

# Isolation of antagonistic *Lactobacillus* from cow dung and its nutritional improvement as probiotic strain

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Different strain of *Lactobacilli* spp. were isolated from fresh cow dung and designated as A1 to A10. Selected *Lactobacillus* isolates were then inoculated with different nutritional sources like MRS broth, Packed milk, Sugarcane juice, Mousammi juice and MRS broth + Sugarcane juice and MRS + Mousammi juice. Their antagonistic effect was tested against three selected pathogenic bacteria namely *S. aureus*, *E. coli* and *Pseudomonas* spp. The result clearly showed that some isolates when inoculated with MRS broth + sugarcane juice inhibited the growth of pathogenic strains in varying degree. Isolate A-6 and A-10 were found to be more effective in reducing growth of all three pathogenic species from 10 to 30 mm. Other isolates (A1 and A2) from the same source, were effective against *E. coli* and *Pseudomonas* spp. with a zone of inhibition of 15 to 10, respectively but no zone of inhibition was seen against *S. aureus*.

Key words : Cow dung, *Lactobacillus*, Probiotic

## INTRODUCTION

All free living animals including man do hosts for a society comprise an enormous number of individual. The normal microflora differs between animal species, body sites of individuals within the same species (Gilliland and Speck, 1977).

Human as well as animals normally are born sterile, but shortly after birth colonization begins and every location is filled up with the fittest microbes from the environment, thus creating a balanced ecological system. (Olotu Olanrewaju, 2007).

In recent years there is a great interest for viable microorganisms that promote or support a beneficial balance of autochthonous microbial population of gastrointestinal tract. The weight of intestinal flora is between 1 to 2 kg, housing  $10^{14}$  bacteria which means that there are more living bacteria than there are cells in a normal body, which plays an important role in equilibrium of the intestinal ecosystem.

The word probiotic is a compound of Latin and Greek word, means "Favorable to life". Probiotics are live microorganisms, which are considered "Friendly germs" due to benefits to the colon and the immune system, when administrated in adequate amount confirm a health benefit on the host.

The *Lactobacilli* group of bacteria was the first probiotic. Milk, which contains *Lactobacilli* bacterium called as *Lactobacillus acidophilus* was also recommended in 1920s and 1930s for the treatment of

constipation and diarrhea. This species of probiotic adheres to the intestinal mucus and epithelial cells and boosts beneficial bacteria.

## MATERIALS AND METHODS

The present study was designed to isolate antagonistic *Lactobacilli* from fresh cow dung, which is undigested residues of herbivorous matter and passed through the animal gut.

A total of 10 sample of fresh cow dung were collected from different locations of Dehradun, Uttarakhand, India.

Isolation of *Lactobacilli* was done by serial dilution method. Dilutions were made up to  $10^{-5}$ , 100 ml from each tube was spreaded on the MRS medium as this is selective media for *Lactobacilli*. These plates were then incubated at 37°C for 24 to 48 hrs.

Selected *Lactobacilli* isolates were inoculated in tubes containing sterilized milk. The tubes were observed for coagulation. Only those isolates which produce smooth curding from milk were selected for further study.

Indicator organisms, which include *S. aureus*, *E. coli*, and *Pseudomonas* spp. were used to assess the amount of antagonistic activity produced by isolates. Cell free filtrate preparation of isolates was done by centrifugation method and then was transferred to fresh sterilized tubes to check antagonistic activity against the pathogenic organisms by the agar well diffusion assay as described by Schillenger and Lucke (1998).

Selected *Lactobacilli* isolates were then inoculated

Table 1: Effect of different nutrients on selected isolates

Lactobacilli Species Isolates	MRS broth supplement			Milk supplement			Mousammi juice			Sugarcane juice			MRS+ Mousammi broth juice			MRS + sugarcane broth		
	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>	<i>E. Coli</i>	<i>Pseudomonas</i>
A <sub>1</sub>	—	—	10mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>2</sub>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>3</sub>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>4</sub>	—	—	10mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>5</sub>	—	—	8mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>6</sub>	—	7mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>7</sub>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>8</sub>	—	6mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>9</sub>	—	—	8mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A <sub>10</sub>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

(-): No zone of inhibition

with different nutritional sources like MRS broth, Packed milk, Sugarcane juice, Mousammi juice, MRS broth + Sugarcane juice and MRS + Mousammi juice to enhance the activity of Lactobacilli isolates.

## RESULTS AND DISCUSSION

The antagonistic activities observed are summarized in Table 1. From the table it is clear that when the isolates were inoculated in MRS broth antagonistic activity was observed against *E. coli*, (6 mm - 7 mm) but no zone of inhibition was shown against *S.aureus* and *Pseudomonas* spp. When they were inoculated in milk supplement, antagonistic activity was again observed against *E. coli* (8-10 mm) but no zone of inhibition was shown against *S.aureus* and *Pseudomonas* spp., than these isolates were inoculated with Mousammi juice supplement and then the antagonistic activity against *E.coli* (12 to 14 mm) was good but no antagonistic activity was found against *S.aureus* and *Pseudomonas* spp. (Table 1). When these isolates were further inoculated into Mousammi juice supplemented with MRS broth, it was found satisfactory against *E.coli* (10-15 mm). *S.aureus* (8-13 mm) and *Pseudomonas* spp. (10-11 mm) (Table 1).

When these isolates were further inoculated with the sugarcane juice supplement, produced good results of antagonistic activity against *S.aureus* (8-12 mm), *Pseudomonas* spp. (8-20 mm) and *E. coli* (8 to 12 mm). Further these isolates were inoculated in MRS broth supplemented with sugarcane juice, an excellent increase in antagonistic activity was observed against *E.coli* (12 to 30 mm), *Pseudomonas* spp. (8-15 mm) and *S.aureus* (12 to 13 mm). Jin *et al.* (1996) had earlier recorded the inhibition zones of between 2.5 mm and 18.5 mm. in the eight pathogens and concluded that *Lactobacillus brevis* C-17 isolated from caecum was the least effective in their inhibitory activity.

## REFERENCES

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