

## Isolation and Biochemical Profiles of Numerous Strains of Lactic Acid Producing Bacteria from Various Parts of a Domestic West African Goat (*Capra Hircus*)

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**Abstract:** In this study, a good number of lactic acid producing bacteria was isolated and subsequently identified from different parts of a domestic goat species (*Capra hircus*), commonly found in the Western part of Africa. Overall, three hundred and sixty eight strains ( $n = 368$ ) of lactic acid producing bacteria were identified from the muscle, liver, lung and intestine, with a view to selecting suitable ones as starter cultures for processing *kundi*, a popular semi dry meat product in West Africa. Identification was based on their morphological, physiological and biochemical characteristics. The numbers of identified strains include 48, 77, 11, 180 and 52 of respective genera *Pediococcus* (13%), *Lactococcus* (21%), *Leuconostoc* (3.6%), *Lactobacillus* (53.6%) and *Tetragenococcus* (14%). Despite effort made towards the characterization of the strains, some of the species of the genera could not be identified to the species level, based on available taxonomic schemes. The numbers of strains that could not be identified to the species level are 22, 12, 6, 18 and 49 of the respective genera of the lactic acid producing bacteria. Analysis of the distribution of the cocci shaped strains of the bacteria showed that out of the total of one hundred and eighty eight, 25, 23, 27, 51 and 62 were obtained from the muscle, liver, lung, intestine and skin respectively of the domestic goat species. Similarly one hundred and eighty strains of rod shaped lactic acid producing bacteria were isolated from the parts of the goat, among which 65, 37, 16, 47 and 15 were from the respective parts. The strains *Ped. pentosaceus*, *Tetragenococcus halophilus* and *Lactobacillus plantarum* were found to be dominant among their respective genera having numbers identified as 12, 21 and 41 respectively. Out of the total three hundred and sixty eight isolated strains of the lactic acid producing bacteria, only 39 strains were heterofermentative, producing gas from glucose. Our research findings indicated that there is great diversity of lactic acid producing bacteria in the various parts of the domestic goat species (*Capra hircus*), probably present as natural flora or contaminants. The bacteria could thus be exploited for use in bioprocessing of fermented food products.

**Key words:** *Kundi*, lactic acid producing bacteria, domestic goat, food bioprocessing

### INTRODUCTION

Goats were one of the earliest animals to be domesticated and are underrated as farm livestock. Goat meat is the primary source of protein in many parts of the world especially Africa and are also an important source of fiber and skins. Its meat is a close textured meat like beef but has far less fat than lamb and is an extremely healthy meat. The fat content of goat is lower than chicken and the saturated fats is also lower than chicken, protein content is as high as beef and iron content is highest of all meats (Table 1.1). The domestic West African goat (*Capra hircus*) is very common in most of the countries of West Africa, including Nigeria, Ghana, Republic of Benin, Cameroun etc. They are one of the main sources of meat in the region, and could be processed into various forms including *kundi*, *suya* and *tsire*.

**Table 1:** Comparison of goat meat to those of other animals

For 85g of roasted meat:	Calories	Fat (g)	Saturated Fat (g)	Protein (g)	Iron (mg)
Goat	122	2.58	0.79	23	3.2
Beef	245	16	6.8	23	2.9
Pork	310	24	8.7	21	2.7
Lamb	235	16	7.3	22	1.4
Chicken	120	3.5	1.1	21	1.5

**Source:** USDA, 2006:

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Lactic acid bacteria (LAB) have a long history of safe use in fermented foods. Today, LAB still play an essential role in the majority of food fermentations and one of the most important contributions of these microorganisms is the extended shelf life of fermented products. However, they also have beneficial influence on nutritional and sensory characteristics as well as on the standardization of end products (De Vuyst and Leroy, 2007). Lactic acid bacteria (LAB) are industrially important organisms because of their fermentative ability as well as health and nutritional benefits. Moreover, they are generally regarded as safe for incorporation into food products (Olaoye and Onilude, 2008). Identification of LAB mostly depends on traditional phenotypic analyses, although molecular biology-based methods have become available (Hertel *et al.*, 1993; Pot *et al.*, 1993; Vermeiren *et al.*, 2004). Hence, until now modern identification techniques have not been used to a large degree for the identification to the species level of LAB from Nigerian foods. Lactic acid bacteria (LAB) have played a long and important role in food technology. The LAB include a wide variety of cell types and physiological and biochemical characteristics (Yanagida *et al.*, 2005). Phenotypic methods relying on physiological and biochemical criteria have been widely used for LAB identification (Montel *et al.*, 1991). The use of phenotypic means of identification of LAB from different sources has been reported (Leisner *et al.*, 1999; Guessas and Kihal, 2004; Conter *et al.*, 2005; Nair and Surendran, 2005). Vermeiren *et al.* (2004) reported the isolation and characterization of LAB strains from cooked meat while Bromberg *et al.* (2004) also reported the isolation and identification of bacteriocin producing LAB from different meat products. In the same vein, Patil *et al.* (2007) were able to isolate bacteriocinogenic LAB from the intestine of rat. Moreover, Olaoye and Onilude, 2008 also reported the isolation of lactic acid bacteria from parts of cow's meat.

Bioprocessing of food products has gained increasing attention as a means of naturally controlling the shelf life and safety of meat products. Some LAB, among those commonly associated with meats, demonstrate antagonism towards pathogenic and spoilage organisms (Vermeiren *et al.*, 2004). Antagonistic cultures added to meat products to inhibit pathogens and/or prolong the shelf life, while changing the sensory properties as little as possible, are termed protective cultures (Lucke, 2000). The meat industry is going through significant technological change, pressured by consumer's demands. The addition of LAB to meat products can improve safety and stability of the products, extending shelf life by inhibiting undesirable changes brought about by spoilage microorganisms or abiotic reactions. It can also provide modifications of the raw material to obtain new sensory properties and health benefits through positive effects on the intestinal microbiota (Oliveira *et al.*, 2008). The identification of LAB that dominates the microbiota of meat is an important step in the search for potential new starter cultures and bacteriocins for meat preservation and fermentation.

In West Africa, most especially Nigeria, reports on the isolation and characterization of lactic acid producing bacteria, especially from domestic goat species (*Capra hircus*), with potential use in bioprocessing of meat products, have not been known. It was therefore the aim of this study to isolate numerous range of lactic acid producing bacteria strains from different part of a domestic goat species, some of which could be useful in the processing of some food products commonly found in West Africa.

## MATERIALS AND METHODS

### **Sampling:**

Fresh samples of the domestic goat were collected from African shops in the city of Nottingham, United Kingdom and taken to the laboratory on ice blocks in clean polyvinylchloride (PVC) bags for analysis.

### **Cultivation and isolation of lactic acid producing bacteria:**

Isolation of lactic acid producing bacteria was carried out from respective part of the goat meat. Ten grams of each meat sample was immersed in sterile 10% (w/v) sucrose solution for about seven minutes, to stimulate LAB growth (Olaoye and Onilude, 2008). Ten grams of this was then homogenized in 90 ml sterile distilled water in a stomacher (Standard bags, Stomacher LAB system, Seward circular 400, UK) for about 2 minutes. Serial dilution of this was then made and 1 ml was pour plated in MRS and M17 agar media and incubated in anaerobic jar at 37°C for 48-72 hours. Resulting colonies were subjected to catalase and oxidase tests (Sharpe, 1979) and successful colonies were sub-cultured repeatedly on respective agar plates until pure colonies were obtained (Guessas and Kihal, 2004). Pure strains were maintained in MRS and M17 broths containing 20% glycerol at -80°C. Sub-culturing into fresh broth media was done every six months.

### **Characterization and subsequent identification of lactic acid producing bacterial strains:**

The modified methods of Onilude *et al.* (2002) and Olaoye and Onilude (2008) were used for the biochemical characterization of the lactic acid producing bacteria. The LAB strains were subcultured twice overnight in MRS or M17 broth at 37°C. The following biochemical tests were conducted: Gram reaction; spore formation (Harrigan and McCance, 1976); oxidase-reaction; reduction of 1% (wt/vol) nitrate in MRS broth; phase-contrast microscopy of cell shape; production of NH<sub>3</sub> from arginine (Leisner *et al.*, 1994);

production of acid and gas from glucose (MRS without beef extract) (Difco, 1984); growth on MRS agar with 4.5 and 6.5% NaCl, for 5 days; growth on MRS agar at 15, 37, and 45°C. Some other phenotypic tests were conducted including acid production from carbohydrates (1%w/v) - arabinose, lactose, mannitol, raffinose, sorbitol, sucrose, dextrin, maltose, melibiose, rhamnose, xylose, trehalose, melizitose, salicin, fructose, galactose, mannose and ribose. This was performed in MRS broth (devoid of glucose and beef extract) containing respective sugars with chloramphenicol red as indicator.

## RESULTS AND DISCUSSION

Table 3.1 shows the phenotypic profiles of the lactic acid producing bacteria isolated from various parts of the domestic goat. The lactic acid bacteria identified consist of both rod and cocci shaped strains, with a total of three hundred and sixty eight isolates. The basis of identification was on their ability to utilize wide range carbon sources in their physiological and biochemical activities. Guessas and Kihal (2004) as well as Yanagida *et al.*, (2005) were able to identify various LAB strains from goat based on their biochemical reactions. Some of the LAB strains isolated by the authors include *Lactobacillus buchneri*, *Lb. plantarum* and *Leuconostoc mesenteroides*, all of which strains were also identified in this study. All isolated strains of LAB were gram positive, catalase negative and non spore forming. They were able to form acid from glucose, and, expectedly, able to grow at 37°C. They also tested positive to methyl red, but negative to gelatin hydrolysis as well as nitrate reduction tests. Confirmation of identities of LAB strains was carried out by reference to Bergey's Manual of Systematic Bacteriology (1986), based on their phenotypic profiles.

**Table 3.1:** Phenotypic profiles of the isolated strains of lactic acid producing bacteria.

	Cocci (n = 188)			Rods (n = 180)		
	+ve	wk	-ve	+ve	wk	-ve
Gas production (glucose)	10	-	178	26	-1	54
Glucose	174	14	-	177	3	-
Arabinose	62	34	92	54	13	113
Lactose	66	28	94	53	21	106
Mannitol	40	58	90	61	25	94
Raffinose	18	26	144	49	24	107
Sorbitol	26	52	110	31	29	120
Sucrose	30	54	104	90	19	71
Dextrin	14	74	100	17	41	122
Maltose	62	50	68	117	16	47
Melibiose	40	56	92	69	23	88
Rhamnose	38	36	114	26	25	129
Xylose	50	52	86	57	27	96
Trehalose	36	50	102	71	25	84
Melizitose	26	54	108	41	25	114
Salicin	24	36	128	57	29	94
Fructose	30	38	120	109	7	64
Galactose	24	44	120	83	24	73
Mannose	30	42	116	98	16	76
Ribose	32	42	114	81	11	88
Ho/He	180(Ho),	8(He)		149(Ho)	31 (He)	
4% NaCl	52	20	116	161	-	19
6.5% NaCl	26	6	156	152	-	28
Growth at 15°C	96	-	92	5	-	175
Growth at 37°C	188	-	-	180	-	-
Growth at 45°C	32	-	156	129	-	51
Methyl Red	188	-	-	180	-	-
Gelatin hydrolysis	-	-	188	-	-	180
Nitrate reduction	-	-	188	-	-	180
NH <sub>3</sub> production (from arginine)	52	-	136	nt	nt	nt

Ho / He = Homofermenter / Heterofermenter strains; +ve = number of positive strains; wk = number of weak strains; -ve = number of negative strains; nt = not tested

The most predominant of the five genera of lactic acid bacteria (LAB) identified from the different parts of the domestic goat species (*Capra hircus*) was *Lactobacillus*. Among the rod shaped lactic acid producing bacteria, *Lactobacillus*, only strains of *Lb. plantarum* was able to grow at 15°C, while growth at 45°C varied among other species. The cocci shaped LAB however displayed varying levels of growth at 15 and 45°C, while the same observation was noted for growth in 4% and 6.5% NaCl. Most of the rod shaped bacteria were all

able to grow in the presence of 4 and 6.5% NaCl, a very vital factor that could be employed in selecting candidates of starter cultures for the bioprocessing of meat products. Most of the processed meat products in Nigeria, including *tsire*, *kundi*, *suya*, may contain salts in varying concentrations depending on taste of individuals, and hence the starter cultures to be used in processing such product should be able to tolerate such salt levels. Besides, such organisms when used on such food products must be able to grow and produce required metabolites at such salt levels (Olaoye and Onilude, 2008). On the other hand, knowing the salt tolerant level of a particular organism would greatly assist in the optimization process of the product on which the organism will ultimately be used as protective culture. Hence, further studies are required in order to be able to exploit the maximum potential of strains of lactic acid producing bacteria to be used as starter candidates.

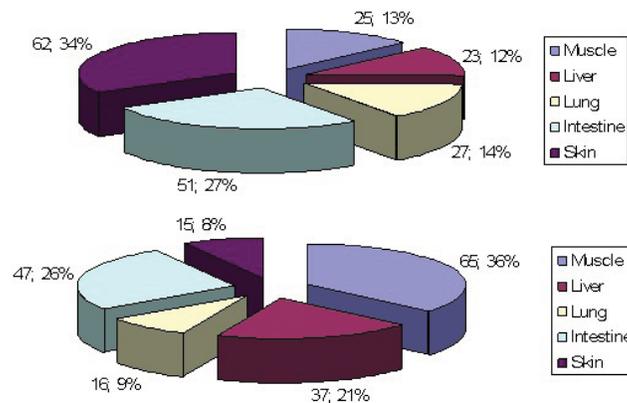
**Table 3.2.** Strains of lactic acid bacteria(LAB) isolated from the domestic goat species (*Capra hircus*)

Genus / species	no	Genus / species	no
<b><i>Pediococcus</i></b> (48 species)		<b>Tetragenococcus</b> (52 strains)	
<i>Ped. pentosaceus</i>	12	<i>T. halophilus</i>	21
<i>Ped. acidilactici</i>	8	<i>T. muriaticus</i>	13
<i>Ped. halophilus</i>	2	Unidentified to species level	18
<i>Ped. urinae-equi</i>	2		
<i>Ped. damnosus</i>	2	<b><i>Lactobacillus</i></b> (180 species)	
Unidentified to species level	22	<i>Lb. plantarum</i>	41
		<i>Lb. brevis</i>	15
		<i>Lb. trichodes</i>	3
		<i>Lb. delbrueckii</i>	5
<b><i>Lactococcus</i></b> (77 species)		<i>Lb. leichmanni</i>	3
<i>Lc. lactis</i> subsp <i>lactis</i>	9	<i>Lb. jensenii</i>	3
<i>Lc. piscium</i>	12	<i>Lb. lactis</i>	4
<i>Lc. plantarum</i>	12	<i>Lb. coprophilus</i>	3
<i>Lc. raffinolactis</i>	11	<i>Lb. bulgaricus</i>	11
<i>Lc. lactis</i> subsp. <i>cremoris</i>	3	<i>Lb. helveticus</i>	5
<i>Lc. garvieae</i>	11	<i>Lb. acidophilus</i>	3
<i>Lc. lactis</i> subsp <i>hordniae</i>	7	<i>Lb. xylosum</i>	3
Unidentified to species level	12	<i>Lb. curvatus</i>	3
		<i>Lb. cellobiosus</i>	11
<b><i>Leuconostoc</i></b> (11 species)		<i>Lb. buchneri</i>	5
<i>Leuc. mesenteroides</i>	5	<i>Lb. salivarius</i>	13
Unidentified to species level	6	Unidentified to species level	49

The LAB isolates generally displayed different patterns in terms of acid formation from different carbon sources, as dictated by to their physiological characteristics. This was very useful in their biochemical characterization and subsequent identification. Represented in Table 3.2 are the genera and species of the LAB isolates identified from different meat samples. The strains belong to five main genera, including *Pediococcus*, *Lactococcus*, *Leuconostoc*, *Tetragenococcus* and *Lactobacillus*, having respective percentage distributions of 13%, 21%, 3%, 14% and 49%. This implies that the common groups of LAB were represented in the genera of LAB isolated in this study. The presence of these genera in meat and meat products has been reported (Aymerich *et al.*, 1998; Bromberg *et al.*, 2004). The research workers were able to detect bacteriocin producing strains among the isolated LAB in their study. This indicates that there is high potential of detecting such properties in the strains of LAB obtained in this study, as some of the identified strains are intended to be screened and later used use as starter cultures on processed meat products. Twenty six isolates were identified to the species level among the genus *Pediococcus* and these include *Ped. pentosaceus* (twelve strains), *Ped. acidilactici* (eight strains), *Ped. halophilus* (two strains), *Ped. urinae-equi* (two strains) and *Ped. damnosus* (two strains) while twenty two strains of the genus could not be identified to species level. Seventy seven species of the genus *Lactococcus* were isolated, among which sixty five were identified to the species level. The species identified include *Lc. lactis* subsp. *lactis* (nine strains), *Lc. piscium* (twelve strains), *Lc. plantarum* (twelve strains), *Lc. raffinolactis* (eleven strains), *Lc. lactis* subsp. *cremoris* (3 strains), *Lc. lactis* subsp. *hordniae* (seven strains) and *Lc. garvieae* (eleven strains). Five strains of *Leuconostoc mesenteroides* were identified while six species of the genus could not be assigned to any specific species. The genus *Tetragenococcus* consisted of two main species among the strains that were identified and these include *T. halophilus* and *T. muriaticus* consisting of 21 and 13 strains respectively. Among the genus *Lactobacillus*, the identified species include *Lb. plantarum* (41 strains), *Lb. brevis* (15 strains), *Lb. trichodes* (3 strains), *Lb. delbrueckii* (five strains), *Lb. leichmanni* (3 strains), *Lb. jensenii* (3 strains), *Lb. lactis* (4 strains), *Lb.*

*coprophilus* (3 strains), *Lb. bulgaricus* (11 strains), *Lb. helveticus* (5 strains), *Lb. acidophilus* (3 strains), *Lb. xylophilus* (3 strains), *Lb. curvatus* (3 strains), *Lb. cellobiosus* (11 strains), *Lb. buchneri* (5 strains) and *Lb. salivarius* (13 strains). Forty nine strains belonging to this genus could not be identified to species level.

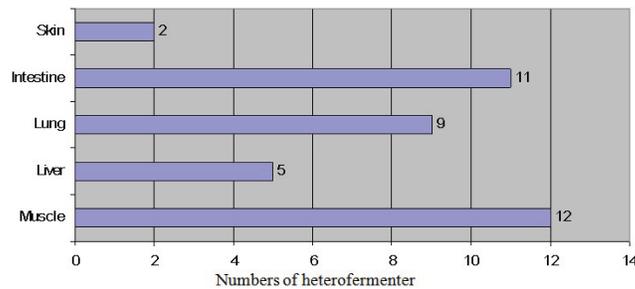
The distributions of isolated LAB strains in respective samples of the domestic goat are presented in Figure 3.1. Out of total of one hundred and eighty eight cocci shaped LAB strains isolated, 25, 23, 27, 51 and 62 were isolated from muscle, liver, lung, intestine and skin respectively, representing percentage (%) distributions of 13, 12, 14, 27 and 34 for the respective samples. Also among the one hundred and eighty rod shaped LAB strains, the highest number of 65 strains was obtained from the muscle while the skin had the lowest number of 15. Overall 65, 37, 16, 47 and 15 strains of LAB were isolated from muscle, liver, lung, intestine and skin respectively, indicating that LAB commonly contaminate inhabit the muscle of the domestic goat. The reason for this could be due to the chemical composition of the muscle, encouraging the growth of the lactic acid producing bacteria. This was in support of the findings of Olaoye and Onilude (2008) who reported similar observations in their findings on isolation of a number of LAB strains from parts of cow. Furthermore it could also be due to handling patterns of the meat products by the processors as well as the retailers.



**Fig 3.1:** Distribution of isolated strains of lactic acid producing bacteria from various parts of the domestic goat species (*Capra hircus*). A - Cocci shaped lactic acid producing bacteria; B -Rod shaped lactic acid producing bacteria

Some of the LAB strains identified in this study were hetero-fermenters. The result shows that thirty one of the identified hetero-fermentative strains belong to the genus *Lactobacillus* while the remaining eight belong to genus *Leuconostoc*, confirming the heterofermentative nature of the genus (Hemme and Foucaud-Scheunemann, 2004; Bjorkroth and Holzapfel, 2006). Presented in Figure 3.2. is the distribution of such strains in the respective domestic goat samples, showing that 2, 11, 9, 5, and 12 heterofermentative LAB were identified from skin, intestine, lung, liver and muscle of the animal. Heterofermentative lactic acid bacteria produce other metabolites besides lactic acid, unlike the homofermentative LAB which produce lactic acid as the main metabolite of sugar fermentation. Heterofermentative strains of LAB are not suitable for food fermentations because the formation of large amounts of carbon dioxide leads to holes of different sizes in the product (Buckenhushkes, 1993). In addition, these LAB produce concentrations of acetic acid that cause a pungent off flavours in food products (Ammor and Mayo, 2007). Hence such strains of LAB need to be carefully screened, if they are to be used as starter cultures for preservation of meat products. A very high percentage of the three hundred and sixty eight strains of lactic acid bacteria isolated in this study belong to the genus *Lactobacillus* followed by genera *Lactococcus*, *Tetragenococcus*, *Pediococcus* and *Leuconostoc* respectively.

Judging from the strains of lactic acid bacteria obtained in this study, there is very likelihood of obtaining candidates of starter cultures for later use for bioprocessing of processed food products especially meat. Some of the isolated strains of LAB have been reported for use as in biopreservation of foods. Onilude *et al.* (2002), Guererro *et al.* (1995) and Kalalou *et al.* (2004) have reported the successful use of LAB strains in the of some meat products.



**Fig. 3.2:** Numbers of isolated heterofermentative strains of lactic acid producing bacteria from the domestic goat species (*Capra hircus*).

**Conclusion:**

Our research findings have shown that a wide variety of lactic acid bacteria could be obtained from the domestic goat species (*Capra hircus*) and these strains could be exploited in the bioprocessing of food products most especially those obtained from goat and other animals. There is the need to subject the LAB strains to physiological performance in order to evaluate their abilities to produce antimicrobial agents including lactic acid, acetic acid as well as diacetyl. Their rate of production of these agents as well as how early such are produced would be very useful in the selection of any of the strains as candidates of starter cultures for food bioprocessing. We therefore intend to address these in our subsequent studies.

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