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Study the effects of seed germination and plant growth promoting activity of *Lactobacillus sp.*

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Abstract
Lactobacillus species are group of bacteria that are generally used to prepared probiotic foods and stimulate the growth of plants. In the present study, bacteria were isolated from curd on MRS agar medium. The isolates were identified by cultural, morphological and biochemical characterization. *Lactobacillus* isolates were found to be rod shaped, gram positive, non-motile. In case of seed germination on the growth of Bengal gram (*Cicer arietinum*), Peas (*Pisum sativum*) and Mustered seeds(*Bassica hirta*) crop plants, it was noted that enhanced growth rate was obtained; hence *Lactobacillus sp.* can be used as bioinoculant.

Keywords: *Lactobacillus*, seed germination, bioinoculant

1. Introduction
Lactic acid bacteria (LAB) include a large number of bacteria with lactic acid production capacity and have many industrial applications. They are Gram-Positive and non-motile organisms. They show Indole, Methyl-red and nitrate reduction tests as positive and Voges-Proskauer, Catalase and Oxidase production and Citrus-utilization tests as negative. They forms white colonies on MRS Agar plates and appear as non-spore forming *bacilli* or *cocci*, which yields lactic acid on carbohydrate fermentation. Due to their ability to withstand high acidic conditions, LAB extends their application in various products development under different conditions [1]. The LAB members of industrial importance are *Lactobacillus* and *Leuconostoc* (Food industry), *Carnobacterium* and *Pediococcus* (Bacteriocin production), *Aerococcus* and *Vagococcus* (Biopharmaceutical industry), *Weissella* and*Enterococcus* (Food and Health Industry), *Tetragenococcus* (Probiotics), *Streptococcus* and *lactococcus* (Diary industry). These organisms contribute to the production of several compounds which are essential for taste, smell, color and texture of various fermented products [2]. The characterization of LAB activities is of great importance to enhance their function at Industrial level. LAB produces a variety of antimicrobial compounds and effective substances such as lactic and acetic acids, probiotics, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide [3-4]. Strains that are used as probiotics for man have been isolated from the human gastrointestinal tract and usually belong to species of the genera *Lactobacillus* and *Bifidobacterium*. LAB plays an important role as preservatives in milk and milk products [5].
In the present study, *Lactobacillus* was isolated and characterized from the curd and study the different seed germination and pot trial were conducted to assess the potential activity of isolated *Lactobacillus* for improving the growth and yield of local crops.

2. Material and methods
2.1 Isolation of Lactobacillus spp.
Curd is the best source for *lactobacillus spp*. Among the other dairy products such as milk, buttermilk etc. Curd is taken in sterilized flask. Under the aseptic conditions curd was serially diluted from 10^{-2} to 10^{-5} from this dilutions 10^{-4}, are selected. Spread plate technique further with streak plate technique is done on MRS medium. They are incubated in incubator 37^0C which is optimum temperature for *Lactobacillus* broth. Incubation at 37^0C for 24 hrs. Broth after 24-48 hrs shown *Lactobacillus* species growth and these species for 24 hours. After the period of incubation the specific isolated colonies were grown. Colony characterization is done for this colonies found to be *Lactobacillus* species. One colony shows 100% resemblance with *Lactobacillus acidophilus*. The isolated colony formed on the MRS agar plates was identified using gram stain, biochemical tests. The identification was performed according to Bergey's manual of determinative of bacteriology. The culture was kept in MRS agar slant and stored at 4^0C for long term storage [6].

2.2 Biochemical characterization of the isolated bacterial strain
Identification of the isolated bacteria as *Lactobacillus* species was performed according to their morphological, cultural, and physiological and biochemical characteristics by the procedures as described in Bergey's Manual of Systematic Bacteriology [7]. The tests carried out were Gram staning, Capsul staning motility test, production of catalase, Indole, Methyl Red, Voges-Proskauer, Citrate, Starch Hydrolysis, endospore test, milk coagulation activities and NaCl and phenol tolerance test.

2.3 Effect of Rhizobium on seed germination
Different seeds namely Bengal gram (*Cicer arietinum*), Peas (*Pisum sativum*) and Mustered seeds(*Bassica hirta*) were

surface sterilized by 70% ethanol and then treated with 1% sodium hypochlorite for 2 min followed by repeated washing with sterile water. After this, the seeds were soaked in the *Lactobacillus* culture broth, while seeds which were soaked in normal nutrient broth kept as a control. Ten seeds of each treatment were kept equidistance in sterilized petriplates containing moist filter paper and the petriplates were incubated at 30°C. Seed germination and percent seedling emergence was calculated using following formula [8].

$$\% \text{ Emergence} = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sown}} \times 100$$

3. Results and Discussion

3.1 Cultural morphological and biochemical characters

Colonies of *Lactobacillus* were obtained on MRS agar after incubation for three days at 37°C (Figure1). The colonies were entire, opaque with wave margin, milky white, translucent, circular in shape, shiny, raised (convex), mucoid surface, flat elevation. These characteristics are similar with the standard characteristics of the *Lactobacillus* which indicates that the isolated microorganisms are *Lactobacillus* species. The isolated bacterium was aerobic, non spore forming, violet coloured gram positive rods and non motile. *Lactobacillus* showed negative chemical reaction for Indole, Methyl red, Voges-Proskaur, Catalase test, Starch Hydrolysis, Lactose Utilization and showed positive chemical reaction Milk Coagulation Assay, Phenol and Nacl test.(Table1.)

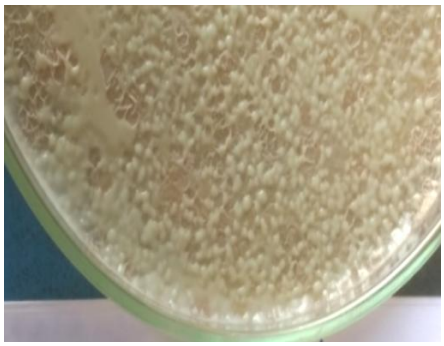

Fig 1: *Lactobacillus* colonies observed on MRS media.

Table 1: Biochemical characterization of the isolated bacterial strain.

Tests	Results
Catalase test	-ve
Indole	-ve
Methyl red	-ve
Voges-Proskauer	-ve
Citrate Utilization Test	+ve
Starch Hydrolysis	- ve
Casein Hydrolysis	-ve
Lactose Utilization	-ve
Phenol (0.4%) test	+ ve
4% Nacl test	+ ve
Milk Coagulation Assay	+ve

3.2 Effect of Rhizobium on seed germination

All the seeds showed maximum germination after 48 h. In comparison of the control (non-treated seeds) and test (seeds coated with *Lactobacillus* culture), highest seed germination was obtained in the test. The number and length of the sprouts were significant in the test as compared to the control (Table 2, 3 and Figure 2).

Table 2: Effect of *Lactobacillus* on the measurement the growth of seed germination.

Scientific names of seeds	Initial growth after 3 days(cm)	Final growth after 10days(cm)
Peas (*Pisum sativum*)	1.5	8.8
Bengal gram (*Cicer arietinum*)	1.6	8.5
Mustered seeds(*Bassica hirta*)	1.2	7.8

Table 3: Effect of *Lactobacillus* on seed germination

	% of seed germination	
Scientific names of seeds	Control	Test
Peas (*Pisum sativum*)	37.5	85
Bengal gram (*Cicer arietinum*)	40	88
Mustered seeds(*Bassica hirta*)	41	84

(a) Peas (*Pisum sativum*)

(b) Bengal gram (*Cicer arietinum*)

(c) Mustered seeds(*Bassica hirta*)

Fig 2: Effect of *Lactobacillus* on seed germination

4. Conclusion

Isolated bacterium was proved as *Lactobacillus* based on their cultural, morphological and biochemical characteristics. Seeds dressed with *Lactobacillus* and plant grown showed high seed germination and stimulatory growth over the control. These results summarized that *Lactobacillus* can be effectively used as a bioinoculant or biofertilizer to enhance the yield of crops.

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