Development of autochthonous starter culture for coconut yogurt

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Introduction

- Presently, preferences tend to shift towards vegetable milk as alternatives for yogurt production due to problems of cost, allergenicity and desire for vegetarian alternatives (Afaneh, 2011)

- Coconut milk is higher in nutrient content compared to cow milk (Yaakob *et al*., 2012)

- Production of yoghurt from coconut milk as the only substrate has been attempted by several researchers (Yaakob *et al*., 2012)

Introduction

- Commercial starter cultures are commonly used for coconut yogurt

- To the best of our knowledge, there is no information on the use of indigenous isolates from coconut milk as starter culture

- Use of autochthonous starter cultures with proven technological capabilities might guarantee improvement of product quality as well as preservation of characteristics that define the identity of coconut yogurt

Objectives

- To isolate, characterize and identify LAB isolated from fermented coconut milk using a combination of morphological, biochemical and molecular techniques
- To screen the isolates for important technological properties relevant to yoghurt starter cultures
- To develop starter cultures for coconut yoghurt production
- To evaluate the sensory properties of the yoghurt produced

Methodology

Sample collection

- Dwarf variety - *Cocus nuciifera var. nana* from Akwa Ibom State

Coconut Milk Extraction

- Coconut milk was extracted following the method of Edem and Elijah (2016)

Isolation characterization and identification of bacterial isolates

- From coconut milk supplemented with 2% sucrose and left on the laboratory bench to ferment for 3-5 days

Methodology

- Man Rogosa Sharpe (MRS) agar was used for isolation and maintenance of culture
- Incubation was done anaerobically at 37^{o}C for 48 h using the Gas Pack system (Merck Anaerocult type A)
- Morphological and biochemical characterization of the isolates using standard methods (Harrigan and McCane, 1976)

Methodology

- **DNA extraction**

Overnight cultures in LB broth using the Dneasy extraction kit (Qiagen, USA)

- **PCR Amplification**

Partial 16SrRNA gene amplified using the 27F and 1492R primers

- **Sequencing**

Bi-directional sequencing of PCR products (with primers 27F and 1492R) by using the Big Dye terminator sequencing cycle v3 kit

Methodology

- **Technological properties of isolates**

Acid production (Soomro and Masud, 2007), Exopolysaccharide production (Guiraud,1998), Proteolytic activity (Thapa *et al*., 2006), Acetaldehyde production (Bennama *et al*., 2012), Syneresis (Guzman-Gonzalez *et al*., 2000)

- **Preparation of yoghurt starter**

Using mixed selected strains (Lm1, Lm2, Lb1 and Lb2 and control) at a rate of 2% inoculum 1:1 ratio and incubated at 42 ºC for 8 h (Table 1)

- **Sensory Evaluation**

20 trained panelists evaluated appearance, colour, aroma, taste, texture and overall acceptability based on 9-point hedonic scale

Experimental Setting

Combinations and proportions of LAB for development of starter culture

Sample	Strain	Ratio
1	Lm1 +Lb1	1:1
2	Lm1 + Lb2	1:1
3	Lm2+ Lb1	1:1
4	Lm2+Lb2	1:1

Lm, *Leuconostoc mesenteroides* subsp. *dextranicum*; Lb, *Lactobacillus plantarum* subsp. *plantarum*
[12]Strain

Results and Discussion

Table 1. Characteristics of LAB strains isolated from fermented coconut milk

Isolate code	Lm1	Lm2	Lm3	Lm4	Lm5	Lm6	Lm7	Lm8	Lb1	Lb2	Lb3	Lb4	Ed1	Ed2	Ed3
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Morphology	B	B	B	B	B	B	B	B	B	B	B	B	C	C	C
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Production of gas from MRS broth	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Methyl red test	+	+	-	-	+	-	+	-	-	-	-	+	-	-	-
Voges prokaeur test	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	+	-	+	-	-	+	-	-	-	+	-	-	-

Lm1-8, *Leuconostoc mesenteroides*; Lb1-2, *Lactobacillus plantarum*; Lb3-4, *Lactobacillus pentosus*, Ed1, *Enterococcus durans*, Ed2, *Enterococcus faecium*, Ed3, *Enterococcus lacts*

Results and Discussion

Table 1. Characteristics of LAB strains isolated from fermented coconut milk

Isolate code	Lm1	Lm2	Lm3	Lm4	Lm5	Lm6	Lm7	Lm8	Lb1	Lb2	Lb3	Lb4	Ed1	Ed2	Ed3
Galactose	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Melibiose	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-
Arabinose	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Ribose	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Trehalose	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-
Salicin	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-

Lm1-8, *Leuconostoc mesenteroides*; Lb1-2, *Lactobacillus plantarum*; Lb3-4, *Lactobacillus pentosus*, Ed1, *Enterococcus durans*, Ed2, *Enterococcus faecium*, Ed3, *Enterococcus lacts*

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Lactose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Cellobiose	-	-	-	+	+	+	+	+	+	+	-	+	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
Growth at 15°C	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+
Growth at 45°C	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Growth at pH 3.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at pH 9.6	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+
Growth at 4.5%NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 6.5% NaCl	+	+	+	+	+	+	+	+	-	-	-	-	p	+	+

Lm1-8, *Leuconostoc mesenteroides*; Lb1-2, *Lactobacillus plantarum*; Lb3-4, *Lactobacillus pentosus*, Ed1, *Enterococcus durans*, Ed2, *Enterococcus faecium*, Ed3, *Enterococcus lacts*

Results and Discussion

Table 2. Identity of LAB from coconut milk based on 16S rRNA Sequence Analysis

Isolate code	Strain name	Accession number	Sequence length(bp)	% identity
Lm1	*Leuconostoc mesenteroides* subsp. *dextranicum*	CP012009	1423	99.9%
Lm2	*Leuconostoc mesenteroides* subsp. *dextranicum*	AB023244	1411	99.9%
Lm3	*Leuconostoc mesenteroides* subsp. *suionicum*	CP015247	1479	99.9%
Lm4	*Leuconostoc mesenteroides* subsp. *dextranicum*	AB681193	1446	99.9%
Lm5	*Leuconostoc mesenteroides* subsp *mesenteroides*	CP000414	1438	99.9%
Lm6	*Leuconostoc mesenteroides* subsp *cremoris*	ACKV01000113	1457	99.7%
Lm7	*Leuconostoc mesenteroides* subsp *mesenteroides*	D31671	1328	99.9%

Results and Discussion

Table 2. Identity of LAB from coconut milk based on 16S rRNA Sequence Analysis

Isolate code	Strain name	Accession number	Sequence length(bp)	% identity
Lm8	*Leuconostoc mesenteroides* subsp *mesenteroides*	AB023242	1126	99.9%
Lb1	*Lactobacillus plantarum* subsp. *plantarum*	ACGZ01000098	1429	99.7%
Lb2	*Lactobacillus plantarum* subsp. *plantarum*	AJ640078	1486	99.7%
Lb3	*Lactobacillus pentosus*	AZCU01000047	1357	99.8%
Lb4	*Lactobacillus pentosus*	AF375905	1215	99.8%
Ed1	*Enterococcus durans*	BCQB01000108	869	100%
Ed2	*Enterococcus faecium*	AJKH01000109	862	99.8%
Ed3	*Enterococcus lactis*	GU983697	936	99.6%

Results and Discussion

Table 3. Technological properties of lactic acid bacteria isolated from fermented coconut milk

Isolate code	Acidity (% lactic acid)	Exopolysaccharide production	Proteolytic activity	Acetaldehyde production (µmol)	Syneresis (%w/w)
Lm1	$0.77^{a} \pm 0.02$	4+	$2.81^{c} \pm 0.20$	$198.00^{a} \pm 35.00$	$9.98^{a} \pm 1.20$
Lm2	$0.75^{a} \pm 0.14$	3+	$6.20^{a} \pm 0.01$	$150.00^{c} \pm 28.00$	$8.86^{b} \pm 2.11$
Lm3	$0.56^{b} \pm 0.05$	2+	$0.98^{e} \pm 0.05$	$72.07^{e} \pm 4.60$	$7.75^{c} \pm 0.98$
Lm4	$0.45^{c} \pm 0.10$	-	$1.76^{d} \pm 0.15$	$54.22^{ef} \pm 6.54$	$6.95^{cd} \pm 1.05$
Lm5	$0.32^{d} \pm 0.01$	-	$5.60^{a} \pm 0.31$	$42.56^{f} \pm 2.08$	$6.80^{d} \pm 0.77$
Lm6	$0.28^{e} \pm 0.03$	2+	$3.83^{b} \pm 0.05$	$26.67^{g} \pm 5.54$	$6.80^{d} \pm 1.32$
Lm7	$0.63^{b} \pm 0.11$	-	$2.12^{c} \pm 0.26$	$67.05^{e} \pm 7.86$	$7.90^{bc} \pm 2.05$
Lm8	$0.25^{e} \pm 0.01$	-	$0.45^{f} \pm 0.02$	$75.18^{e} \pm 6.23$	$6.78^{d} \pm 2.01$

Proteolytic activities expressed as Unit mL^{-1} and data represent the means of three determinations ± standard error. EPS, Exopolysaccharide; 2+, 3+, 4+ (degree of opacity, EPS production); 2+, slightly opaque; 3+, opaque; 4+, very opaque. Means having different superscript within the same column differ significantly ($p < 0.05$).

Results and Discussion

Table 3. Technological properties of lactic acid bacteria isolated from fermented coconut milk

Isolate code	Acidity (% lactic acid)	Exopolysaccharide production	Proteolytic activity	Acetaldehyde production (µmol)	Syneresis (%w/w)
Lb1	$0.71^{a} \pm 0.13$	3+	$6.03^{a} \pm 0.10$	$172.00^{b} \pm 46.00$	$8.20^{b} \pm 1.95$
Lb2	$0.76^{a} \pm 0.10$	3+	$2.55^{c} \pm 0.22$	$218.00^{a} \pm 54.00$	$9.12^{a} \pm 2.00$
Lb3	$0.36^{d} \pm 0.02$	-	$0.15^{f} \pm 0.02$	$81.64^{e} \pm 9.68$	$6.86^{d} \pm 1.24$
Lb4	$0.44^{c} \pm 0.05$	-	$3.18^{b} \pm 0.23$	$108.00^{d} \pm 15.28$	$6.91^{cd} \pm 2.02$
Ed1	$0.51^{c} \pm 0.09$	-	$0.97^{e} \pm 0.09$	$52.00^{f} \pm 8.93$	$7.10^{c} \pm 1.17$
Ed2	$0.41^{c} \pm 0.06$	-	$0.22^{f} \pm 0.03$	$46.97^{f} \pm 5.02$	$6.90^{cd} \pm 0.94$
Ed3	$0.33^{d} \pm 0.07$	-	$1.53^{d} \pm 0.31$	$31.05^{fg} \pm 2.32$	$6.84^{d} \pm 1.26$

Proteolytic activities expressed as Unit mL^{-1} and data represent the means of three determinations ± standard error. EPS, Exopolysaccharide; 2+, 3+, 4+ (degree of opacity, EPS production); 2+, slightly opaque; 3+, opaque; 4+, very opaque. Means having different superscript within the same column differ significantly ($p < 0.05$).

Results and Discussion

•Important technological properties in yogurt starter cultures include acidity, proteolytic activity, acetaldehyde production, exopolysaccharide production and syneresis (Gurakan & Altay, 2010)

•The International Dairy Federation has recommended that the minimum value of acidity in yogurt is 0.70% (Obi *et al*., 2010)

Results and Discussion

• Exopolysaccharide functions as viscosifying agents, stabilizers, emulsifiers, gelling agents or water binding agents and play important role in the texture of the product (De Vuyst et al., 2001)

•The proteolytic activity of dairy LAB is essential for the growth of the organisms in milk and it is involved in the development of organoleptic properties of different fermented products (Hassaine *et al*., 2007).

Results and Discussion

Table 4. Sensory evaluation of coconut yogurt produced using indigenous starter culture

Sample	Taste	Colour	Aroma	Appearance	Texture	Overall Acceptability
Lm1+Lb1	$6.8^{d} \pm 1.7$	$7.3^{c} \pm 0.8$	$5.3^{d} \pm 1.3$	$6.7^{b} \pm 0.6$	$6.0^{d} \pm 0.7$	$6.5^{c} \pm 1.0$
Lm1+Lb2	$7.2^{c} \pm 1.3$	$7.1^{c} \pm 1.4$	$5.7^{c} \pm 1.5$	$5.8^{c} \pm 1.8$	$6.2^{c} \pm 1.1$	$6.2^{d} \pm 1.2$
Lm2+Lb1	$7.1^{c} \pm 0.7$	$8.0^{b} \pm 1.1$	$5.8^{c} \pm 1.1$	$6.5^{b} \pm 1.3$	$6.3^{c} \pm 1.5$	$7.3^{b} \pm 1.4$
Lm2+Lb2	$8.5^{a} \pm 1.6$	$8.4^{a} \pm 1.8$	$6.5^{b} \pm 0.6$	$7.2^{a} \pm 1.0$	$6.5^{b} \pm 1.0$	$8.0^{a} \pm 0.9$
Control	$8.0^{b} \pm 1.1$	$8.0^{b} \pm 0.5$	$7.1^{a} \pm 0.8$	$7.2^{a} \pm 1.5$	$6.9^{a} \pm 0.5$	$8.2^{a} \pm 1.2$

Means having different superscript within the same column differ significantly ($p < 0.05$).

Conclusions & Recommendations

- LAB associated with coconut milk have been identified molecularly
- *Leuconostoc mesenteroides* is the dominant species
- *Lactobacillus plantarum* subsp. *plantarum* and *Leuconostoc mesenteroides* subsp. *dextranicum* exhibited favourable technological properties relevant to yogurt production
- Yoghurt produced with mixed culture of *Lactobacillus plantarum* subsp. *Plantarum* Lb2 and *Leuconostoc mesenteroides* subsp. *dextranicum* Lm2 was preferred by panelist indicating their possible use as starter culture for coconut yogurt
- The physicochemical, proximate, fatty acid composition, flavour analysis of the yoghurt should be determined

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