

Screening of Indigenous Strains of Lactic Acid Bacteria from Indian Poultry

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ABSTRACT

This study was aimed to isolate indigenous strains of Lactic Acid Bacteria from Indian backyard chickens. Lactic Acid Bacteria are known for good probiotic potential and commonly used to improve intestinal health of human and animals. A total of 18 Isolates were isolated from chicken fecal sample collected from backyard poultry. 05 out of 18 Isolates showed antagonistic effect against poultry pathogenic *S. Entertidis* and *E. coli*. The morphological, biochemical properties and carbohydrate fermentation pattern indicated that, the presence of species belongs to Genus *Lactobacillus*, *Enterococcus* and *Streptococcus*. All these genera are part of Lactic Acid Bacteria group. Further probiotic potential studies would strengthen the use of these strains as possible alternative for antibiotic growth promoters.

Keywords: Lactic Acid Bacteria, Chicken, Probiotic, Biochemical Characterization

INTRODUCTION

The indigenous intestinal micro-flora is a protective barrier against pathogens in animals and it strongly affect nutrient digestion and absorption. In recent time, role of gram positive Lactic Acid Bacteria in health and functionality of human and other animals has been well studied, mainly due to its ability to produce acids and some antimicrobial agents like bacteriocin (Torshizi et al., 2008). Lactic acid bacteria (LAB) are widely distributed in nature and include a huge number of bacterial genera, some of the best known are *lactobacilli*, *lactococci*, *enterococci*, *streptococci*, *leuconostoc*, and *pediococci* (Pessione, 2012).

Lactic acid bacteria (LAB) includes gram-positive, non spore formers, catalase negative, facultative anaerobes, fermentative bacteria. They are known for fermentation of hexoses into lactic acid. Lactic Acid Bacteria is among the common microflora of chicken gastrointestinal tract. LAB colonizes in the small intestine and caeca of chicken. They

are also shaded through excretion and commonly found in fecal matter of healthy birds (Heravi et al., 2011). Due to their multi-benefits, LABs are commonly used as Probiotics in human, animals and chickens. The application of Probiotics in poultry has gained substantial attention during last few years as a substitute to antibiotic growth promoters (Muhammad et al., 2009). Many LAB strains screened from different sources are being used as Probiotics in poultry but it is unlikely that each strain possesses every desired character that will make them a suitable probiotic candidate. In present study, we aimed to isolate and biochemically characterize some indigenous species of Lactic Acid Bacteria from fecal samples of free range Indian chickens, which further intended to characterize their probiotic potential.

MATERIAL METHODS

Enrichment and Isolation of Lactic Acid Bacteria

The fecal samples were collected from indigenous chickens from backyard poultry in and around Latur (Maharashtra). The collected samples were first inoculated in MRS broth and incubated at 37°C for 24 Hrs. Subsequently, the enriched bacterial consortium was inoculated on de Man Rogosa Sharp (MRS) agar. The inoculated plates were incubated at 37°C for 24-48 Hrs in anaerobic jar (Noohi et al., 2014 and Gunasekaran et al., 2012). After incubation, distinct colonies with Gram Positive and Catalase Negative nature were selected for further investigation.

Antimicrobial Activity of Lactic Acid Bacteria

For effective and concise study, Lactic Acid Bacteria isolates with antagonistic potential (an essential probiotic property) were only selected for further set of experiments. Here, antimicrobial activity of LAB isolates

was studied against common poultry pathogens including *S. Entertidis* and *E. coli* as per protocol given by Jouzan et al., 2016.

Morphological and Biochemical Characterization

The cultures were identified according to their morphological and biochemical characteristics. Morphological tests including Gram nature and spore forming ability of selected isolated were studied. Biochemical tests like Catalase test, Oxidase test, MR-VP test and Citrate utilization test were carried out as per the standard protocols given by Nair et al., 2005. Carbohydrate fermentation pattern was also studied by using sugars like D-Glucose, D-Fructose, Lactose, Sucrose, Mannitol and Starch (Patil et al., 2016).

RESULTS & DISCUSSION

A total of 18 isolates were isolated from fecal samples of indigenous chicken on LAB specific MRS medium. All isolates showed typical colony morphologies of Lactic Acid Bacteria. All isolates were Gram Positive and were unable to produce Catalase enzyme. Out of 18 LAB isolates only 05 isolates viz Isolate SS02, SS05, SS09, SS11 and SS16 showed antagonistic effect against both poultry pathogens *S. Entertidis* and *E. coli*. The LAB isolates with antimicrobial activity were further selected for morphological and biochemical characterization.

In the morphological characterization, all 05 Isolates showed typical Gram Positive nature and non-spore forming ability. Isolate SS02 and SS05 were rod shaped bacterium whereas remaining 03 Isolates i.e. SS09, SS11 and SS016 showed cocci shape. Isolate SS16, bacterial cells were observed in pair and chain, which is typically found in *Streptococci* genus.

Table 01: Morphological and Biochemical Characterization of Lactic Acid Bacteria Isolates

Isolate Code	Gram Nature	Sugar Fermentation Test	Glucose	Fructose	Lactose	Sucrose	Mannitol	Starch	Biochemical Properties	Catalase Test	Oxidase Test	Methyl Red Test	Voges Proskauer	Citrate Utilization
SS02	Positive Rods		+	+	+	+	+	+		-	-	-	-	-
SS05	Positive Rods		+	+	+	+	+	+		-	-	-	-	-
SS09	Positive Cocci		+	+	+	+	+	-		-	-	-	+	-
SS11	Positive Cocci		+	+	+	+	+	-		-	-	-	+	-
SS16	Positive Cocci		+	+	+	V	-	-		-	-	-	+	-

As per carbohydrate fermentation pattern and biochemical properties, Isolate SS02 and SS05 found to ferment all 06 Sugars used in experiment. Whereas, those isolates have no ability to produce Catalase and Oxidase enzymes. The Isolates showed negative MR-VP tests and unable to utilize citrate. These are typical properties of *Lactobacillus spp.* Results of biochemical tests and sugar fermentation pattern match with *Lactobacillus salivarius*. This is in agreement with Tinrat et al., 2011.

Isolate SS09 and SS11 were also able to utilize all sugars except Starch. Biochemically, they showed negative results to Catalase test, Oxidase test, Methyl Red test and Citrate utilization test. However, they showed positive test result for Voges- Proskauer test. Similar results were also shown by Manero and Blanch, 1999 for organism *Enterococcus faecium*. The morphology, biochemical properties and sugar fermentation pattern of SS16 matches with a few *Streptococcus spp.* Although, it needs more biochemical and molecular exploration for species level identification.

Overall, all 05 Isolates were found to be in a group of Lactic Acid Bacteria. Out of which, probiotic potential of *Lactobacillus salivarius* is widely studied phenomenon. The isolated and characterized strains can further be used to study probiotic potential.

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