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Screening of Antagonistic Activity and Antibiotic Resistance of Microflora Isolated from *Idli* Batter

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Main objective of this work was to examine the antimicrobial activity against the selected food borne pathogens and antibiotic susceptibility of selected microflora isolated from idli batter. Ten representative isolates tested shown the inhibition against all the tested food pathogens, such as, Escherichia coli (6-16mm), Salmonella typhi (10-17mm), Staphylcoccus aureus (6-22mm), Serratia marcescens (6mm), Listeria monocytogens (6-21mm), Bacillus cereus (6mm), Shigella flexnari (6mm and 10mm). The isolates also revealed resistance towards antibiotics such as vancomycin, erythromycin, ciproflaxin and tetracyclin. This work documents the functional characteristics of these selected cultures intended for potential probiotic use.

Key words*:* *Idli* batter, Antimicrobial activity, LAB, Antibiotics, Probiotics.

Idli is a very popular fermented breakfast food consumed in the Indian subcontinent, traditionally prepared from pre-soaked parboiled rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*) (Agrawal et al., 2000). Various reports on Idli batter fermentation in the aspects related to optimization of ingredients, microbiological, physico-chemical and nutritional aspects (Thyagaraja et al., 1992). Microorganisms from fermented foods have been screened for probiotic traits mainly as antimicrobial activity against pathogenic microorganisms (Ouwehand et al., 2002). The probiotics assist the equilibrium between harmful and favourable bacteria in the intestine thus conserving a healthy digestive system. (Botic et al., 2007). The inhibitory action of microflora is due to the production of primary metabolites such as lactic and acetic acids, ethanol, and carbon dioxide. Microorganisms are also known to produce antimicrobial compounds such as bacteriocins, benzoic and formic acids, diacetyl, acetoin and hydrogen peroxide (Mahnaz Kazemipoor and Che, 2012). Antimicrobial resistance is a worldwide health problem that threatens the effective treatment for bacterial infections in human and animals. On the other hand, widespread use of antibiotics in animal farming and agriculture is also a cause for supplementing antibiotic resistance that is consequently revealed in the food system (Schwaiger et al. 2011). The aim of the study to identify the microflora which possess the antimicrobial and antibiotic resistance from *idli* batter.

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MATERIALS AND METHODS

Sample collection

Idli batter samples were collected from different household in Pondicherry. All the samples were collected in sterile containers with proper hygienic conditions. Collected samples were brought to the laboratory for further analysis.

Isolation and Identification of microflora from *idli* batter

1g of *Idli* batter sample were dissolved in 9ml of phosphate buffer saline and serially diluted upto 10^{-10} and spread on nutrient agar and incubated at 30°C for 24hrs. MRS agar were incubated in anaerobic jar at 30°C for 48hrs, isolate the distinct colony and stored at -4°c as a glycerol stock. The DNA was isolated through using the spin column kit obtained from Himedia Pvt. Ltd., India. The rRNA gene was polymerized with primers U1F 52 - AGA GTT TGA TCC TGG CTC AG - 32 and U1R 52 - GGT TAC CTT GTT ACG ACT T - 32 . PCR reaction was performed. The PCR products were separated on a 1% (W/V) agarose gel at a constant power of 120 V in 1×TAE-buffer for 30 min. The amplified products were visualized under UV transilluminator for identification (Singh & Ramesh., 2009).

Antimicrobial activity

The antimicrobial activity of selected isolates was tested against food pathogen such as *E.coli* (MTCC 727), *Salmonella typhi* (MTCC 8767), *Staphylcoccus aureus* (MTCC 1144), *Serratia marcescens* (MTCC 97*)*, *Listeria monocytogens* (MTCC 1143), *Bacillus cereus* (MTCC 1272), *Shigella flexnari* (MTCC 1457) by well diffusion method (Gonzalez et al. 2007). Muller-Hinton media was prepared for the propagation of food pathogens. The food pathogenic organisms were cultured in nutrient broth at 37°C for 24 h. After incubation, inoculum was spread using sterile cotton swab on Muller-Hinton agar. About 50µl of broth supernatant from selected isolates were added to the well for testing antimicrobial activity. The plates were incubated at 37°C for 24 h. After incubation, the inhibition zones were observed. The inhibition zones diameters were measured in millimetre (Gonzalez et al. 2007).

Antibiotic sensitivity test

Selected isolates were tested for resistance to antibiotics such as ampicillin (10 µg), cephalotoxin (30 µg), vancomycin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg) and kanamycin (30 µg) by disc diffusion method. The isolates were grown on MRS and nutrient broth at 30°C for 24 h. About 50µl of isolates were spread on MRS and nutrient agar. After 5-10 minutes, 6mm antibiotic disc were kept on the specific agar plates using disc dispenser (Herreros et al. 2005).

RESULTS AND DISCUSSION

Isolation of microflora

Total of 300 pure colonies were isolated from different *idli* batter out of which ten isolates were randomly selected and identified as *Bacillus subtilis* (7), *Bacillus amyloliquefaciens* (2), and *Leuconostoc lactis* (1) through 16s rRNA sequencing (Table 1). These ten isolates were used in the further study.

Antimicrobial activity against food pathogens

Majority of the isolates tested showed antimicrobial activity against the food pathogen (Figure 1 and Table 1). The zone of inhibition for *E. coli* ranged from 6mm to 16mm diameter. Whereas, 4 isolates (PUFSTFMId08, PUFSTFMId10, PUFSTFMId15, PUFSTMId31) shown 6mm zone, 3 isolates (PUFSTFMId13, PUFSTFMId02, PUFSTFMId14) range from 11-18mm, and remaining have no zone formation against *E. coli*. The zone of inhibition ranged from 10mm to 17mm against *Salmonella typhi*, 3 isolates (PUFSTFMId12, PUFSTFMId13, and

Table 1. Antimicrobial activity against food pathogens

Isolates	*E.coli*	*S. typhi*	*S. aureus*	*S. marcesens*	*L. monocytogens*	*B. cereus*	*Shigella*sp.
PUFSTFMId01	Nil	Nil	6mm	Nil	6mm	Nil	Nil
PUFSTFMId02	16mm	17mm	Nil	Nil	6mm	Nil	Nil
PUFSTFMId08	6mm	16mm	Nil	Nil	11mm	6mm	6mm
PUFSTFMId10	6mm	Nil	Nil	Nil	Nil	Nil	Nil
PUFSTFMId11	Nil	13mm	17mm	Nil	10mm	Nil	Nil
PUFSTFMId12	Nil	11mm	22mm	Nil	10mm	6mm	10mm
PUFSTFMId13	11mm	11mm	14mm	Nil	13mm	Nil	6mm
PUFSTFMId14	18mm	10mm	14mm	Nil	Nil	Nil	Nil
PUFSTFMId15	6mm	14mm	19mm	6mm	21mm	Nil	Nil
PUFSTFMId31	6mm	11mm	11mm	Nil	10mm	Nil	Nil

PUFSTFMId31) formed 11mm, isolates (PUFSTFMId14, PUFSTFMId11, PUFSTFMId15, PUFSTFMId08, PUFSTFMId02) indicated 10-17mm zone of inhibition and remaining have no zone of inhibition. 7 isolates (PUFSTFMId01, PUFSTFMId31, PUFSTFMId14, PUFSTFMId11, PUFSTFMId15, PUFSTFMId12) shown antimicrobial activity against *Saphylococcus aureus* ranged from 6mm to 22mm. PUFSTFMId15 formed range from 6mm-11mm zone of inhibition against *Serratia sp.* and remaining have no zone formation.8 isolates shown antimicrobial activity against *L. monocytogens*. 2 isolates shown zone of inhibition against *B, cereus* and 3 isolates shown against *Shigella.spp.*

The inhibitory activity revealed by these strains might be due to the existence of bacteriocins or bacteriocin-like metabolites. Production of bacteriocins by lactic acid bacteria is commonly observed and has been considered to contribute to their colonization ability and competitive ability to survive in the intestinal environment (Garriga et al. 1993). Similar findings have been reported in an earlier study (Vijai et al. 2005) where out of 25 colonies, 10 randomly picked colonies exhibited good antimicrobial activity against Gram-positive bacteria *B. cereus, S. aureus* and *L. monocytogenes* as well as Gram negative bacteria. In a similar study (Simsek et al. 2006), antimicrobial activity of 20 identified and 8 unidentified strains (all the microflora isolated from sour dough) and two control *L. sake* strains (Lb706 and Lb706-A) was determined against certain bacterial strains (*L. sake* Lb790, *Listeria monocytogenes* Li6, *B. licheniformis* NRRL-B1264, *E. coli* ATCC39403, *S. aureus* ATCC29213, *C. perfringens* 4TTK, *Saccharomyces cerevisiae*) showing comparable results.

Table 2. Antibiotic susceptibility to various antibiotics

Isolate	AMP (10µg)	CHLO (30µg)	VAN (30µg)	STREP (10µg)	CIP (5µg)	TET (30µg)	KAN (30µg)	ERYT (15µg)
PUFSTFMId12	S	S	S	S	S	S	S	S
PUFSTFMId08	S	S	S	S	S	S	S	S
PUFSTFMId02	S	S	S	S	S	S	S	S
PUFSTFMId10	S	S	S	S	S	S	S	S
PUFSTFMId15	S	S	S	S	S	S	S	S
PUFSTFMId13	R	S	R	S	S	S	S	R
PUFSTFMId31	R	S	R	S	S	S	S	R
PUFSTFMId11	R	S	R	S	S	S	S	S
PUFSTFMId14	R	S	R	S	S	R	S	S
PUFSTFMId01	S	S	R	S	R	S	S	S

Table 3. Microflora involved in antimicrobial and antibiotic resistance test

Isolates	Strain and Accession no.
PUFSTFMId12	*Bacillus subtilis* KC834384
PUFSTFMId08	*Bacillus subtilis* KC834380
PUFSTFMId02	*Bacillus subtilis* KC213820
PUFSTFMId10	*Bacillus subtilis* KC834382
PUFSTFMId15	*Bacillus subtilis* KC834387
PUFSTFMId13	*Bacillus subtilis* KC834385
PUFSTFMId31	*Bacillus subtilis* KC855546
PUFSTFMId11	*Bacillus amyloliquefaciens* KC834383
PUFSTFMId14	*Bacillus amyloliquefaciens* KC834386
PUFSTFMId01	*Leuconostoclactis*KC117496

Antibiotics susceptibility

Among the 10 selected isolates, antibiotic resistance were shown against amphicillin, vancomycin, ciproflaxin, tetracycline, erythromycin, and chloramphenicol (Figure 2 and Table 2). Four of the isolates were resistant to amphicillin (10µg), 5 isolates were resistance to vancomycin (30µg), 2 isolates were resistance to erythromycin (15µg), 1 isolate is resistance to ciproflaxin (5µg), 1 isolate is resistance to tetracyclin (30µg). Earlier work reported that the resistance profiles of Enterococci (*E. faecium*) isolated from food comprising numerous acquired traits (Mathur et al. 2005). A probable baseline was providing by *E. faecium* strain 68, which was used as a probiotic for man as well as for animal, and as

silage inoculants. It was susceptible to erythromycin (15 µg disc), framycitin (100 µg), streptomycin/penicillin (streptopen 35 µg), gentamicin (10 µg), penicillin, tetracycline (30 µg) and chloramphenicol (30 µg). It was resistant to kanamycin (30 µg), streptomycin (10 µg) and oxacillin (5 µg).

Lactobacillus, *Pediococcus* and *Leuconostoc* spp. have been reported to have natural resistance to vancomycin, a property that was suitable to distinct them from all other Gram-positive bacteria (Hamilton et al. 1998). Specific lactobacilli have enormous natural resistance to cefoxitin, bacitracin, fusidic acid, ciprofloxacin, gentamicin, streptomycin, kanamycin, nitrofurantoin, metronidazole, norfloxacin, sulphadiazine, teicoplanin, trimethoprim and vancomycin (Danielsen and Wind 2003) as also reported in the present study. The selected isolates are having strong antimicrobial activity and antibiotic resistance and can act as a potent probiotics. Furthermore, these cultures can be

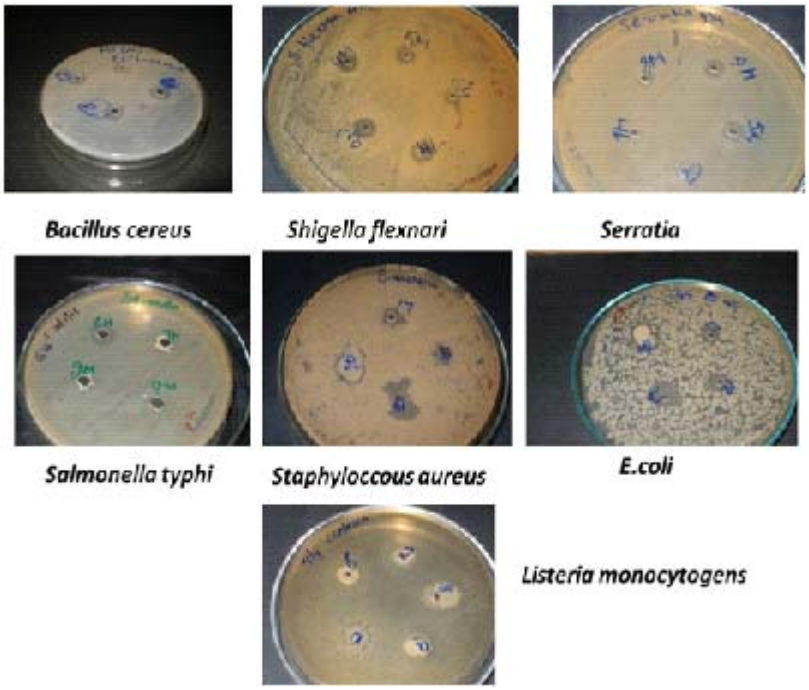

Fig. 1. Antimicrobial activity against various pathogens

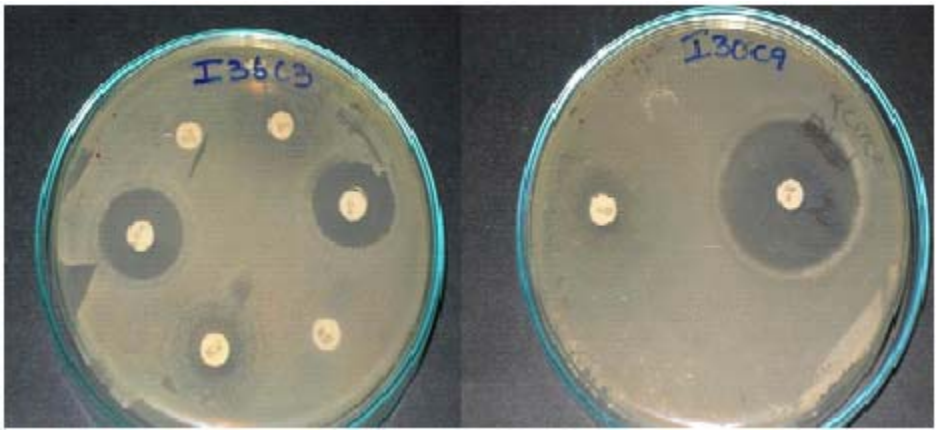

Fig. 2. Antibiotic susceptibility against different antiboitcs

used as candidates for the development of functional starter cultures in the food fermentation.

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