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Short Communication

Phenotypic characterisation and assessment of the inhibitory potential of *Lactobacillus* isolates from different sources

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Six strains of *Lactobacillus spp*. were isolated from fermenting corn slurry, fresh cow milk, and the faeces of pig, albino rat, and human infant. Their inhibitory action was tested against some spoilage and pathogenic bacteria. *Lactobacillus acidophilus* isolated from milk was found to display a higher antagonistic effect with zones of inhibition of 6 and 15 mm against *E. coli* and *Pseudomonas aeruginosa* respectively. This isolate was incapable of inhibiting the other indicator bacteria. The other isolates have zones of inhibition ranging between 1 to 4 mm. Characterisation of the microbial metabolic product for antimicrobial agents reveals that lactic acid may be responsible for the inhibition of the indicator organisms.

Key words: *Lactobacilli,* biopreservatives, pathogenic bacteria.

INTRODUCTION

The use of chemical preservatives and salt in foods is being frowned at because of their probable adverse effects on the health of consumers. However, lowering of the salt content of food or omission of preservatives will encourage growth of microorganisms (Nykanen et al., 1998). To ensure microbiological safety of foods, alternative means of preservation must be developed.

Lactobacilli have been found to produce metabolic products that play important role in controlling undesirable microflora in the gut (Itoh et al., 1995). They were able to prevent an increase of pathogenic bacteria by production of antimicrobials such as organic acids, hydrogen peroxide, and bacteriocins (Jin et al., 1996). Hence, they can be used as biopreservative agent

Lactobacillus species obtained from chicken caecum had been found to display antagonistic effect against other bacteria such as *Escherichia coli* and *Salmonella species*. Lactic acid was found to be the metabolic product responsible for the inhibition of other bacteria (Jin et al., 1996). Jay (1986) reported that the antimicrobial properties of lactic acid results from the undissociated lactic acid molecule and the reduction of pH below the level at which growth of many bacteria is inhibited. Lactic acid is widely used in foods as such or through the use of lactic acid bacteria fermentation.

The inhibition of the growth of various Gram positive and negative bacteria by *L. sake* from meat and *Lactobacillus* isolates from faeces of chicken had been reported by Schillinger and Lucke (1989) and Jin et al. (1996), respectively. Recently, Chang et al. (2001) reported the inhibition of the growth of some pathogens by *Lactobacillus reuteri* BSA 13, obtained from pig faeces.

This paper reports characterisation and preliminary antagonistic effect of six *Lactobacillus spp* from different sources against some spoilage and pathogenic bacteria. The characterisation of the metabolic product responsible for the inhibitory effect was also investigated.

MATERIALS AND METHODS

Sources of Isolates

Lactobacilli were obtained from fermenting corn slurry, fresh cow milk and faeces of human infant, albino rat and pig. The infant's faeces was collected from a day care centre, while faeces of pig and albino rat were obtained from the piggery and Rat house, Federal university of Technology, Akure.

Source of indicator bacteria

Pure cultures of indicator bacteria were obtained from Department of Microbiology, Obafemi Awolowo University, Ile Ife. These bacteria are: *Bacillus cereus* NCIB 6349, *E. coli* Type 1 NCIB 86, *Pseudomonas aeruginosa* NCIB 950, *Klebsiella pneumoniae* NCIB

Table 1. Phenotypic characterisation of *Lactobacillus* isolates.

Characteristics	1B	1M	2P	5S	1A	3A
Colour on MRS	Light brown	Light brown	Off white	Off white	Light brown	Off white
Edge	Serrated	Serrated	Entire	Entire	Serrated	Entire
Texture	Sliming	Sliming	Smooth	Smooth	Sliming	Smooth
Elevation	Raised	Raised	Raised	Raised	Raised	Raised
Gram reaction	+	+	+	+	+	+
Shape of cell	Long rods	Long rods	Rods	Rods	Long rods	Rods displayed separately
Catalase	-	-	-	-	-	-
Motility	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-
Indole	-	-	-	-	-	-
Glucose	-	-	G	-	-	G
Ribose	-	-	A	A	-	A
Arabinose	-	-	-	A	-	A
Sucrose	A	A	A	A	A	-
Mannitol	-	-	-	A	-	-
Growth at 15^0C	-	-	-	+	-	+
Growth at 37^0C	+	+	+	+	+	-
Tentative identity	*Lactobacillus acidophilus*	*Lactobacillus acidophilus*	*Lactobacillus fermentum*	*Lactobacillus plantarum*	*Lactobacillus acidophilus*	*Lactobacillus brevis.*

-: Negative. +: Positive. G: Gas formation. A: Acid formation. 1B : Isolate from human neonate faeces. 2P: Isolate from pig. 1A and 3A : Isolates from albino rat. 1M : Isolate from fresh cow milk. 5S : Isolate from fermenting corn slurry.

14070, *Staphylococcus aureus* NCIB 8588 and *Shigella dysenteriae.* They were maintained on agar slant throughout the duration of the study.

Isolation of *Lactobacillus* spp.

Samples of the faeces were homogenised and serially diluted using the method of Taylor (1962) and 0.1 ml of 10^{-4} and 10^{-5} dilutions were transferred into sterile petri-dishes. Also, fermenting corn slurry and fresh cow milk were serially diluted and 0.1 ml of the same dilutions above was plated. About 18-20 ml of sterile deMann Rogosa and Sharpe (MRS) agar (LAB M) was poured on the petri dishes. The plates were allowed to set and were incubated under anaerobic environment of $C0_2$ (generated inside a hermetically sealed dessicator) at 37 ^{0}C for 24 h.

Phenotypic Characterisation of Isolates

Discreet colonies of the isolates were sub-cultured to get a pure culture. The isolates were examined macroscopically for the colonial features and microscopically for cells morphological appearance. A battery of tests such as catalase, oxidase, indole, Nitrate reduction, and ability to utilise different sugars were investigated (Parker and Collier, 1990).

Detection of antagonistic activity

The well diffusion assay of Schillinger and Lucke (1989) was used to detect the inhibitory effect of the *Lactobacillus* isolates against the six indicator bacteria. This involves seeding petri dishes with the test bacteria and introducing 50 µl *Lactobacillus* isolates into holes bore with 3 mm cork borer. The plates were incubated aerobically at 37^0C for 24 h after which the plates were examined for clear zones of inhibition.

Characterisation of inhibitory substance(s)

The culture supernatants were assayed for organic acids and bacteriocins using the method described by Jin et al. (1996). The agar diffusion assay earlier described was used. To assay for organic acids, the culture supernatants were neutralised to pH 6.5 with addition of 1 M NaOH. For bacteriocin, trypsin from beef extract (1mg/ml) (BDH) was used to treat the supernatant for 12 h at 37^0C. The treated supernatant (50 µl) were transferred into wells bore in agar and appropriately labelled. Evidence of inhibition was observed after incubation for 24 h at 37^0C.

RESULTS AND DISCUSSION

The phenotypic characteristics tentatively revealed that isolates 1A, 1B and 1M were different strains of *L. acidophilus* while, isolates 3A, 5S and 2P are *L. brevis, L. plantarum* and *L. fermentum,* respectively (Table 1). *L. acidophilus* (1 M) from fresh cow milk was found to be most active in inhibiting *E. coli* and *P. aeruginosa* with the zones of inhibition of 6 and 15 mm, respectively. The other isolates had between 1–4 mm zones of inhibition (Table 2). The isolate from human baby faeces, *L. acidophilus* (1B), was the only isolate that was able to

Table 2. Antagonism of some indicator bacteria by *Lactobacillus* isolates using agar diffusion method.

Indicator Bacteria	Zone of Inhibition (mm)					
	1B	**1M**	**2P**	**5S**	**1A**	**3A**
B	3.0±1.0	NI	2.0±1.0	4.5±0.5	3.5±1.0	1.0±1.0
E	2.5±0.0	6.0±1.7	1.0±0.0	4.0±1.2	1.5±1.0	1.0±1.0
P	NI	15.0±2.5	2.0±1.0	3.0±0.0	NI	NI
KL	4.0±0.1	NI	3.0±1.0	4.0±2.0	2.0±1.0	1.5±2.0
ST	3.5±1.3	NI	NI	NI	NI	NI
SH	4.0±2.0	NT	2.0±0.0	NT	2.0±0.5	3.0±1.7

*Values are means of three replicates (Mean ± SD). **1B : Isolate from human neonate faeces. 2P: Isolate from pig . 1A and 3A : Isolates from albino rat. 1M : Isolate from fresh cow milk. 5S : Isolate from fermenting corn slurry.**

B: *Bacillus cereus* NCIB 6349
E: *E coli* Type 1 NCIB 86
P: *Pseudomonas aeruginosa* NCIB 950
KL: *Klebsiella pneumoniae* NCIB 14070
ST: *Staphylococcus aureus* NCIB 8588
SH: *Shigella dysenteriae* Clinical isolate
NI: No Inhibition.
NT: Not tested.

inhibit *S. aureus* while the other isolates did not show any inhibitory action against this organism.

Jin et al. (1996) had earlier reported the inhibition of *E. coli* and *Salmonella* strains by *Lactobacillus spp* from chicken intestine. Itoh et al. (1995) also reported the inhibition of food borne bacteria by bacteriocins from *L. gasseri.* They observed that several strains of *L. gasseri* showed wide inhibitory activity against *Listeria monocytogenes, Bacillus cereus, S. aureus*, and *E. coli.* Two commercial *Lactobacillus* strains, *L. casei,* and *L. lactis,* were reported to inhibit the growth of six *Salmonella* serotypes (Oyarzabal and Conner, 1995). Ehrmann *et al.* (2002) recently reported the inhibition of faecal strains (*E. coli* CTC 1028, *Salmonella enteritidis* CTC 039 and *Salmonella typhimurium* CTC 1037) by *Lactobacilli* isolated from crops and intestine of ducks.

The antibacterial effect of the isolates was demonstrated against both Gram positive and negative bacteria. This observation contradicts the report of Gilliland and Speck (1977) which indicates that *lactobacilli* showed stronger antibacterial effect against Gram positive than Gram negative bacteria.

The inhibitory zones remained unchanged when the supernatant was treated with trypsin, indicating that bacteriocin was not responsible for the inhibition of the bacteria. However, when the supernatant was treated with NaOH and adjusted to pH 6.5, there was no inhibition. This is an indication that the organic acid produced by the *Lactobacillus* isolates may be actually responsible for the inhibition of the indicator bacteria. Jin et al. (1996) had earlier reported that organic acids produced by the *Lactobacillus* isolates might be responsible for their inhibitory action.

The present report shows that *Lactobacilli* which can produce antibacterial agent against some major food spoilage and pathogenic bacteria are present in fresh cow milk, fermenting corn slurry, and the faeces of human neonates, pig and albino rat. These sources could be of great interest in the production of biopreservatives for the food industries.

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