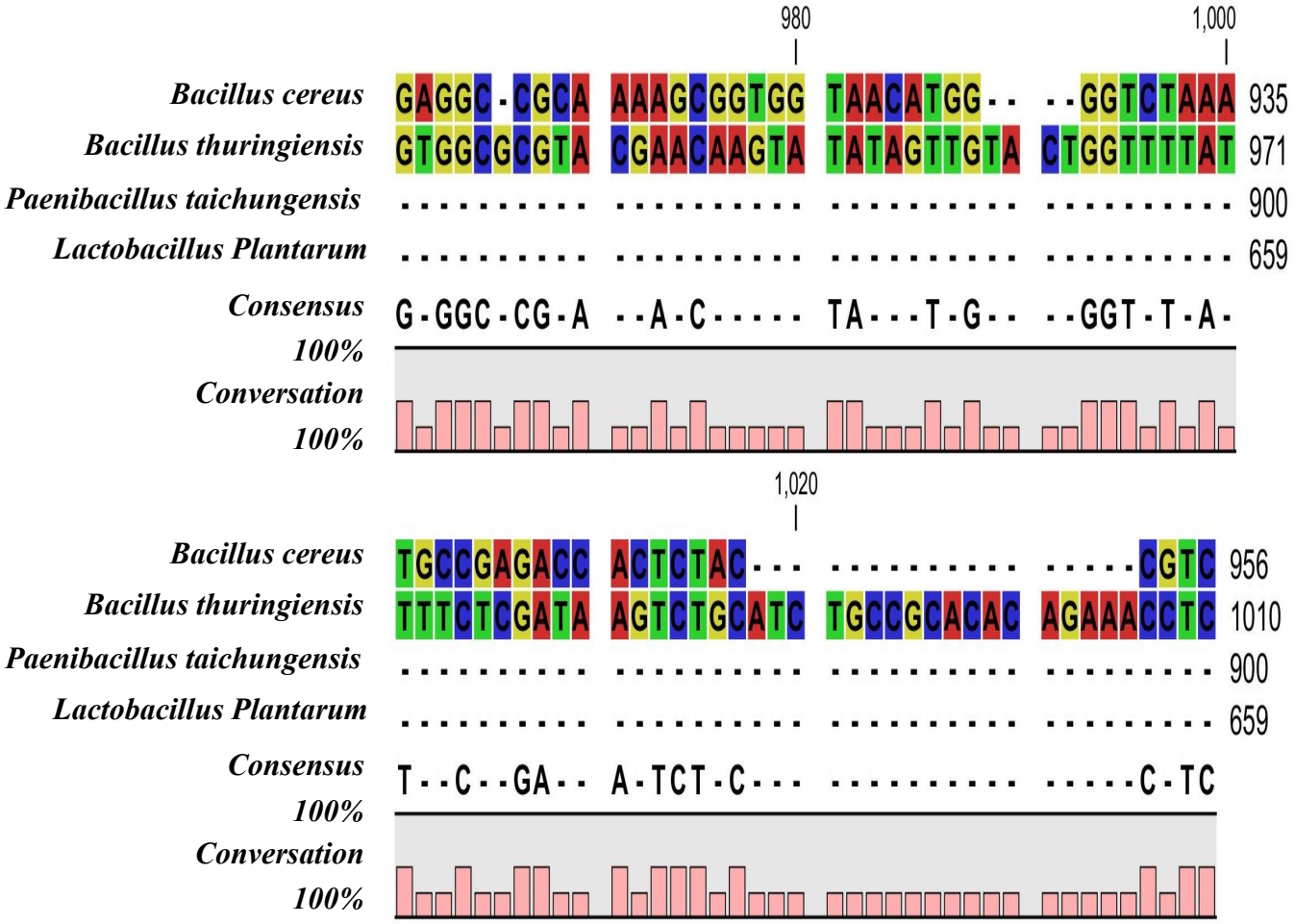

20
40
Bacillus cereus
Bacillus thuringiensis
Paenibacillus taichungensis
Lactobacillus Plantarum
Consensus
NGNGGGCCGT NCTTAA---T GCAGTCGAGC --GCACGNNN
100%
Conversation
100%
60
80
Bacillus cereus
Bacillus thuringiensis
Paenibacillus taichungensis
180
200
Bacillus cereus
Bacillus thuringiensis
Paenibacillus taichungensis
Lactobacillus Plantarum
Consensus
GGT-CGAGAT TGAAAGTCNG CTTCGGCTGT CACTTATGGA
100%
Conversation
100%
220
240
Bacillus cereus
Bacillus thuringiensis
Paenibacillus taichungensis
Lactobacillus Plantarum
Consensus
TNGNCCNGCG TCGCATTAGC TAGTTGGTGA GGTAACGGCT
100%
Conversation
100%
260
280
Bacillus cereus
Bacillus thuringiensis
Paenibacillus taichungensis
Lactobacillus Plantarum
Consensus
CACCAAGGCA ACGATGCGTA GCCGACCTGN GAGGGTGATC
100%
Conversation
100%
300
320


340
360
Bacillus cereus AGGCAGCAGT AGGGAATCTT CCGCAATGGA CGAAAGTCTG 351
Bacillus thuringiensis AGGCAGCAGT AGGGAATCTT CCGCAATGGA CGAAAGTCTG 357
Paenibacillus taichungensis AGGCAGCTGT GAGGAATCTT CCGCAATGCG CGAAAGCCTG 338
Lactobacillus Plantarum AGGCGGTT-- -----AGCTT CT-------- CGAGTACCT- 193
Consensus AGGCAGCNGT AGGGAATCTT CCGCAATGGA CGAAAGNCTG
100%
Conversation
100%
380
400
Bacillus cereus ACGGAGCAAC GCCGCGTGAG TGATGAAGGT TTTCGGGTCG 391
Bacillus thuringiensis ACGGAGCAAC GCCGCGTGAG TGATGAAGGC TTTCGGGTCG 397
Paenibacillus taichungensis ACGGAGCATT GCCGCGTGAG TGATGATGGT TTTCGGATCG 378
Lactobacillus Plantarum -------ACC ACCACGGGGG TCA----AGT TCT------G 216
Consensus ACGGAGCAAC GCCGCGTGAG TGATGAAGGT TTTCGGGTCG
100%
Conversation
100%
420
440
Bacillus cereus TAAAACTCTG TTGTAAGGGA AGAACAAGTG CTAGTTGAAT 431
Bacillus thuringiensis TAAAACTCTG TTGTTAGGGA AGAACAAGTG CTAGTTGAAT 437
Paenibacillus taichungensis TAGATCTCTG GTGGCAGGGA AGAAC----G CTCAGGAGAG 414
Lactobacillus Plantarum TTGGTTTCTA AGGCTAACGA AAA------- -CAAGTACAG 248
Consensus TANANCTCTG TTGTTAGGGA AGAACAAGTG CTANNTNAAN
100%
Conversation
100%
460
480
Bacillus cereus TGGCTGTCCC TT--TGCGGG AACCTAACCA AAAGGCCACG 469
Bacillus thuringiensis AAGCTGGCAC CT--TGACGG TACCTAACCA GAAAGCCACG 475
Paenibacillus taichungensis TAACTTTTCC TAGGTGACGG TTTCTGAGAT GAAAGCCGCG 454
Lactobacillus Plantarum CGGCATCAGC GCCTTGAAGG GAGCCACGTT GGGTGCCCAA 288
Consensus TNGCTNTCCC TT--TGACGG TACCTAANCN GAAAGCCACG
100%
Conversation
100%


500
520
Bacillus cereus GTTACTTCCG GCCAGGAAGC CCGGAAAATC CTAGGGTGCA 509
Bacillus thuringiensis GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGTGGC 515
Paenibacillus taichungensis GCTAACTACG TGTCAGCAGC CGCGGTAATA CGTAGGGGGC 494
Lactobacillus Plantarum CAATCCTCCG AACAAGAAAC GATCG----- -------GGA 316
Consensus GCTANCTNCG TGCNAGNAGC CGCGGTAATA CGTAGGTGGN
100%
Conversation
100%
540
560
Bacillus cereus AGGGATTATC CGGATTTATG GGGTGTA-AA GGCGGGTCGG 548
Bacillus thuringiensis AAGCGTTATC CGGAATTATT GGGCGTA-AA GCGCGCGCAG 554
Paenibacillus taichungensis AAGCGTTGCC CGGAATTATT GGGCGTATAA GCGCGCGCAG 534
Lactobacillus Plantarum AGTCCCTTTC CGGGGTAA-- ---------- --------AG 336
Consensus ANGCGTTATC CGGAATTATT GGGCGTA-AA GCGCGCGCAG
100%
Conversation
100%
580
600
Bacillus cereus ATGGATTCTT GTGTCGGAGT TGAACTCCCC CGCGCCAA-- 586
Bacillus thuringiensis GTGGTTTCTT AAGTCTGATG TGAAAGCCCA CGGCTCAA-- 592
Paenibacillus taichungensis GCGGTCATTT AAGTCTGGTG TTTAATCCCG GGGGCTCAAC 574
Lactobacillus Plantarum TTGGTCACCG AAAGTTCAAT CACGACCCTG TCCCAAGAGG 376
Consensus GTGGTNNCTT AAGTCTGATN TGAAATCCCG CGNNCCAA--
100%
Conversation
100%
620
640
Bacillus cereus CTGGGGTGGT GATTGTGAAA CTTGAGAAAC TTAGAGGGCA 626
Bacillus thuringiensis CCGTGGAGG- GTCATTGGAA ACTGGGAGAC TT-GAGTGCA 630
Paenibacillus taichungensis CCCCGGAATC GCACTTGAAA TCTGGGTGAC TTTGATCGCA 614
Lactobacillus Plantarum TGAAGGCGGG GACTTTGGA- --TGGGTTGA TCTTAGCCCA 413
Consensus CCGNGGAGGN GACTTTGNAA NCTGGGNGAC TTTGAGCGCA
100%
Conversation
100%


660
680
Bacillus cereus AAAAAAGAAC CGCGAATTCC AG-GGGTACG CGTGAA-T-G 663
Bacillus thuringiensis GAAGAGGAAA GTGGAATTCC AT-GTGTAGC GGTGAAAT-G 668
Paenibacillus taichungensis GAAGAAGAGA GTGGAATTCC CCCGTGTAGC GGTGAAAATTG 654
Lactobacillus Plantarum GA-------- ------CCTC GGCGGACGCC TTTG---TGG 436
Consensus GAAGAAGAAA GTGGAATTCC AGCGNGTANC GGTGAAAT-G
100%
Conversation
100%
700
720
Bacillus cereus GGTAGAGGAA TATGAGGTAC CCCGGTGGCG AGGTGGGACT 703
Bacillus thuringiensis CGTAGAGATA TG-GAGGAAC ACCAGTGGCG AAG-GCGACT 706
Paenibacillus taichungensis CGGAAAGATG TG-GAGGAAC CCCAGTGGCG AAAGGCGACT 693
Lactobacillus Plantarum CTGAACACCC TAATGATTAC GCCATCGCCA AGAACATTAC 476
Consensus CGNANAGATA TN-GAGGNAC CCCAGTGGCG ANNNGCGACT
100%
Conversation
100%
740
760
Bacillus cereus TTCTGGGTCT GTA-CTGACA CCTAAAGCCG GAAAGCTTGC 742
Bacillus thuringiensis TTTCTGGTCT GTAACTGACA C-TGAGGCTC GAAAGCGTG- 744
Paenibacillus taichungensis CTTCTGGGCT GTAACTGA-A CCTGAGGCGG AAAAGCGGG- 731
Lactobacillus Plantarum CTTCAAGATT AGCGCCAAAA CTTCTGACCC GCGGGTCGTG 516
Consensus NTTCTGGTCT GTAACTGACA CCTGAGGCCN GAAAGCGNG-
100%
Conversation
100%
780
800
Bacillus cereus GAGACTACAT GGCATT-GGT TACCCTGGGT AGTCCAACGC 781
Bacillus thuringiensis GGGAGCAAAC AGGATT-AGA TACCCTGG-T AGTCCA-CGC 781
Paenibacillus taichungensis GGGAGCAAAC GGGATTTAGA TACCCTTGGA AGTCCA-CGC 770
Lactobacillus Plantarum GTTCGCAAGT CCGAT-AAGG CCCTCTTAAA GGTCGTCAAC 555
Consensus GGGAGCAAAN GGGATT-AGA TACCCTNGGN AGTCCA-CGC
100%
Conversation
100%


820
840
Bacillus cereus CTTAAAC--G ATGAGGTGCT AGGGGTTAGG GGTTTTTCAG- 818
Bacillus thuringiensis CGTAAAC--G ATGAAGTCTA AGTGTTAAAA GGGTTTCGC- 818
Paenibacillus taichungensis CGTAAAACGA ATGAATGCTA GGTGTTAGGG GGTTTTCGAT 810
Lactobacillus Plantarum AAGACAATCA AGGACGCCAA GAAGTCCGGC GAATTAGACA 595
Consensus CGTAAAN--N ATGAAGTCTA NGTGTTANGG GGTTTTCNC-
100%
Conversation
100%
860
880
Bacillus cereus -CCCATGTGT GCCTG--AAC TTAACGCATT ATGGCGTCGC 855
Bacillus thuringiensis -CCTTTTAGT TGCTGGAAAT TTAACGCATT TAAGCAACTC 857
Paenibacillus taichungensis ACCCTTTGGG TGCCGAAAGT TAAACACTTT TAAGGCATTC 850
Lactobacillus Plantarum AGCTCTTCAA AGAAGCCAA AAGCTACAAT ACAGCAACAA 635
Consensus ACCNTTTNGT TGCTGNAAAT TNAACNCATT NAAGCAACTC
100%
Conversation
100%
900
920
Bacillus cereus C--T---GGC GGAGTAT--G GTCGCAAAGA -TTGAA---- 883
Bacillus thuringiensis CGCT---TGC GGAGTAC--G GCCGTAAGGT -TTGAAAACT 891
Paenibacillus taichungensis CGCCCTGGGG GGAGTAACGG GCCGCAAAGA ATTGAAAACT 890
Lactobacillus Plantarum C--------- -CAATAAACG ATCG-ATAGA ATTAA----- 659
Consensus CGCT---GGC GGAGTAA--G GNCGCAAAGA ATTGAAAACT
100%
Conversation
100%
940
960
Bacillus cereus ---------- ---------- ---TCTCAAG GAAATTGACC 900
Bacillus thuringiensis TCAATATAAA TTGAACGGCG GCCTGCCAAG AAGATACGTT 931
Paenibacillus taichungensis TAA------- ---------- -------AAG GAAA------ 900
Lactobacillus Plantarum ---------- ---------- ---------- ---------- 659
Consensus T-A------- ---------- ---T--CAAG GAAAT-----
100%
Conversation
100%



Appendix 4 b: Pigeon-pea Isolates Alignment at 28±2 ^{o}C

Appendix 5 a

16SrRNA Sequence of Pigeon-pea Isolates at 37±1^{o}C

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGsT
GGCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTAC
CTAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCC
ACCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCG
TTTGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAA
TGATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAAT
AATTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTT
AGTTGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCC
CAGGTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGC
CAACGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAG
AATTTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTC
AGATTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGG
CTTAACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAG
CTGTTTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCC
TAGATGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACA
TGGATAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTA
CGATAACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCC
GGATTCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATT
GATATGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGA
CGTGGCCTT.

Lactobacillus fermentum CP011536.1

CGAGTGGGCCAATTTAAGCGTCGTCAGTTACTACAAGCTTTCCGCCACTCTCTACGC
CCTCGGGGTCATCAGCTTAGTGACCATTTGGTGGGTGGTGAACCAATTTGGCCAGTG
GCGGGGGAACCTGCGGATTATGCACGGGGTGGCAACGTATGCCTACCGCGCTTACC
TAGGCAATGTCTTTTGGCAGACCTTGCTTTGGGATTGGTGGGGTCGTCAATTAGCCA
CCACGCACCCATGGTTAGCGTTGGCGCTCCTCTGGCCGGCTACTTGGTTGTTAGCGTT
TGGTTTTGCCTACCTGTTACACCTGATATGGGGGCGCCGGCCGGTTAAACAAAAATG
ATTCAAGAACCACTAATTGATTGAAAGCGTTTAATTATCTGGTTTGAAAGGAAATAA
TTAAAGTAGACCACTTGACGAATCGACCAAAGACCGTTATGGTGAGGGTAGTTTAGT
TGCCTAGCCAGAATCGTTGGAGGGATTATGCTCAATCTTAATACAACTGCCGCCCAG
GTTCCCCAAGAAGTGGCCCGCTTAGACGCCACCACCCAGCGCCAGCTAAACGCCAA
CGCCGCGGTGCTCGTGCGGGGGCTGCGCCAGGACCTGGACATGACCACGGGAGAAT
TTGCGACATACGTAGGCTTAACGCCAACTTTAATTTCGTCCATTGAAGAGGTTCAGA
TTAACGTCTCCTACGCCCTGGTGGCTGACATCGCACACCGGGCGGGAAAACGGCTTA
ACATTGAGTATCGGTGATTTAAGAGAGTGATAGCAAGGGACTGGGAAAAGAGCTGT
TTTTCCGGTCCCTTTTTTATATACATTTAACGATAACGACATAAAGTTGTATCCTAGA
TGTGTCGATAACGTCATAAAAAGGAGAGATATCATGGCACAATTAAACCACATGGA
TAAGCAATTTAAGACCCTCGCTGACTTTTTGGGGACCCACTTTATTTACACCTACGAT
AACGGCTGGGAATACGAATGGTACGCTAAAAACGACCACACCGTTGACTCCCGGAT
TCACGGTGGGATGGTCGCCGGCCGCTGGGTGAAGGACCAAGAAGCCCACATTGATA
TGCTGACTGAAGGAGTATACAAGGTTGCTTGGACGGAACCGACTGGGACCGACGTG
GCCTT.

Lactobacillus fermentum CP005958.1

AAAAACATACAAATAGACGAGGAGTGCTTAATTATGTTATCAGTACCTGATTATGAG
TTTTGGTTTGTTACCGGTTCACAACACCTTTATGGTGAAGAACAATTGAAGTCTGTTG
CTAAGGATGCGCAAGATATTGCGGATAAATTGAATGCAAGCGGCAAGTTACCTTAT
AAAGTAGTCTTCAAAGATGTTATGACGACGGCTGAAAGT

ATCACCAACTTTATGAAAGAAGTTAATTACAATGATAAGGTAGCCGGTGTTATTACT
TGGATGCACACATTCTCACCAGCCAAGAACTGGATTCGTGGAACTGAACTGTTACAA
AAACCATTATTACACTTAGCAACGCAATATTTGAATAATATTCCATATGCAGACATT
GATTTTGATTACATGAACCTTAACCAAAGTGCGCATGGC

GACCGTGAATATGCCTACATTAACGCCCGGTTGCAGAAACATAATAAGGTTGTCTAC
GGCTATTGGGGCGATGAAGATGTGCAAGAACAGATTGCGCGTTGGGAAGACGTCGC
AGTAGCGTACAATGAGAGCTTTAAAGTTAAGGTTGCTCGTTTTGGCGACACGATGCG
TAATGTGGCCGTTACTGAAGGTGACAAGGTTGAAGCTCAA

ATTAAGATGGGCTGGACAGTTGACTATTATGGTATCGGTGACTTAGTTGAAGAGATC
AATAAGGTTTCGGATGTTGATATTGATAAGGAATACGCTGACTTGGAGTCTCGGTAT
GAAATGGTCCAGGGCGATAACGATGC

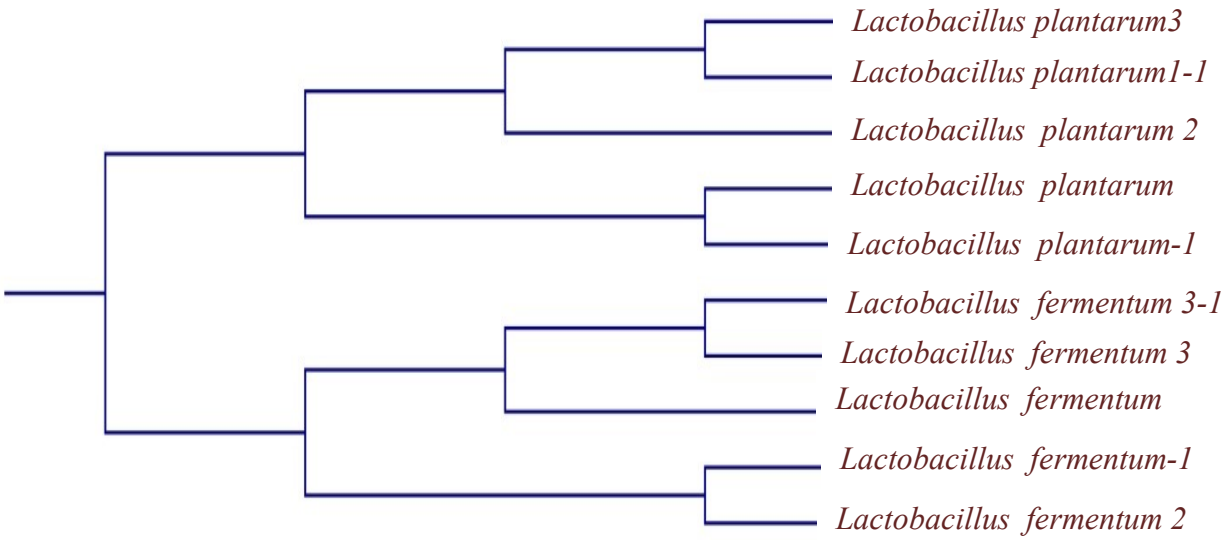
*Lactobacillus plantarum*CP012122.1

TGGTTCGGAAGGCTTCTACGGCTGATGTGAATGCGTACCGCAACCTGGCGTTCAATC
CGCATGATCAAGTAGCAGATACAGGAGCTTATCCGCACGCAAGCTCCAATGTTGCG
GAAGAAACTAATCCAACATTCATTGCACAGAATGCCATTGATGGCAAGCTAGCCAA
TATTTCGCATGGGTCATATCCGTATGGATCGTGGGGTATTC

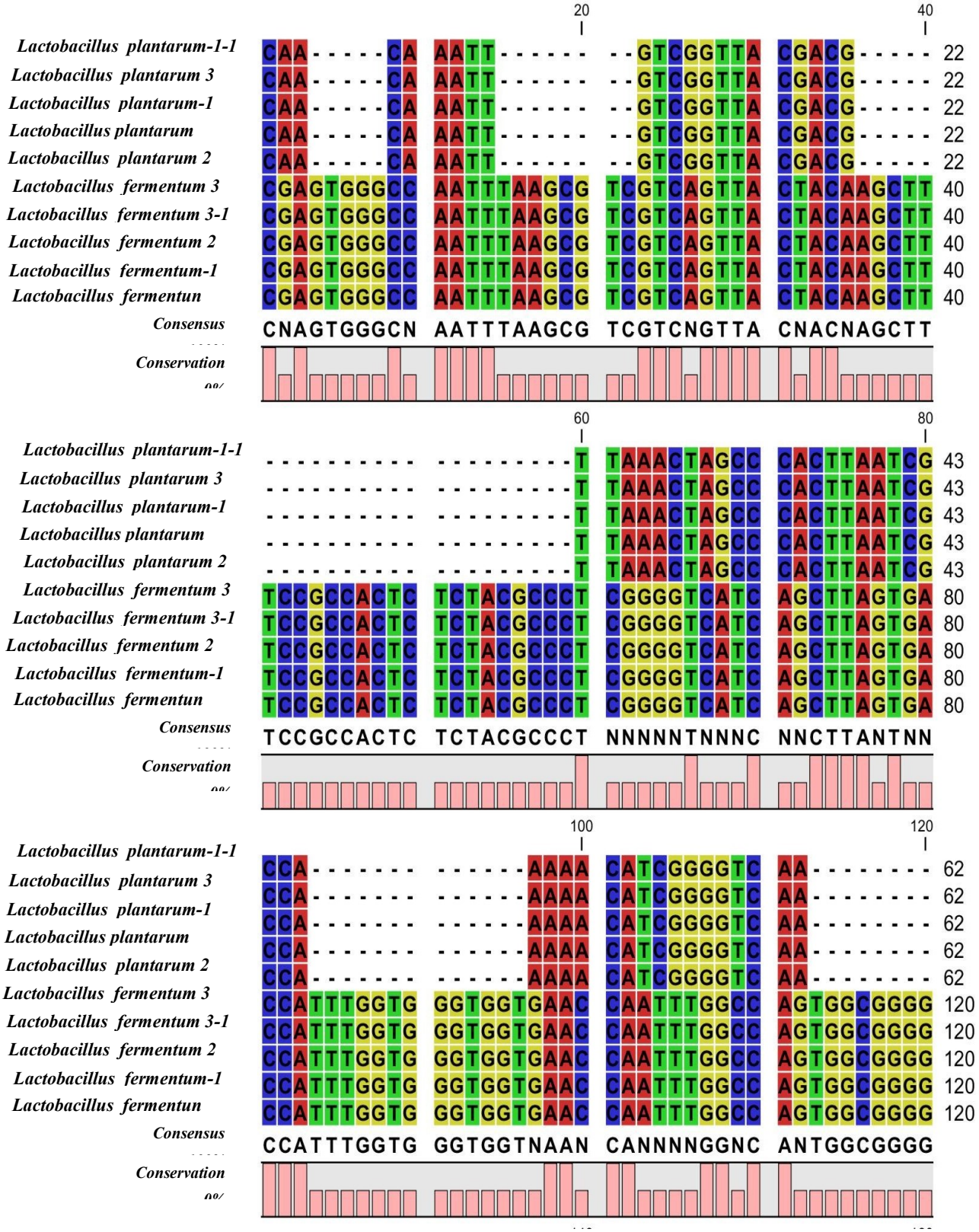
ATGAACGAGCGGATGCCGCATTGACTATTGACTTTGGTCGCCCAGTCGAAATTAATA
TGGTCAAGCTTTTAGTTCGTAGTGACCACTTAGAACGGCCGCATGATGGTTATTGGA
ATCAAGGCACGCTGGAATTCTCTGATGGCAGTCAACAAGTGGTTGTAATGGATGATT
CAGATACGTTTCAAACAGTTAGATTCGCACCTAAAGTGA

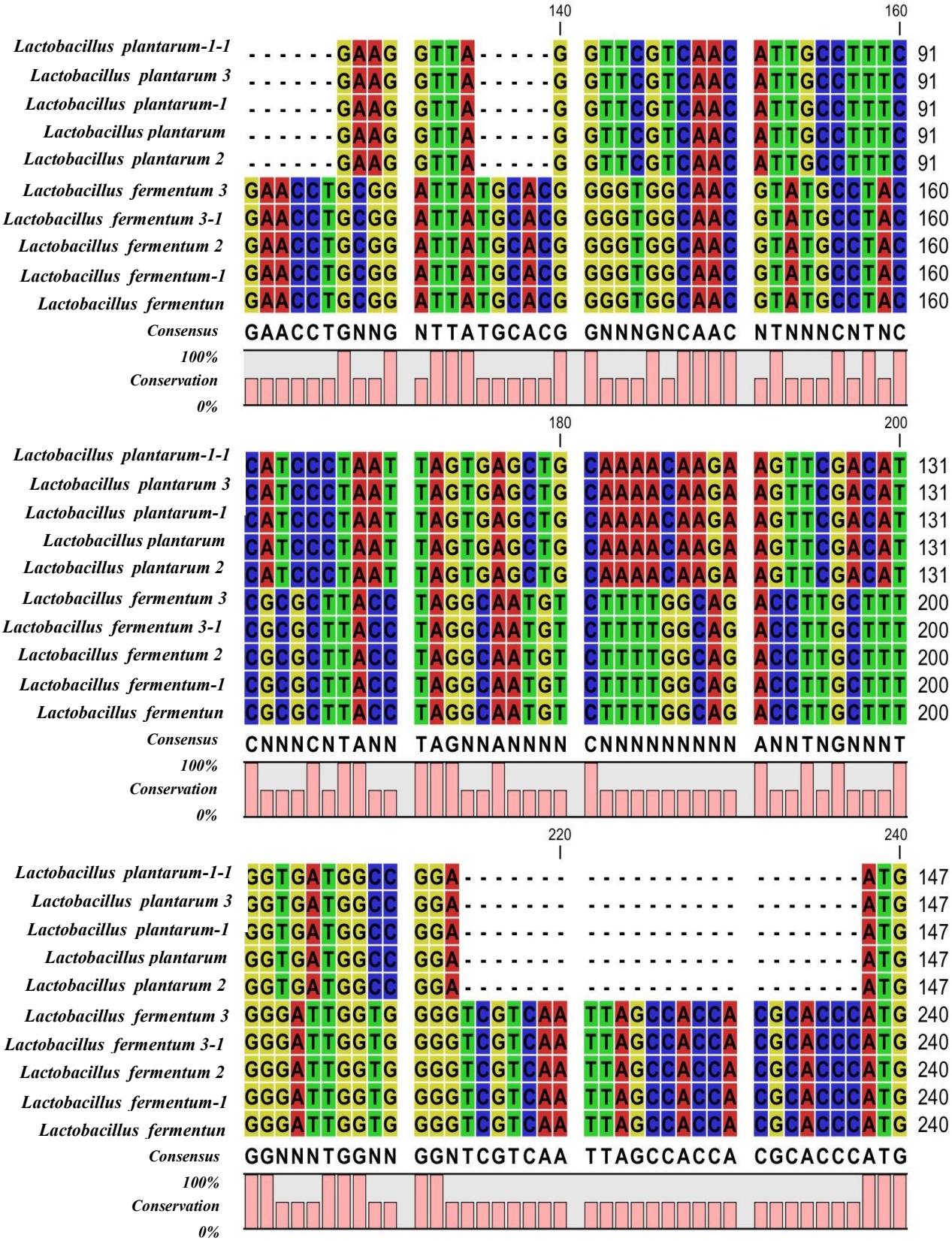
CTTCGACGCTAATACTAAAGGATCTAGTACCCGCTGAAGATAGTGCAAGGTTTAAGG
CACTAACTCAGATTGAAGCCTATGGGTATGCTCGGAACTAGGATAGATGCAATGAG
AATGTCAAACGGATAAATTAATCAGAT

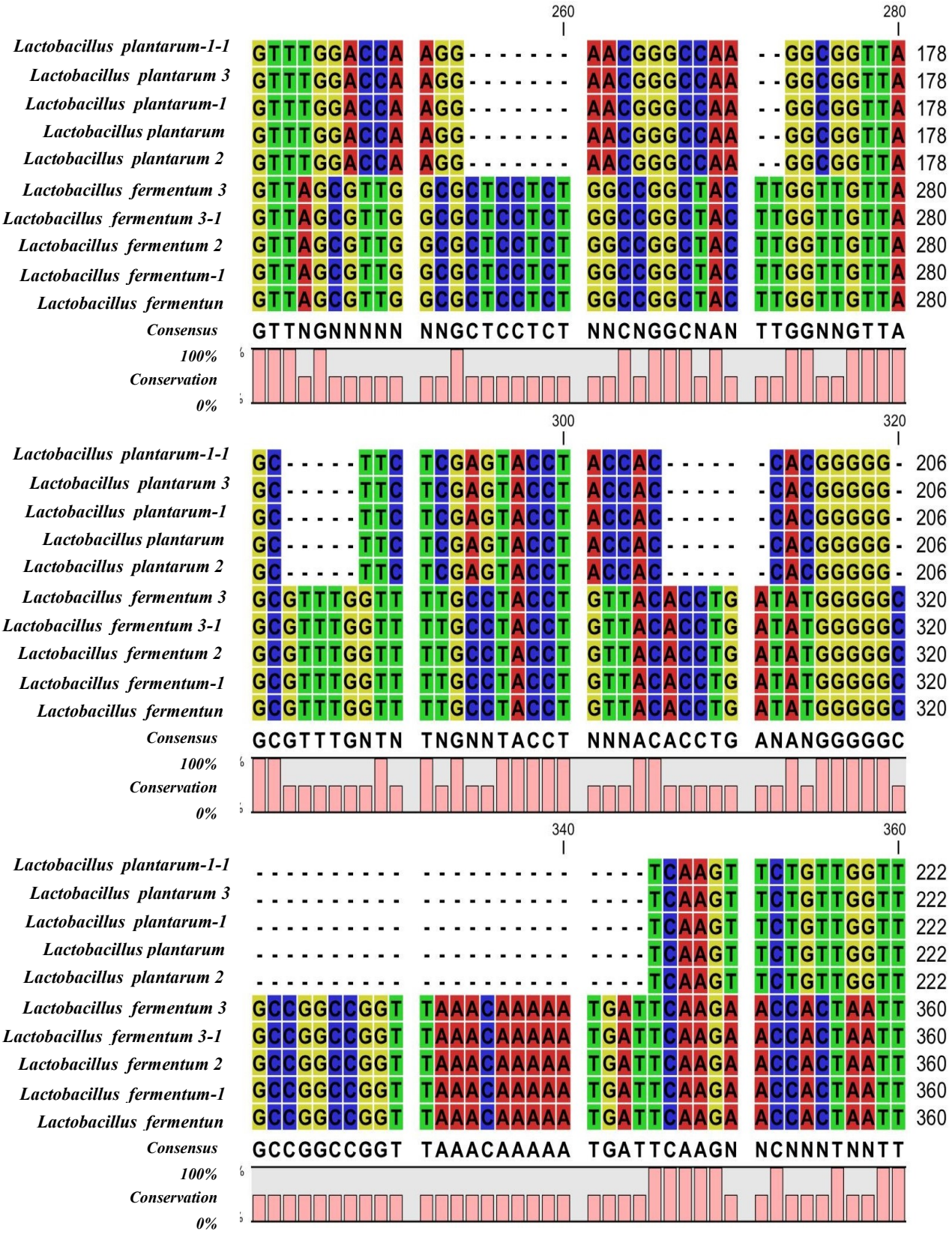
*Lactobacillusplantarum*CP016071.1



Appendix 5 b: **Phylogenetic Tree of Pigeon-pea Isolates at 37**±1^{o}**C**


20
40
Lactobacillus plantarum-1-1
Lactobacillus plantarum 3
Lactobacillus plantarum-1
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum 2
Lactobacillus fermentum-1
Lactobacillus fermentun
Consensus
Conservation
60
80
Lactobacillus plantarum-1-1
Lactobacillus plantarum 3
Lactobacillus plantarum-1
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum 2
Lactobacillus fermentum-1
Lactobacillus fermentun
Consensus
Conservation
100
120
Lactobacillus plantarum-1-1
Lactobacillus plantarum 3
Lactobacillus plantarum-1
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum 2
Lactobacillus fermentum-1
Lactobacillus fermentun
Consensus
Conservation


140
160
Lactobacillus plantarum-1-1 ------GAAG GTTA-----G GTTCGTCAAC ATTGCCTTTC 91
Lactobacillus plantarum 3 ------GAAG GTTA-----G GTTCGTCAAC ATTGCCTTTC 91
Lactobacillus plantarum-1 ------GAAG GTTA-----G GTTCGTCAAC ATTGCCTTTC 91
Lactobacillus plantarum ------GAAG GTTA-----G GTTCGTCAAC ATTGCCTTTC 91
Lactobacillus plantarum 2 ------GAAG GTTA-----G GTTCGTCAAC ATTGCCTTTC 91
Lactobacillus fermentum 3 GAACCTGCGG ATTATGCACG GGGTGGCAAC GTATGCCTAC 160
Lactobacillus fermentum 3-1 GAACCTGCGG ATTATGCACG GGGTGGCAAC GTATGCCTAC 160
Lactobacillus fermentum 2 GAACCTGCGG ATTATGCACG GGGTGGCAAC GTATGCCTAC 160
Lactobacillus fermentum-1 GAACCTGCGG ATTATGCACG GGGTGGCAAC GTATGCCTAC 160
Lactobacillus fermentun GAACCTGCGG ATTATGCACG GGGTGGCAAC GTATGCCTAC 160
Consensus GAACCTGNNG NTTATGCACG GNNNGNCAAC NTNNNCNTNC
100%
Conservation
0%
180
200
Lactobacillus plantarum-1-1 CATCCCTAAT TAGTGAGCTG CAAAACAAGA AGTTCGACAT 131
Lactobacillus plantarum 3 CATCCCTAAT TAGTGAGCTG CAAAACAAGA AGTTCGACAT 131
Lactobacillus plantarum-1 CATCCCTAAT TAGTGAGCTG CAAAACAAGA AGTTCGACAT 131
Lactobacillus plantarum CATCCCTAAT TAGTGAGCTG CAAAACAAGA AGTTCGACAT 131
Lactobacillus plantarum 2 CATCCCTAAT TAGTGAGCTG CAAAACAAGA AGTTCGACAT 131
Lactobacillus fermentum 3 CGCGCTTACC TAGGCAATGT CTTTTGGCAG ACCTTGCTTT 200
Lactobacillus fermentum 3-1 CGCGCTTACC TAGGCAATGT CTTTTGGCAG ACCTTGCTTT 200
Lactobacillus fermentum 2 CGCGCTTACC TAGGCAATGT CTTTTGGCAG ACCTTGCTTT 200
Lactobacillus fermentum-1 CGCGCTTACC TAGGCAATGT CTTTTGGCAG ACCTTGCTTT 200
Lactobacillus fermentun CGCGCTTACC TAGGCAATGT CTTTTGGCAG ACCTTGCTTT 200
Consensus CNNNCNTANN TAGNNANNNN CNNNNNNNNN ANNTNGNNNT
100%
Conservation
0%
220
240
Lactobacillus plantarum-1-1 GGTGATGGCC GGA-------- ---------- -------ATG 147
Lactobacillus plantarum 3 GGTGATGGCC GGA-------- ---------- -------ATG 147
Lactobacillus plantarum-1 GGTGATGGCC GGA-------- ---------- -------ATG 147
Lactobacillus plantarum GGTGATGGCC GGA-------- ---------- -------ATG 147
Lactobacillus plantarum 2 GGTGATGGCC GGA-------- ---------- -------ATG 147
Lactobacillus fermentum 3 GGGATTGGTG GGGTCGTCAA TTAGCCACCA CGCACCCATG 240
Lactobacillus fermentum 3-1 GGGATTGGTG GGGTCGTCAA TTAGCCACCA CGCACCCATG 240
Lactobacillus fermentum 2 GGGATTGGTG GGGTCGTCAA TTAGCCACCA CGCACCCATG 240
Lactobacillus fermentum-1 GGGATTGGTG GGGTCGTCAA TTAGCCACCA CGCACCCATG 240
Lactobacillus fermentun GGGATTGGTG GGGTCGTCAA TTAGCCACCA CGCACCCATG 240
Consensus GGNNNTGGNN GGNTCGTCAA TTAGCCACCA CGCACCCATG
100%
Conservation
0%


260
280
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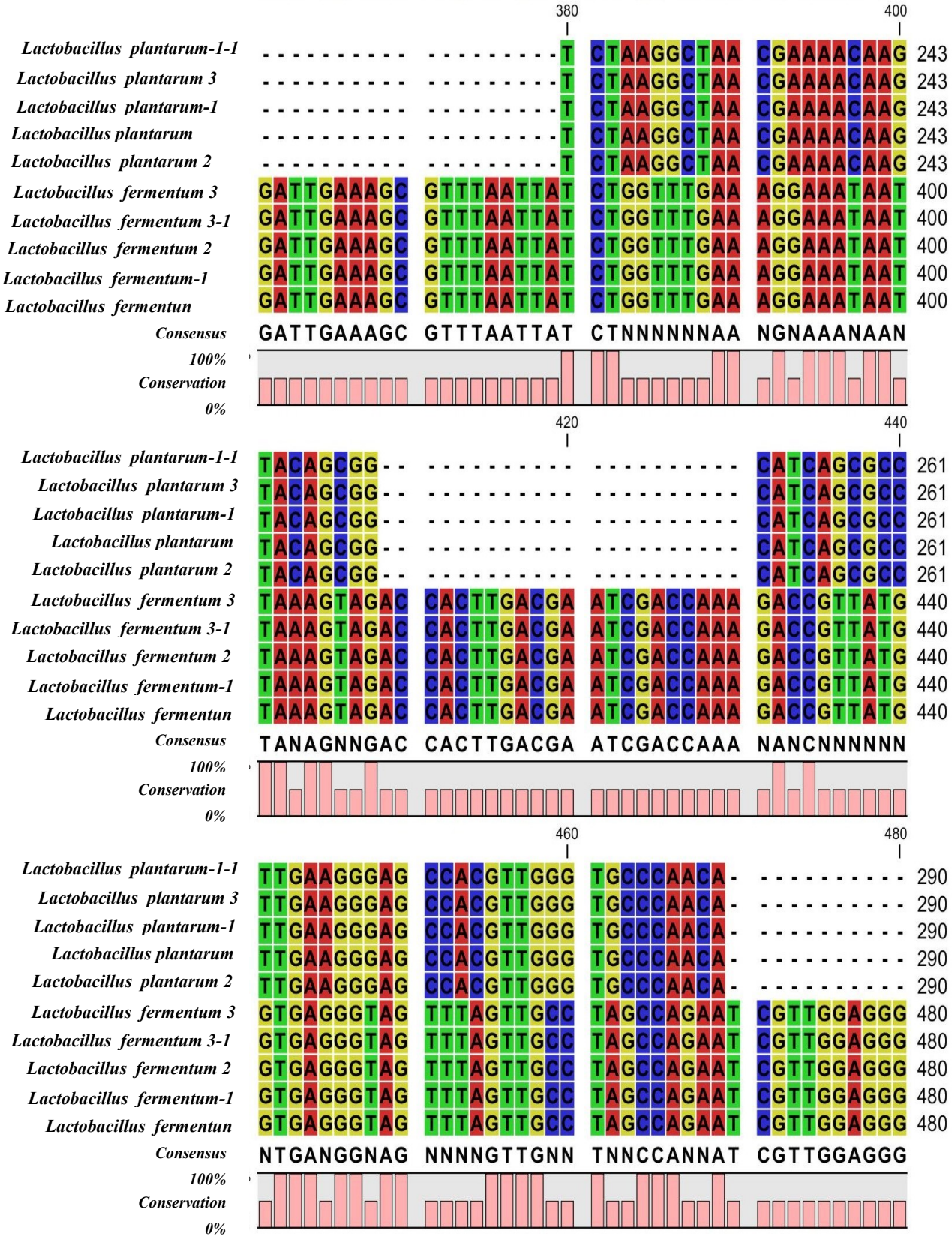
Lactobacillus plantarum 3 GTTTGGACCA AGG------- AACGGGCCAA --GGCGGTTA 178
Lactobacillus plantarum-1 GTTTGGACCA AGG------- AACGGGCCAA --GGCGGTTA 178
Lactobacillus plantarum GTTTGGACCA AGG------- AACGGGCCAA --GGCGGTTA 178
Lactobacillus plantarum 2 GTTTGGACCA AGG------- AACGGGCCAA --GGCGGTTA 178
Lactobacillus fermentum 3 GTTAGCGTTG GCGCTCCTCT GGCCGGCTAC TTGGTTGTTA 280
Lactobacillus fermentum 3-1 GTTAGCGTTG GCGCTCCTCT GGCCGGCTAC TTGGTTGTTA 280
Lactobacillus fermentum 2 GTTAGCGTTG GCGCTCCTCT GGCCGGCTAC TTGGTTGTTA 280
Lactobacillus fermentum-1 GTTAGCGTTG GCGCTCCTCT GGCCGGCTAC TTGGTTGTTA 280
Lactobacillus fermentun GTTAGCGTTG GCGCTCCTCT GGCCGGCTAC TTGGTTGTTA 280
Consensus GTTNGNNNNN NNGCTCCTCT NNCNGGCNAN TTGGNNGTTA
100%
Conservation
0%
300
320
Lactobacillus plantarum-1-1 GC-----TTC TCGAGTACCT ACCAC----- -CACGGGGG- 206
Lactobacillus plantarum 3 GC-----TTC TCGAGTACCT ACCAC----- -CACGGGGG- 206
Lactobacillus plantarum-1 GC-----TTC TCGAGTACCT ACCAC----- -CACGGGGG- 206
Lactobacillus plantarum GC-----TTC TCGAGTACCT ACCAC----- -CACGGGGG- 206
Lactobacillus plantarum 2 GC-----TTC TCGAGTACCT ACCAC----- -CACGGGGG- 206
Lactobacillus fermentum 3 GCGTTTGGTT TTGCCTACCT GTTACACCTG ATATGGGGGC 320
Lactobacillus fermentum 3-1 GCGTTTGGTT TTGCCTACCT GTTACACCTG ATATGGGGGC 320
Lactobacillus fermentum 2 GCGTTTGGTT TTGCCTACCT GTTACACCTG ATATGGGGGC 320
Lactobacillus fermentum-1 GCGTTTGGTT TTGCCTACCT GTTACACCTG ATATGGGGGC 320
Lactobacillus fermentun GCGTTTGGTT TTGCCTACCT GTTACACCTG ATATGGGGGC 320
Consensus GCGTTTGNTN TNGNNTACCT NNNACACCTG ANANGGGGGC
100%
Conservation
0%
340
360
Lactobacillus plantarum-1-1 ---------- ---------- ----TCAAGT TCTGTTGGTT 222
Lactobacillus plantarum 3 ---------- ---------- ----TCAAGT TCTGTTGGTT 222
Lactobacillus plantarum-1 ---------- ---------- ----TCAAGT TCTGTTGGTT 222
Lactobacillus plantarum ---------- ---------- ----TCAAGT TCTGTTGGTT 222
Lactobacillus plantarum 2 ---------- ---------- ----TCAAGT TCTGTTGGTT 222
Lactobacillus fermentum 3 GCCGGCCGGT TAAACAAAAA TGATTCAAGA ACCACTAATT 360
Lactobacillus fermentum 3-1 GCCGGCCGGT TAAACAAAAA TGATTCAAGA ACCACTAATT 360
Lactobacillus fermentum 2 GCCGGCCGGT TAAACAAAAA TGATTCAAGA ACCACTAATT 360
Lactobacillus fermentum-1 GCCGGCCGGT TAAACAAAAA TGATTCAAGA ACCACTAATT 360
Lactobacillus fermentun GCCGGCCGGT TAAACAAAAA TGATTCAAGA ACCACTAATT 360
Consensus GCCGGCCGGT TAAACAAAAA TGATTCAAGN NCNNNTNNTT
100%
Conservation
0%


380
400
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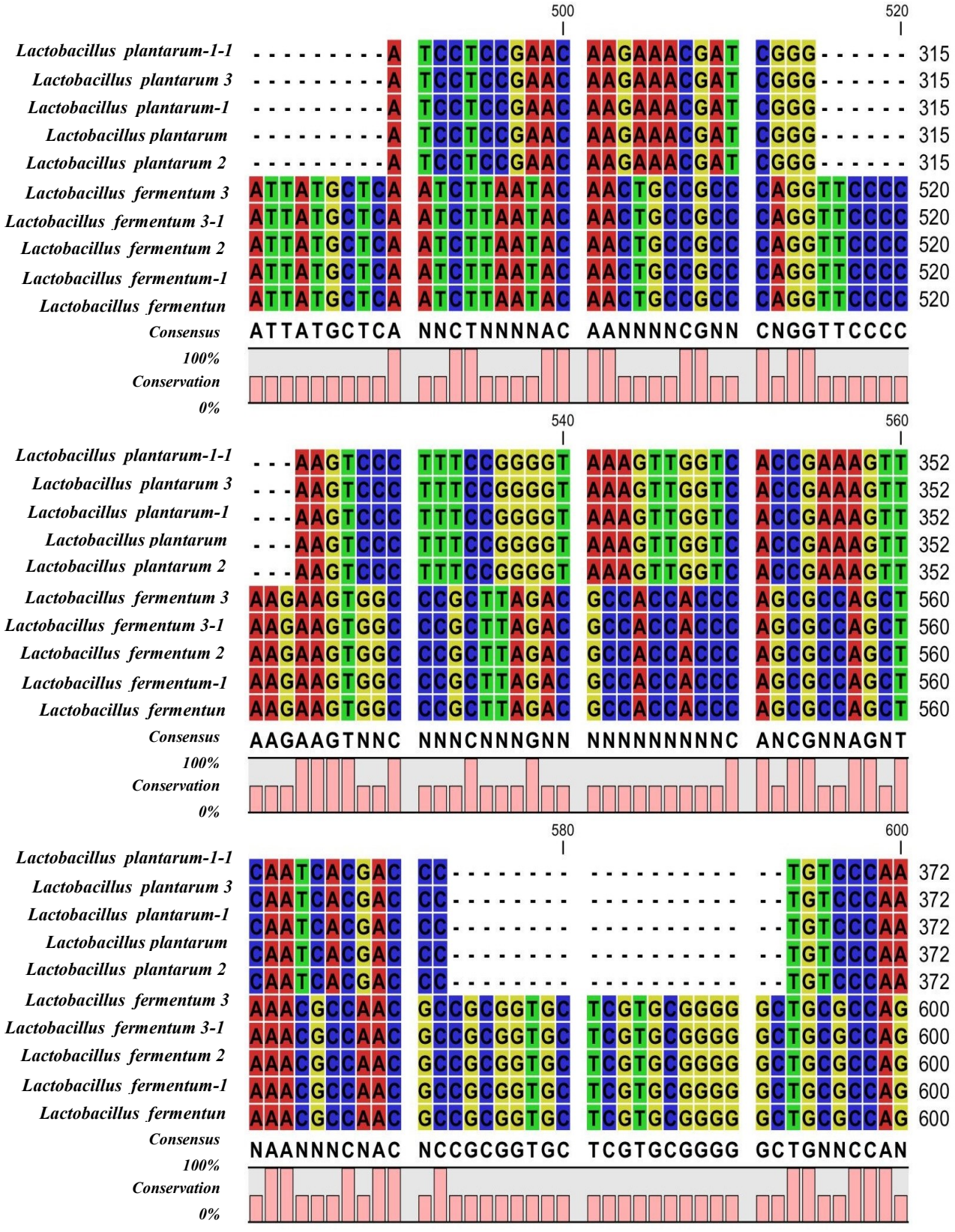
Lactobacillus plantarum-1 T CTAAGGCTAA CGAAAACAAG 243
Lactobacillus plantarum T CTAAGGCTAA CGAAAACAAG 243
Lactobacillus plantarum 2 T CTAAGGCTAA CGAAAACAAG 243
Lactobacillus fermentum 3 GATTGAAAGC GTTTAATTAT CTGGTTTGAA AGGAAATAAT 400
Lactobacillus fermentum 3-1 GATTGAAAGC GTTTAATTAT CTGGTTTGAA AGGAAATAAT 400
Lactobacillus fermentum 2 GATTGAAAGC GTTTAATTAT CTGGTTTGAA AGGAAATAAT 400
Lactobacillus fermentum-1 GATTGAAAGC GTTTAATTAT CTGGTTTGAA AGGAAATAAT 400
Lactobacillus fermentun GATTGAAAGC GTTTAATTAT CTGGTTTGAA AGGAAATAAT 400
Consensus GATTGAAAGC GTTTAATTAT CTNNNNNNAA NGNAAANAAN
100%
Conservation
0%
420
440
Lactobacillus plantarum-1-1 TACAGCGG CATCAGCGCC 261
Lactobacillus plantarum 3 TACAGCGG CATCAGCGCC 261
Lactobacillus plantarum-1 TACAGCGG CATCAGCGCC 261
Lactobacillus plantarum TACAGCGG CATCAGCGCC 261
Lactobacillus plantarum 2 TACAGCGG CATCAGCGCC 261
Lactobacillus fermentum 3 TAAAGTAGAC CACTTGACGA ATCGACCAAA GACCGTTATG 440
Lactobacillus fermentum 3-1 TAAAGTAGAC CACTTGACGA ATCGACCAAA GACCGTTATG 440
Lactobacillus fermentum 2 TAAAGTAGAC CACTTGACGA ATCGACCAAA GACCGTTATG 440
Lactobacillus fermentum-1 TAAAGTAGAC CACTTGACGA ATCGACCAAA GACCGTTATG 440
Lactobacillus fermentun TAAAGTAGAC CACTTGACGA ATCGACCAAA GACCGTTATG 440
Consensus TANAGNNGAC CACTTGACGA ATCGACCAAA NANCNNNNNN
100%
Conservation
0%
460
480
Lactobacillus plantarum-1-1 TTGAAGGGAG CCACGTTGGG TGCCCAACA 290
Lactobacillus plantarum 3 TTGAAGGGAG CCACGTTGGG TGCCCAACA 290
Lactobacillus plantarum-1 TTGAAGGGAG CCACGTTGGG TGCCCAACA 290
Lactobacillus plantarum TTGAAGGGAG CCACGTTGGG TGCCCAACA 290
Lactobacillus plantarum 2 TTGAAGGGAG CCACGTTGGG TGCCCAACA 290
Lactobacillus fermentum 3 GTGAGGGTAG TTTAGTTGCC TAGCCAGAAT CGTTGGAGGG 480
Lactobacillus fermentum 3-1 GTGAGGGTAG TTTAGTTGCC TAGCCAGAAT CGTTGGAGGG 480
Lactobacillus fermentum 2 GTGAGGGTAG TTTAGTTGCC TAGCCAGAAT CGTTGGAGGG 480
Lactobacillus fermentum-1 GTGAGGGTAG TTTAGTTGCC TAGCCAGAAT CGTTGGAGGG 480
Lactobacillus fermentun GTGAGGGTAG TTTAGTTGCC TAGCCAGAAT CGTTGGAGGG 480
Consensus NTGANGGNAG NNNNGTTGNN TNNCCANNAT CGTTGGAGGG
100%
Conservation
0%


500
520
Lactobacillus plantarum-1-1 - - - - - - - - - A T C C T C C G A A C A A G A A A C G A T C G G G - - - - - - 315
Lactobacillus plantarum 3 - - - - - - - - - A T C C T C C G A A C A A G A A A C G A T C G G G - - - - - - 315
Lactobacillus plantarum-1 - - - - - - - - - A T C C T C C G A A C A A G A A A C G A T C G G G - - - - - - 315
Lactobacillus plantarum - - - - - - - - - A T C C T C C G A A C A A G A A A C G A T C G G G - - - - - - 315
Lactobacillus plantarum 2 - - - - - - - - - A T C C T C C G A A C A A G A A A C G A T C G G G - - - - - - 315
Lactobacillus fermentum 3 A T T A T G C T C A A T C T T A A T A C A A C T G C C G C C C A G G T T C C C C 520
Lactobacillus fermentum 3-1 A T T A T G C T C A A T C T T A A T A C A A C T G C C G C C C A G G T T C C C C 520
Lactobacillus fermentum 2 A T T A T G C T C A A T C T T A A T A C A A C T G C C G C C C A G G T T C C C C 520
Lactobacillus fermentum-1 A T T A T G C T C A A T C T T A A T A C A A C T G C C G C C C A G G T T C C C C 520
Lactobacillus fermentun A T T A T G C T C A A T C T T A A T A C A A C T G C C G C C C A G G T T C C C C 520
Consensus A T T A T G C T C A N N C T N N N N A C A A N N N N C G N N C N G G T T C C C C
100%
Conservation
0%
540
560
Lactobacillus plantarum-1-1 - - - A A G T C C C T T T C C G G G G G T A A A G T T G G T C A C C G A A A G T T 352
Lactobacillus plantarum 3 - - - A A G T C C C T T T C C G G G G G T A A A G T T G G T C A C C G A A A G T T 352
Lactobacillus plantarum-1 - - - A A G T C C C T T T C C G G G G G T A A A G T T G G T C A C C G A A A G T T 352
Lactobacillus plantarum - - - A A G T C C C T T T C C G G G G G T A A A G T T G G T C A C C G A A A G T T 352
Lactobacillus plantarum 2 - - - A A G T C C C T T T C C G G G G G T A A A G T T G G T C A C C G A A A G T T 352
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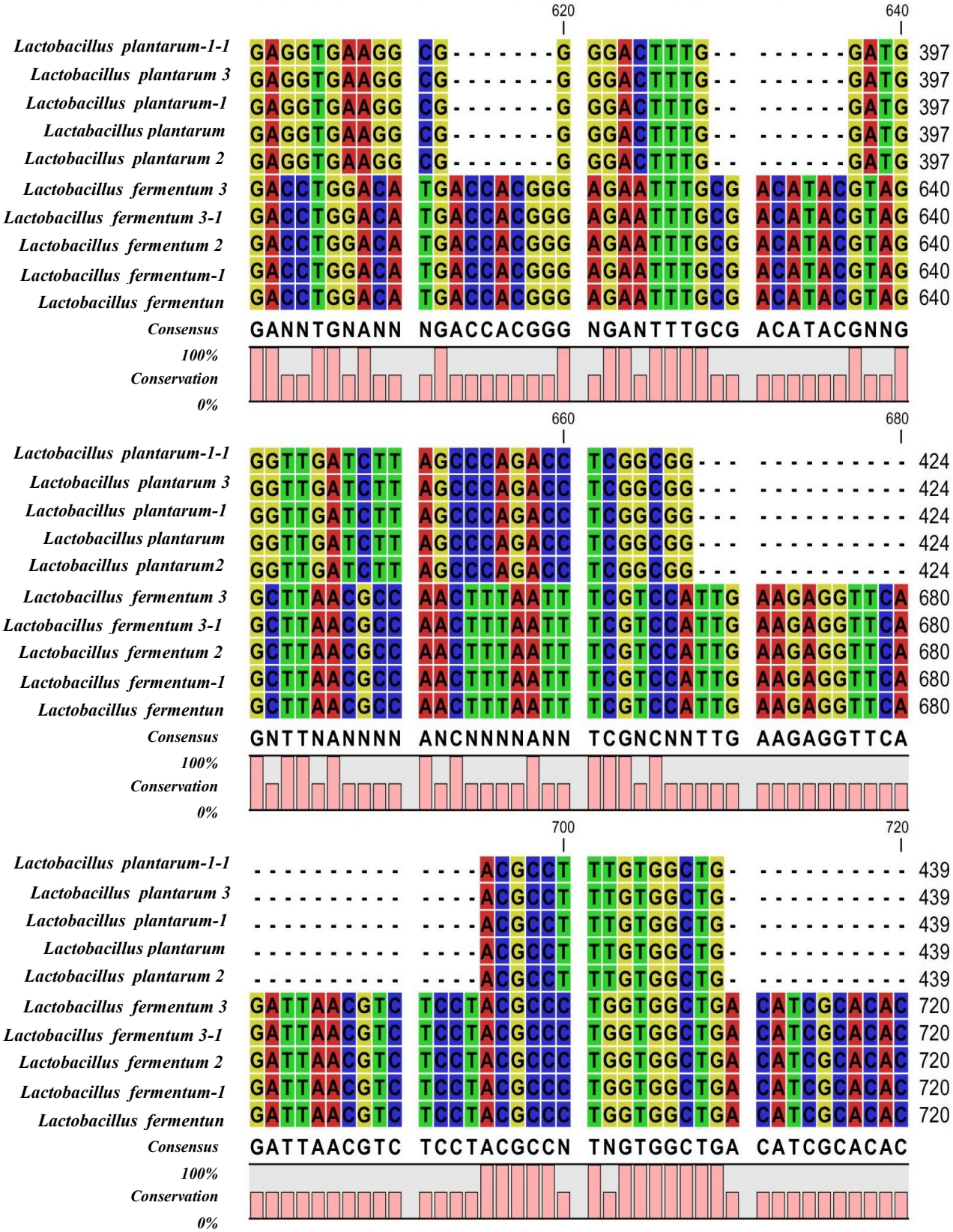
Lactobacillus fermentum 3-1 A A G A A G T G G C C C G C T T A G A C G C C A C C A C C C A G C G C C A G C T 560
Lactobacillus fermentum 2 A A G A A G T G G C C C G C T T A G A C G C C A C C A C C C A G C G C C A G C T 560
Lactobacillus fermentum-1 A A G A A G T G G C C C G C T T A G A C G C C A C C A C C C A G C G C C A G C T 560
Lactobacillus fermentun A A G A A G T G G C C C G C T T A G A C G C C A C C A C C C A G C G C C A G C T 560
Consensus A A G A A G T N N C N N N C N N N G N N N N N N N N N C A N C G N N A G N T
100%
Conservation
0%
580
600
Lactobacillus plantarum-1-1 C A A T C A C G A C C C - - - - - - - - - - - - - - - - - - T G T C C C A A 372
Lactobacillus plantarum 3 C A A T C A C G A C C C - - - - - - - - - - - - - - - - - - T G T C C C A A 372
Lactobacillus plantarum-1 C A A T C A C G A C C C - - - - - - - - - - - - - - - - - - T G T C C C A A 372
Lactobacillus plantarum C A A T C A C G A C C C - - - - - - - - - - - - - - - - - - T G T C C C A A 372
Lactobacillus plantarum 2 C A A T C A C G A C C C - - - - - - - - - - - - - - - - - - T G T C C C A A 372
Lactobacillus fermentum 3 A A A C G C C A A C G C C G C G G T G C T C G T G C G G G G G C T G C G C C A G 600
Lactobacillus fermentum 3-1 A A A C G C C A A C G C C G C G G T G C T C G T G C G G G G G C T G C G C C A G 600
Lactobacillus fermentum 2 A A A C G C C A A C G C C G C G G T G C T C G T G C G G G G G C T G C G C C A G 600
Lactobacillus fermentum-1 A A A C G C C A A C G C C G C G G T G C T C G T G C G G G G G C T G C G C C A G 600
Lactobacillus fermentun A A A C G C C A A C G C C G C G G T G C T C G T G C G G G G G C T G C G C C A G 600
Consensus N A A N N N C N A C N C C G C G G T G C T C G T G C G G G G G C T G N N C C A N
100%
Conservation
0%


620
640
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Lactobacillus plantarum 3 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum-1 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactabacillus plantarum GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus plantarum 2 GAGGTGAAGG CG-------G GGACTTTG-- ------GATG 397
Lactobacillus fermentum 3 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum 3-1 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum 2 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentum-1 GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Lactobacillus fermentun GACCTGGACA TGACCACGGG AGAATTTGCG ACATACGTAG 640
Consensus GANNTGNANN NGACCACGGG NGANTTTGCG ACATACGNNG
100%
Conservation
0%
660
680
Lactobacillus plantarum-1-1 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum 3 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum-1 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus plantarum2 GGTTGATCTT AGCCCAGACC TCGGCGG--- ---------- 424
Lactobacillus fermentum 3 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum 3-1 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum 2 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentum-1 GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Lactobacillus fermentun GCTTAACGCC AACTTTAATT TCGTCCATTG AAGAGGTTCA 680
Consensus GNTTNANNNN ANCNNNNANN TCGNCNNTTG AAGAGGTTCA
100%
Conservation
0%
700
720
Lactobacillus plantarum-1-1 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum 3 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum-1 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum ---------- ----ACGCCT TTGTGGCTG- ---------- 439
Lactobacillus plantarum 2 ---------- ----ACGCCT TTGTGGCTG- ---------- 439
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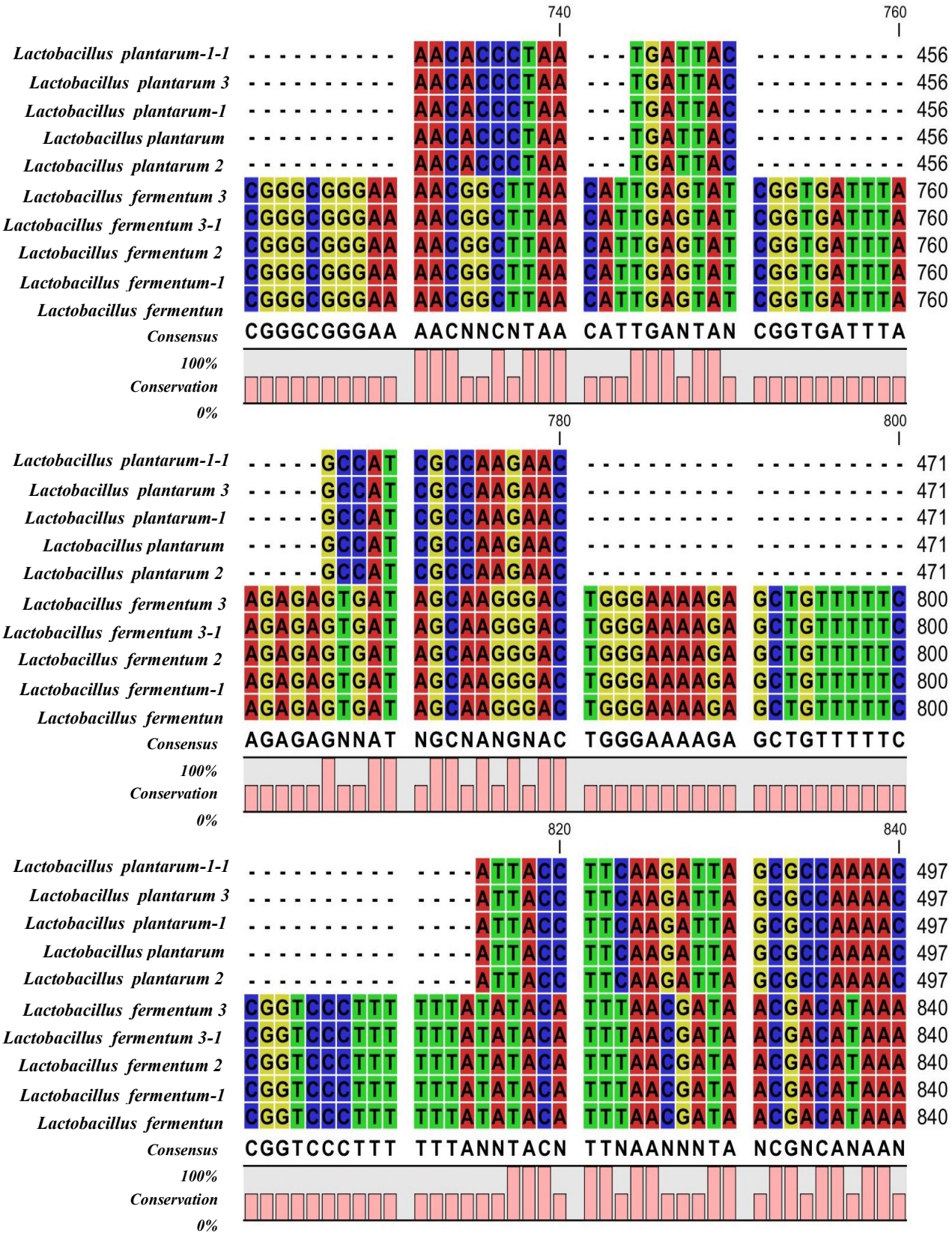
Lactobacillus fermentum 3-1 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentum 2 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentum-1 GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Lactobacillus fermentun GATTAACGTC TCCTACGCCC TGGTGGCTGA CATCGCACAC 720
Consensus GATTAACGTC TCCTACGCCN TNGTGGCTGA CATCGCACAC
100%
Conservation
0%


740
760
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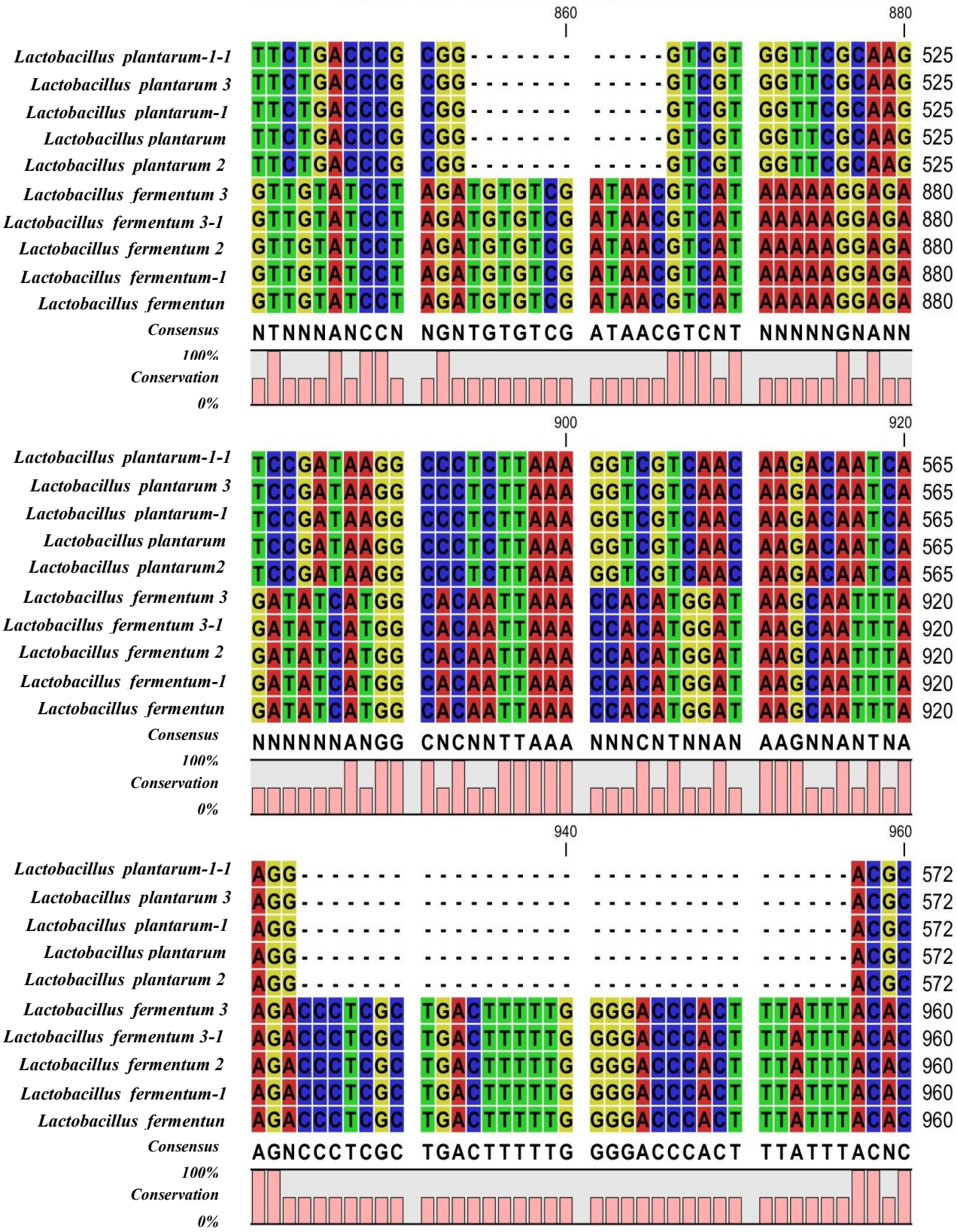
Lactobacillus plantarum-1 - - - - - - - - - - AACACCCTAA - - - TGATTAC - - - - - - - - - - 456
Lactobacillus plantarum - - - - - - - - - - AACACCCTAA - - - TGATTAC - - - - - - - - - - 456
Lactobacillus plantarum 2 - - - - - - - - - - AACACCCTAA - - - TGATTAC - - - - - - - - - - 456
Lactobacillus fermentum 3 CGGGCGGGAA AACGGCTTAA CATTGAGTAT CGGTGATTTA 760
Lactobacillus fermentum 3-1 CGGGCGGGAA AACGGCTTAA CATTGAGTAT CGGTGATTTA 760
Lactobacillus fermentum 2 CGGGCGGGAA AACGGCTTAA CATTGAGTAT CGGTGATTTA 760
Lactobacillus fermentum-1 CGGGCGGGAA AACGGCTTAA CATTGAGTAT CGGTGATTTA 760
Lactobacillus fermentun CGGGCGGGAA AACGGCTTAA CATTGAGTAT CGGTGATTTA 760
Consensus CGGGCGGGAA AACNNCNTAA CATTGANTAN CGGTGATTTA
100%
Conservation
0%
780
800
Lactobacillus plantarum-1-1 - - - - - GCCAT CGCCAAGAAC - 471
Lactobacillus plantarum 3 - - - - - GCCAT CGCCAAGAAC - 471
Lactobacillus plantarum-1 - - - - - GCCAT CGCCAAGAAC - 471
Lactobacillus plantarum - - - - - GCCAT CGCCAAGAAC - 471
Lactobacillus plantarum 2 - - - - - GCCAT CGCCAAGAAC - 471
Lactobacillus fermentum 3 AGAGAGTGAT AGCAAGGGAC TGGGAAAAGA GCTGTTTTTC 800
Lactobacillus fermentum 3-1 AGAGAGTGAT AGCAAGGGAC TGGGAAAAGA GCTGTTTTTC 800
Lactobacillus fermentum 2 AGAGAGTGAT AGCAAGGGAC TGGGAAAAGA GCTGTTTTTC 800
Lactobacillus fermentum-1 AGAGAGTGAT AGCAAGGGAC TGGGAAAAGA GCTGTTTTTC 800
Lactobacillus fermentun AGAGAGTGAT AGCAAGGGAC TGGGAAAAGA GCTGTTTTTC 800
Consensus AGAGAGNNAT NGCNANGNAC TGGGAAAAGA GCTGTTTTTC
100%
Conservation
0%
820
840
Lactobacillus plantarum-1-1 - - - - - - - - - - - - - - ATTACC TTCAAGATTA GCGCCAAAAC 497
Lactobacillus plantarum 3 - - - - - - - - - - - - - - ATTACC TTCAAGATTA GCGCCAAAAC 497
Lactobacillus plantarum-1 - - - - - - - - - - - - - - ATTACC TTCAAGATTA GCGCCAAAAC 497
Lactobacillus plantarum - - - - - - - - - - - - - - ATTACC TTCAAGATTA GCGCCAAAAC 497
Lactobacillus plantarum 2 - - - - - - - - - - - - - - ATTACC TTCAAGATTA GCGCCAAAAC 497
Lactobacillus fermentum 3 CGGTCCCTTT TTTATATACA TTTAACGATA ACGACATAAA 840
Lactobacillus fermentum 3-1 CGGTCCCTTT TTTATATACA TTTAACGATA ACGACATAAA 840
Lactobacillus fermentum 2 CGGTCCCTTT TTTATATACA TTTAACGATA ACGACATAAA 840
Lactobacillus fermentum-1 CGGTCCCTTT TTTATATACA TTTAACGATA ACGACATAAA 840
Lactobacillus fermentun CGGTCCCTTT TTTATATACA TTTAACGATA ACGACATAAA 840
Consensus CGGTCCCTTT TTTANNTACN TTNAANNNTA NCGNCANAAN
100%
Conservation
0%


860
880
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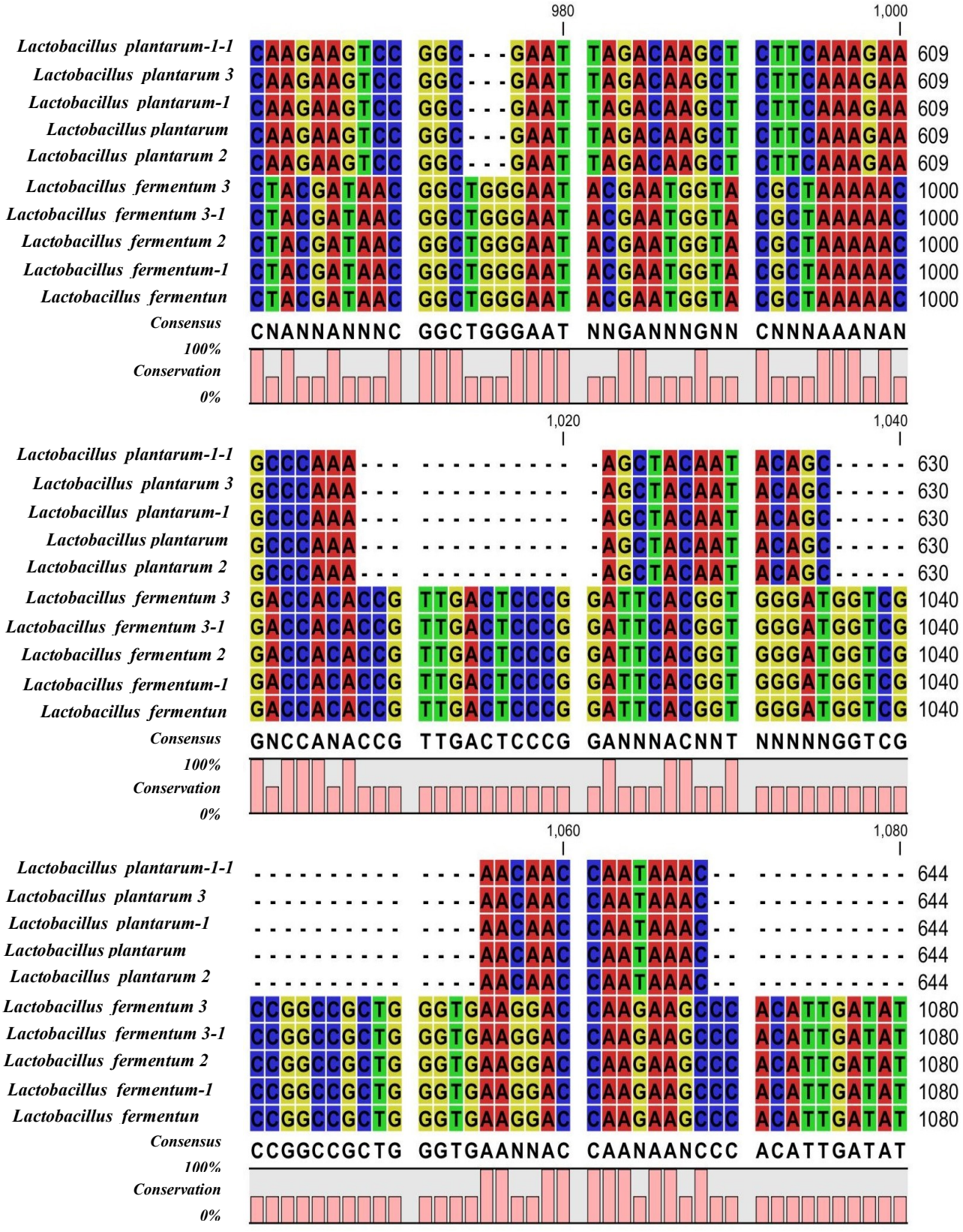
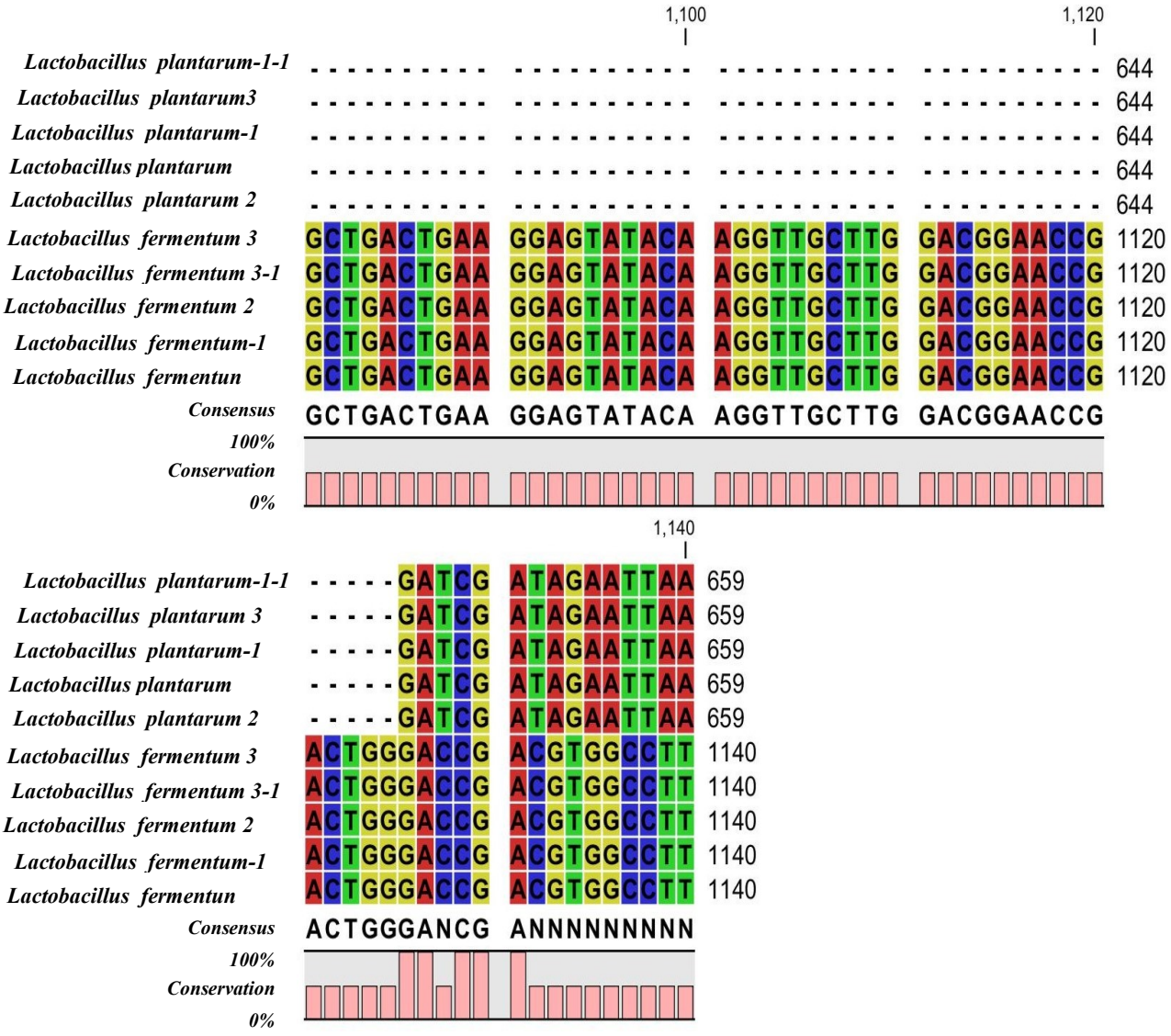
Lactobacillus plantarum-1 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus plantarum 2 TTCTGACCCG CGG------- -----GTCGT GGTTCGCAAG 525
Lactobacillus fermentum 3 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum 3-1 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum 2 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentum-1 GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Lactobacillus fermentun GTTGTATCCT AGATGTGTCG ATAACGTCAT AAAAAGGAGA 880
Consensus NTNNNANCCN NGNTGTGTCG ATAACGTCNT NNNNNGNANN
100%
Conservation
0%
900
920
Lactobacillus plantarum-1-1 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum 3 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum-1 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus plantarum2 TCCGATAAGG CCCTCTTAAA GGTCGTCAAC AAGACAATCA 565
Lactobacillus fermentum 3 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum 3-1 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum 2 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentum-1 GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Lactobacillus fermentun GATATCATGG CACAATTAAA CCACATGGAT AAGCAATTTA 920
Consensus NNNNNNANGG CNCNNTTAAA NNNCNTNNAN AAGNNANTNA
100%
Conservation
0%
940
960
Lactobacillus plantarum-1-1 AGG------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum 3 AGG------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum-1 AGG------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum AGG------- ---------- ---------- ------ACGC 572
Lactobacillus plantarum 2 AGG------- ---------- ---------- ------ACGC 572
Lactobacillus fermentum 3 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum 3-1 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum 2 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentum-1 AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Lactobacillus fermentun AGACCCTCGC TGACTTTTTG GGGACCCACT TTATTTACAC 960
Consensus AGNCCCTCGC TGACTTTTTG GGGACCCACT TTATTTACNC
100%
Conservation
0%


980
1,000
Lactobacillus plantarum-1-1
Lactobacillus plantarum 3
Lactobacillus plantarum-1
Lactobacillus plantarum
Lactobacillus plantarum 2
Lactobacillus fermentum 3
Lactobacillus fermentum 3-1
Lactobacillus fermentum 2
Lactobacillus fermentum-1
Lactobacillus fermentun
Consensus
100%
Conservation
0%
1,020
1,040
1,060
1,080



Appendix 5 c: Pigeon-pea Isolates Alignment at 37±1^{o}C

Appendix 6

Questionnaire for Sensory properties of Breadfruit-Pigeon-pea meal and pizzelle cookie Samples

Instructions: Please rank the following samples of breadfruit-pigeon-pea products according to the level of likeness or dislike.

Feelings	**Scores**
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Samples	Appearance	Taste / Crispiness	Colour	Aroma	Overall Acceptability
551					
552					
553					
554					
555					
556					
557					
558					

Name :

Date :

Signature:

Appendix 7

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CIAT	Centro International de Agricultural Tropical
CF	Crude Fiber
cm	Centimeter
^{o}C	Degree
DNA	Deoxy Ribonucleic Acid
DMRT	Duncan Multiple Range Test
FAO	Food and Agricultural Organization
G/cm3	Gram per Centimeter Cubed
G/ml	Gram per Milliliter
G/cc	Gram per Centimeter
g	Gram
HCN	Hydrogen Cyanide
HPLC	High Performance Liquid Chromatography
HCL	Hydrogen Chloride
H2SO4	Hydrogen tetraoxosulphate (VI) acid
h	Hour
ICRAF	International Center for Research in Agroforestry.
kj	Kilo Joule
kg/ha	Kilogram per Hectares
kg	Kilogram
kcal	Kilo Calorie
KCN	Potassium Cyanide
LGC	Least Gelation Concentration
LLDPE	Linear Low Density Polyethylene
MC	Moisture Content
mm	Millimeter
m^2	Meter squared

m	Meter
M	Molar
M	Molecular size marker
Mg/g	Milligram/gram
mg	Milligram
Min	Minute
ml	Milliliter
NRCS	National Resources Conservation Service
NCBI	National Centre for Biotechnology Information
No.	Number
OAC	Oil absorption capacity
ppm	Parts per million
p>0.05	Probability greater than 0.05
P<0.05	Probability less than 0.05
PCR	Polymerase Chain Reaction
RVA	Rapid Visco Analyzer
RVU	Rapid Visco Unit
rpm	Revolution per minutes
SD	Standard deviation
spp	Species
Sec	Second
Temp	Temperature
tons/ha	Tonnes per hectares
ug	Micron gram
UV	Ultraviolet
Vol	Volume
w/v	Weight per Volume
WAC	Water Absorption Capacity
WHO	World Health Organization
%	Percentage