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Plasmid Profile Of *Streptococcus lactis* And *Lactobacillus plantarum* Isolated From *Ogi* Encoding For Acetaldehyde In Yoghurt

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Abstract

An investigation was carried out on the plasmid profile of *Streptococcus lactis* and *Lactobacillus plantanum* isolated from a total of 120 *Ogi* samples collected from Oyingbo, a local market in Lagos State. Morphological, cultural, physiological characterization and API kits were employed to isolate and identify *Streptococcus lactis* and *Lactobacillus plantanum*. All the isolates harboured plasmids ranging from 1-7 in numbers and 3kb – 21.5 kb in sizes. Distinct plasmid patterns were seen using the electrophoresis of plasmid DNA method. In this study, single strains and mixed cultures of the isolates were investigated for acetaldehyde production in yoghurt (fermented milk product) with the isolates using column trapping gas liquid chromatography technique. The results show that acetaldehyde production of the isolates were somewhat variable from strain to strain and hence could be used as starter cultures for yoghurt production with market acceptability.

Keywords: *Ogi*, Plasmid profile, *Streptococcus lactis*, *Lactobacillus plantanum*, LAB, Yoghurt.

INTRODUCTION

Ogi is a Nigeria name given to a traditional fermented and often sour starch cake processed exclusively from maize, sorghum, or millet but not from rice or wheat. *Ogi* usually has a smooth texture and is boiled into a porridge called pap before consumption (Onyekwere et al. 2004). Fermented *ogi* pap has a mild to strong sour flavour resembling that of yoghurt and a characteristic aroma which quickly differentiates it from starch and corn flour. The colour of *ogi* depends on that of the cereal used to prepare it – slightly cream for white maize, cream for yellow maize, light brown for sorghum, and greenish to gray for millet.

All of the fermented dairy products are produced by the use of microorganisms, including lactic acid bacteria (LAB), molds and yeasts. The lactic acid bacteria are the most important ones among these microorganisms and are normally added to the milk as milk grown culture also called "Starter Culture". The starters used in dairy industry can be divided into three groups: mesophilic, thermophilic and artisanal starter cultures (Beresford et al. 2001).

The primary function of starter lactic acid bacteria is acid production during the fermentation processes (Beresford *et al*. 2001). In industry, starter cultures can be defined as strains which produce sufficient acid to reduce pH of the milk to 5.3 in 6h at 30°C (Beresford *et al*. 2001).

Lactobacillus plantarum is rod shaped, facultative, anaerobic, non-pathogenic gram positive bacterium naturally existing in human saliva and gastro intestinal tract. As a member of the lactic acid bacteria, it is commonly used in food fermentation. Examples of food containing *Lactoacillus plantarum* include Yoghurt, cheese, pickles, kimchi, Nigerian *ogi*, sourdough and stock fish. Its wide use in food makes it suitable for probiotics development with great potentials. (Marta et al. 2009). *Lactococcus lactis* sp. *Lactis* and *cremoris and Lb. plantrum* are approved as food grade organisms (Savadogo et al.2004).

Streptococcus lactis and *Lactobacillus plantarum* are lactic acid bacteria widely used in the dairy industry for the production of fermented foods i.e. yoghurt, fermented milk r

and cheese. During the fermentation process, the main purpose of the bacteria is the rapid acidification of milk and the sensory properties of the product being the flavour development. (Dana et al. 2011). *Lactococcus lactis* and *Lactobacillus plantarum* are widely used as starter cultures for several types of cheese, and fermented milk products (Bolotin et al. 2001).Some researchers isolated and characterized artisanal strains in order to provide sensorial properties similar to those of fermented milk- products. New strains of lactic acid bacteria have been isolated from different dairy products, such as raw milk (Wouters et al. 2002), fermented milks (Cogan et al. 1997, Ayad et al. 1999; Dana et al. 2011).

One of the basic parameters through which starter cultures are characterized is their ability to produce aroma compounds such as acetaldehyde, diacetyl (Dana et al. 2011). Acetaldehyde is considered as the most typical aroma compound in yoghurt, wine and some beverages (Routary et al. 2011).

Plasmid biology is an important area of investigation in dairy starter cultures since some properties, vital for successful milk fermentation, are coded by genes residing in plasmid DNA of lactic acid bacteria. Plasmid is a double stranded, extra chromosomal DNA of bacterium. Plasmids are important tools in genetic and biotechnology labs where they are commonly used to multiply or express particular genes. Plasmids are also used to make large amounts of proteins. (Ward et al. 2004). Plasmid profiling is an excellent tool for strain characterization and identification and for the characterization of starter culture composition (Mäyra-Mäkinen and Bigret, 1998).Typing based on plasmid analysis is also particularly useful for preliminary screening if it is used in conjunction with other techniques (Mannu and Paba, 2002).

The following functions are plasmid-linked; Plasmid-linked function are as follows; fermentation of lactose, sucrose, galactose, mannose, xylose, and even glucose, proteinase activity, phage resistance, bacteriocin production, citrate utilization, exopolysaccharide production, DNA restriction and modification, which are all found in lactic acid bacteria. (Ward et al.2011*)*.

Streptococcus lactis and *Lactobacillus plantarum* might include a number of plasmids. In different strains, for example, between 1 and 12 plasmids of different sizes (ranging in size from 2 to over 80 kbp) can be found. The following factors are sometimes identified as plasmid encoded properties in *Streptococcus lactis* and *Lactobacillus plantarum* (Mckay, 1983): lactose transport and metabolism, Casein degradation by cell wall protease, citrate and oligopeptide transport from outside the cell, bacteriophage resistance, as well as exopolysaccharides formation, antibiotic resistance and aroma and flavour formation.

Although plasmids encode defined properties, L*actococci* often contain plasmids with no known function. These plasmids are named as cryptic plasmids and they have been used as the basis of cloning systems for analysing non-*Lactococcal* genes (Cogan, 1996).

Most starter cultures used for yoghurt fermentation are imported (Ward et al. 2011*)*; hence there is need for indigenous source. At the moment, there is no report about *Streptococcus lactis* and *Lactobacillus plantarum* isolated from *ogi* (Nigeria maize fermented gruel) to have evidence of acetaldehyde production. Although studies have reported the involvement of *S. lactis* and *L. plantarum* as commercial culture in wine and cheese production (Fumi et al. 2010) and in fermentation of milk to produce yoghurt, but the context isolating them from *ogi* is not known. Their potentials to produce acetaldehyde have not been reported.

Acetaldehyde occurs in all living organisms, sometime in very small amounts as a product of many metabolic processes. The content of the intermediate acetaldehyde increases considerably when alcoholic fermentation is used for the production of food. Pathways of production of acetaldehyde have been summarized by Tamime and Robin, (2007). Even though the importance of acetaldehyde acetone and diacetyl for yoghurt flavour is well established, it is very difficult to evaluate the contribution of several identified volatile compounds because their sensory perception thresholds vary considerably (Zourari et al. 1991).

Therefore, the aim of the study is to evaluate

L. plantarum and S. lactis strains isolated from *ogi* for use as possible yoghurt starter with increased commercial relevance. The objectives are therefore (i) to identify, isolate and characterize indigenous *Streptococcus lactis* and *Lactobacillus plantarum*, among the isolated strains from 120 *ogi* samples collected from Oyingbo market in Lagos Nigeria. (ii)To know whether the presence of the generally regarded as safe (GRAS) organisms will influence the production of acetaldehyde or not in yoghurt (milk fermented product). (iii)To explore the potentials of locally made *ogi*– consumed within Lagos metropolis – in order to develop cheap starter culture for yoghurt production.

MATERIALS AND METHODS

Sample Collection

A total of 120 non-replicate samples of Nigeria fermented maize gruel (*ogi*) were obtained from Oyingbo, a local market in Ebute-Metta, Lagos Mainland local government area of Lagos state. They were bought randomly and collected aseptically from different sellers in the same market. Samples were labeled and cultured after collection at microbiology laboratory of Nigeria Institution of Medical Research (NIMR), Yaba, Lagos for further analysis.

TRADITIONAL PREPARATION OF *OGI*

Ogi preparation was simulated in laboratory using the traditional methods. In brief, one kilo gram of cereal grains (Maize) was cleaned and steeped in water separately for 2 days in a suitable container. The water decanted and the grains wet-milled before sieving with fine wire mesh. The pomace was discarded and the starch suspension was allowed to sediment during which fermentation was carried out for 2-3 days by the natural flora of the grains.

Isolation of *Streptococcus lactis* and *Lactobacillus plantarum*

The samples were aseptically weighed and homogenized. From each sample, a1: 10 dilution was subsequently made using peptone water followed by making a 10 fold serial dilution. A 0.1ml from each dilution was then subculture in duplicate into the MR Sagar (deManRogosa Sharpe) (Merck, Germany) used for isolating *Lactobacilli* and *Lactococcus* according to the method of Badis-Breard et al. (2009). To prevent the growth of yeasts, the media were then supplemented with 100mgL^{-1}ofcycloheximide before being incubated at the appropriate temperatures (35^{o}C) for 2-3 days according to the method of Beukes et al. (2001.Colonies were randomly selected and steak plating was then used to purify the strains which were subsequently kept in two different condition including at 4^{o}C for MRS plates and at -20^{o}C for MRS broths supplemented by 20% glycerol for further use.

Identification of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are functional related group of non-pathogenic, phylogenetically diverse bacteria that produced lactic acid as primary metabolic product from glucose and are often associated with food fermentations. All strains were initially tested for gram reaction, catalase production and spore formation, according to the methods and criteria described by Sharpe (1979). Colonies were characterized on MRS agar. Strains with gram positive and catalase negative reaction were finally used for further identification. Growth at different temperature (15^{o}C and 45^{o}C) for 2-3 days, resistance to 60^{o}C for 30 minutes (Sharman test), growth in the presence of 4.0% NaCl and different pHs (3.9 and 9.6) were considered to identify the strains. Hydrolysis of arginine, (H_2S) Hydrogen sulphide production, utilization of citrate, gas formation from glucose and dextran production from sucrose were also determined. All strains were also tested for fermentation of galactose, glucose, xylose, surcrose, maltose, rafinose, ribose, meicibiose, mannitose, rhamnose, salacin and fructose. The growth of bacterial strains at 15^{o}C and 45oC was visually confirmed by the changes in turbidity of MRS broth after 24, 48 and 72hrs of incubation. The tolerance of microorganisms to the different levels of salt, pH was also visually evaluated. Carbon sources were added individually to this medium as filter sterilized solution to a final concentration of 1%. Carbohydrate utilization was checked at the 24hrs and 48hrs and on the 7th day of the corresponding temperature according to the

method of Marrokiet al. (2011).

API 50CHL

API 50CHL is a standardized system, associating 50 biochemical tests for the study of the carbohydrate metabolism of microorganism.

Inoculation of the Strips

With the aid of sterile micropipette with tips, the bacterial suspension in API 50CHL medium was distributed into the 50 tubes of the strips (tubes only). The capsules of the strips were overlay with mineral oil to create anaerobic conditions. The strips were incubated at the optimum temperature at 37°C for 24hrs and 48hrs respectively. The strips are read after stipulated incubation periods of 24hrs and 48hrs and the reaction of each test was recorded in the record sheet provided. The identification of the bacterial strain was subsequently performed using the Apiweb identification software.

Plasmid DNA isolation

Bacterial cultures were inoculated into 10ml MRS broth and then incubated overnight at 30°C. They were centrifuged for 10 minutes at 5,000rpm. The supernatant was discarded and the pellet was re-suspended in 500ul 1 x TE buffer. After that, the samples were centrifuged for 10mins at 5000 rpm and the supernatant was discarded completely. The pellet was re-suspended in 200l lysozyme buffer (1 x TE containing 25% sucrose and 20mg/ml lysozome). The solution was incubated for 1h at 37°C. After the incubation, 400 l alkaline SDS solution 3% SDS). 0.2N NaOH) was added and incubated at room temperature for 7-mins. After that 300l of ice cold 3M Sodium acetate pH 4.8 were added. The solution was mixed immediately and centrifuged for 20mins at 10,000rpm at 4°C. The supernatant was transferred into the new Eppendorf tubes and 650l propanol was added and mixed. The solution was centrifuged for 30min at 100rpm at room temperature, then the supernatant was removed and the pellet was suspended in 300l 1 x TE. 200l of 7.5 M Ammonium acetate containing 0.5mg/ml ethidium bromide were added into the solution and mixed well. After this step, 350l phenol solution were added and centrifuged for 10 min at 10,00rpm at room temperature. The aqueous phase was transferred to a new eppendorf tube and then 350l chloroform/Isoamyl alcohol (24:1) was added, mixed well and centrifuged for 10 min at 10,000rpm at room temperature. The aqueous phase was again transferred into the new Eppendorf tube and 1ml 99% cold ethanol was added and mixed and the pellet was washed in 500l if 70% ethanol . After the washing step, plasmid DNA was pelleted and dried at 37°C and then re-suspended in 50l 1 x TE incubated for 1hr, 37°C and stored at -20°C.

Electrophoresis of Plasmid DNA

Plasmid DNA was electrophoresed in 0.8% agarose gel. Eight hundred mg of standard agarose was dissolved in 100mlof 1 x TAE buffer by boiling. Fifteen l of ethidum bromide (10mg/ml) were added after cooling the agarose solution to room temperature. After cooling, the agarose solution was poured into the gel casting stand with combs were placed. After the gel was cooled, the comb was removed gently from the casting tray , the agarose gel was placed into the electrophoresis tank and 1 X TAE buffer was added until the buffer cover the gel. 10l of plasmid DNA sample were taken and mixed with 2l of gel loading buffer. After that, the samples and 3 l of DNA (500mg) molecular weight marker were loaded into the wells. The electrophoresis was performed for that 60mA. The images on the gel were visualized using photometer in dark documentation room.

Preparation of Fermented Milk Samples

Microorganisms and strains.

The microorganisms and strains from the Nigerian fermented gruel (*ogi*) were used.

Fermentation:

Fermented milk samples were prepared using the strains of *Lactobacillus plantarum* and *Streptococcus lactis* isolated from *ogi* with fermentation conditions and milk preparation as described by Ott et al. (2000). Fermentation was performed in 2 L closed glass bottles containing 1 Le of milk each. The pH of fermented milk samples was adjusted to the desired values with DL-Lactic acid (Fluka).

Analysis of the samples

Fermented milk samples were analyzed for acetaldehyde using static headspace gas chromatography (S-HS-GC). All sets were performed at least in triplicates.

RESULTS AND DISCUSSION

Enumeration and Isolation of Lactic Acid Bacteria

Lactic acid bacteria were detected in high count in most of the analyzed samples. The presumptive *Lactobacillus plantarum* count ranged 250-320 colonies of 10^5 (1: 100000) in M17 and MRS media and presumptive *Streptococcus lactis* varied from 160-180 colonies of 10^5 (1:100000) after 24hrs. The results showed the presence of LAB in (*Ogi*) was in high count, similar results were reported by Onyekwer *et al. (*2004). Total lactic acid bacteria population count in *ogi* samples are shown in Table 1.

Table 2 shows the isolation and distribution of *Streptococcus lactis* and *Lactobacillus plantarum* isolates recovered from the '*Ogi'* samples.

Table 1. Total lactic acid bacteria population count in *ogi* samples cfu/g.

Sample Name	Lactic acid bacteria count on MRS agar	
Brown Ogi	241 × 106	36 × 107
	233 × 106	30 × 107
Yellow Ogi	189 × 106	42 × 107
	194 × 106	47 × 107
White Ogi	264 × 106	53 × 107
	270 × 106	55 × 107

Table 2. Isolation and distribution of *Streptococcus lactis* and *Lactobacillus plantarum* isolates recovered from the '*Ogi'* samples.

Ogi sample by maize variety	No. (%). of positive sample	Species		
		L. plantarum	***S. lactis***	**Both**
White (n = 33) Yellow (n = 51)	18 (54.5)	10	5	3
Brown (n = 36)	20 (39.2)	15	3	2
Total (N = 120)	17 (47.2) 55 (45.8)	11 36 (65.6)	4 12 (21.8)	2 7 (12.6)

Interpretation:

A total of 55 *ogi* samples were positive for *L. plantarum* and *S. lactis*, representing 45.8% isolation rate of these species of lactic acid bacteria. Further analysis showed that *L. plantarum* and *S. lactis* accounted for 65.6% and 21.8% respectively of these bacterial species when occurred singly and 12.6% when occurred jointly.

The biochemical characteristics of *s. lactis* and *lb. plantarum* isolated from Nigeria *ogi* using API 50 CHL is shown in Table 3.

Table 3. Biochemical characteristics of *S. lactis* and *Lb. plantarum* isolated from Nigeria *ogi* using API 50 CHL

	ISOLATE CODE					
	YL12	YL7	BL11	BL7	WL4	WL1
Colour	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+ve	+ve	+ve	+ve	+ve	+ve
Cellular	Rods	Rods	Rods	Rods	Rods	Rods
Morphology						
Catalase Test	-	-	-	-	-	-
Oxidase Test	-	-	-	-	-	-
Motility Test	-	-	-	-	-	-
MR-methyl Red	+	+	+	+	+	+
VP-vogesprosxane	-	-	-	-	-	-
Growth at 15^{o}C	-	+	-	+	-	+
Growth at 45^{o}C	+	-	+	-	+	-
Growth at PH 3.9	+	+	+	+	+	+
Growth at PH 9.6	-	+	-	+	-	+
Growth in 4% NaCl	-	+	-	+	-	+
Growth in 0.5% Bile Salt	-	+	-	+	-	+
NH_3 From Arginnime	-	-	-	-	-	-
H_2S Production		+		+	-	+
GALACTOSE	+	+	+	+	+	+
GLUCOSE	+	+	+	+	+	+
XYLOSE	-	-	-	-	-	-
SUCROSE	+	+	-	-	+	+
MALTOSE	+	+	+	+	+	+
RAFINOSE	-	+	-	-	-	+
RIBOSE	+	+	+	+	+	+
MECIBIOSE	-	+	-	-	-	+
MANNITOSE	+	+	-	+	+	+
ARABINOSE	-	+	-	+	-	+
TREHALOSE	+	+	+	-	+	+
RHAMNOSE	-	-	-	-	-	-
LACTOSE	+	+	+	+	+	+
SALACIN	-	-	-	-	-	-
FRUCTOSE	+	+	+	+	+	+
PROBABLE IDENTITY	Lactobacillus lactic	Lactobacillus plantarum	Lactobacillus Lactis	Lactobacillus plantarum	Lactobacillus Lactis	Lactobacillus plantarum

Figure 1 shows agarose gel electrophoresis of plasmids recovered from the *S. lactis* and *L. plantarum* isolates.

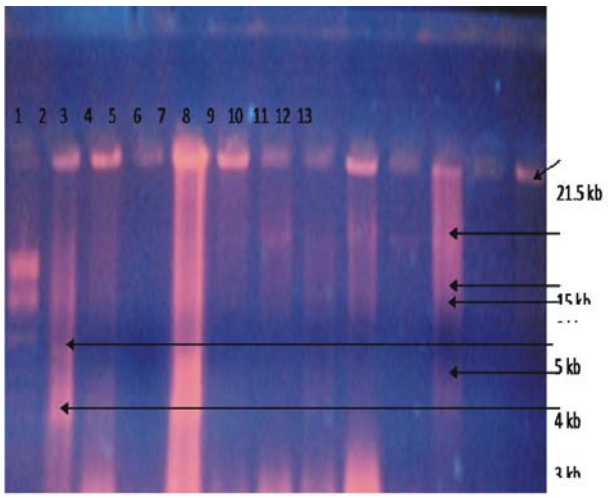

Fig.1. Plasmid profiles of representative isolates

Lane 1 = 10 kb DNA marker + 21.5 kb plasmid marker; Lanes 2 – 7 = *S. lactis* 005, 009, 012, 015, 021, 025; Lanes 8 – 13 = *L. plantarum* 003, 005, 012, 017, 020, 027.

It was proposed that nine strains were found to be very closely related after sma 1 digestion plasmid DNA.

In order to differentiate Streptococcus *lactis* and *Lactobacillus plantarum* isolates, plasmid DNA bands constituted different plasmid groups. Some of the 50 isolates shared a similar plasmid profiles with reference strain of the *Streptococcus lactis* and *Lactobacillus plantarum.* Ward et al. (2004) have also characterized nine strains of *Lactococcus lactis* used in the dairy industry by PFGE and Plasmid Profiling. These results show that our strains exhibit a great diversity based on the origin of *ogi* sample. Table 4 shows the distribution, pattern and sizes of plasmids recovered from *S. lactis* and *L. plantarum* isolates.

Table 4. The distribution, pattern and sizes of plasmids recovered from *S. lactis* and *L. plantarum* isolates.

Lane	No. of isolates	No. of plasmids	Plasmid size, kb
Lane 2	1	7	21.5, 15, 10, 9, 5, 4, 3
Lane 3	3	3	21.5, 15, 3
Lane 4	6	1	21.5
Lane 5	3	4	21.5, 10, 6, 3
Lane 6	4	2	21.5, 15
Lane 7	2	4	21,5, 15, 9, 8
Lane 8	2	6	21.5, 15, 9, 8, 4, 3
Lane 9	7	3	21.5, 15, 9
Lane 10	11	2	21.5, 15
Lane 11	3	5	21.5, 15, 9, 8, 4
Lane 12	10	1	21.5
Lane 13	10	1	21.5

Table 5: Tested samples of fermented milk, their pH and concentration of acetaldehyde.

Trial	Samples	pH	Acetaldehyde (ppm)
1	YL12+MILK	4.1	1.1
2	YL7+MILK	4.2	1.5
3	BL11 +MILK	4.1	1.3
4	BL7 +MILK	3.9	1.4
5	WL4+MILK	4.3	1.8
6	WL1+MILK	4.2	1.9
7	YL12+YL7 + MILK	4.4	2.9
8	BL11+BL7+MILK	4.4	6.0
9	WL4+WLI+MILK	4.4	7.4
10	YL12+BL11+MILK	4.5	2.6
11	YL12 +WL1+Milk	4.3	3.8
12	BL7 +YL7+MILK	4.1	5.4
13	YL7 +WL4+MILK	4.4	11.9

These results show that the isolated microorganism used exhibit great differences in acetaldehyde yield, fermentation of milk with *Streptococcus lactis* alone and *Lactobacillus plantarium* alone (Trails 1-6) gave products that were considerably different from the products obtained in the presence of the two microorganism as starter culture.

These results are in accordance with other research groups in raw goat milk (Mannu and Paba 2002; Marroki et al. 2011).The pH of the different yoghurts was adjusted with lactic acid to 3.9, 4.0, 4.1, 4.2, 4.4, 4.5 to create two series of ranging pH. This range covered the acidity of many different kind of yoghurt, from very mild to very acidic and also to prevent over – acidification pH (>5), applying heat shock (58^{o}C for 5mins) to yoghurt produced which agreed with the standard expressed by Analie and Viljeon (2001).

The production of acetaldehyde by the traditional yoghurt starter culture which comprises of *S. thermophilus* and *L. bulgaricus* agreed with the findings of Routray and Mishra (2011) and Ott et al. (2011). Minor difference could be observed between samples in different trials (8, 9 and 12) respectively. This suggested that the origin of strains investigated gave its characteristics to the final products fermentation; however acetaldehyde was much rapid in the presence of both isolates. However, acetaldehyde production by *S. lactis and L. plantarum* was observed to vary from strain to strain which is in agreement with the trend reported by Ott et al. (2001).

Generally, the following results were obtained from the investigation: (i) The ubiquitous presence of *Streptococcus lactis* and *Lactobacillus plantarum* in nature was confirmed with the identification of different strains from non-dairy environment. (ii) Some of these S. *lactic* and *L. plantarum* harbor plasmid and some do not. (iii) Acetaldehyde production by mixed cultures was detectably moderate. However, a rapid production of acetaldehyde occurred between 2 and 6 hours incubation. The level of this compound then decreased up to 15hr of incubation and leveled off with continued incubation up to 24hrs. (iv) The acetaldehyde production by the rods and bacilli was somewhat variable from strain to strain. In general however, less acetaldehyde was produced and microbial numbers were moderate when compared with the yield in normal yoghurt starter culture as described by Mishra et al. (2001). (v) *S. lactis* and *L. plantarum* isolated from white fermented *ogi* resulted in the production of higher level of acetaldehyde followed by ones isolated from the yellow counterpart. (vi) During refrigerated storage of 1 to 14 days, acetaldehyde concentration decreased considerably.

CONCLUSION

In this study, single strains and mixed cultures of *Streptococcus lactic* and *Lactobacillus plantarum* isolated from *ogi* were investigated for acetaldehyde production in yoghurt bases. The yoghurt base was composed of 2% fat homogenized milk fortified with 2% non-fat dry milk. The volatile compounds produced by the composite yoghurt culture were trapped and chromatographed by column trapping GLC technique. Acetaldehyde was identified by coincidence of retention time with that of the authentic compound and quantitated with the use of methyl acetate as an internal standard. Acetaldehyde production by single strains of *S. Lactis* and *LbPlantarum* and mixed culture of these in yoghurt bases was determined as a function of incubation time. The volatile acidity developed in yoghurt was determined.

RECOMMENDATIONS

In the light of the results obtained in this research, it is recommended that other technological characteristics such as bacteriophage resistance, proteolytic and lipolytic activity, antimicrobial activities and exopolysac charide production should be determined. Genes residing within the plasmids related to such functional properties should also be investigated. Functions, expression, characterization of these isolates should be carried out to provide improved technological methods in dairy industry. Finally, further research is recommended on the strains that were characterized in this study, to determine if they could be used as starter cultures for the fermentation of yoghurt in place of yoghurt starter cultures.

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