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Isolation and Identification of *Brevibacillus lactosporum* From Soil and Evaluation of their Antibiotic Properties

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Abstract

Health promoting microorganisms such as probiotics are recently been used as food additive and therapeutic supplement especially as enhancer of prophylaxis and digestion. Here we report the isolation and identification of from soil and evaluation of their antibiotic properties. Morphological and biochemical test were done in order to establish the genus and species details. The species was found to be *Brevibacillus lactosporum*. The organism was also subjected to commonly available antibiotics for its antibiotic susceptibility for its probiotic credibility.

Keywords: probiotics, antibiotic properties, *Brevibacillus lactosporum.*

Introduction

The bacterium was discovered by Boyd J. O' Donnell in the late 1980's while he was visiting Iceland. He was intrigued by the health of the plants they grew without the fertilizers. Then he concluded with further studies revealed that the soil's growing power to be a species of bacteria that was later named *Bacillus Laterosporus*. *Brevibacillus* is a genus established in 1996 of aerobic, endospore- forming bacteria. This genus was derived by a genetic reclassification of strain previously allotted to the *Bacillus brevis* group. *Bacillus* species are commonly associated with soil and as such are isolated almost ubiquitously from soil, water, dust and air. Some strains produce crystalline inclusion of various shapes and sizes, which are released separately from spores during lysis of the sporangium.

Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. Among them, a bacterium is an important group. *Brevibacillus laterosporus* (Br. laterosporus) (Shida et al., 1996), formerly classified as Bacillus laterosporus (Laubach.1916) *Brevibacillus lactosporum* is a gram positive a bacterium belongs to the family Paenibacillaceae. It is an ubiquitous species that has been isolated from a wide range of materials including soil (Oliveira *et al*.,2004),gemstones (Khan,2001), lahar (Raymundo, 1997), fresh water (Laubach, 1916), sea water (Suslova *et al*., 2012), insect bodies (White, 1912), leaf surfaces (Roy *et al*., 2006), locust beans (Sarkar *et al*.,2002); compost (Adegunloye, *et al*., 2007), milk (Varadaraj., *et al* 1993), cheese (Román-Blanco *et al*.,1999), honey (Iurlina., 2005), starchy foods (Fangio., 2010), gelatin-factory effluents (Sharma., *et al*.1996),animal hide and wool (Chen,2012), quails (Kasmani.,2012).

It has the potential to be used as a biological controlling agent with a very wide spectrum of biological activities - including snails, nematodes and

insects. It has an ability to produce a canoe-shaped lamellar parasporal inclusion adjacent to the spore (Huang *et al*., 2005). In human beings, it survives the stomach acids condition. It does not have to be refrigerated so it will not spoil and grow molds in the body. Once introduced to the intestine the spores begin to germinate and multiply every 32 minutes. However, this is a soil based bacteria and like all soil based bacteria's. I believe it will not set up large long-term colonies of bacteria in the intestine. The effects are transitory and the colonies will begin to die within 3 days or so. Testing has shown it to be completely safe. It contains no dairy, gluten and animal substances. Ren *et al*., 2007 reported that *Brevibacillus lactosporum* represents a particularly resistant to probiotic microorganism, which theoretically should make it the ideal candidate for gastrointestinal passage and subsequent improvement of digestive functions. It is currently considered as a safe and beneficial bacterium for gut wellness, with a number of supplements promising meliorated patterns of food decomposition, bowel motility, and nutrient assimilation. It has shown that such bacteria accelerate the natural enzymatic activities of the body, thus ameliorating metabolic rates. This more regularized nutritional intake could then lead to a maximized form of weight management, as well as better cardiovascular and overall health in time.

Brevibacillus lactosporum is commonly called as that **antibacterial and immune support**. Commonly immune system depends up to 70-80% on the number of live probiotic cultures present in yourgut on a day-to-day basis. This phenomenon is owed to the antiseptic properties of probiotics, which target pathogens entering the organism and inhibit their development (through lactic acid, hydrogen peroxide, etc.). It is extremely important to have such an internal defense mechanism to rely on, especially since viruses and microbes are becoming increasingly more resistant to pharmaceutical grade drugs. *Brevibacillus lactosporum* has been proven to react as an inhibitor for various fungi and similar pathogens which could affect the digestive system and other parts of the body as well. Consequently, *Brevibacillus lactosporum* enhancement should boost your organism's natural immunomodulatory functions so as to combat such issues in the early stages. It extends its antibacterial properties beyond the abdominal area, with the respiratory system also benefiting from this probiotic's anti-germ capacities. The aim of this study was the isolation and identification of a strain of *Brevibacillus laterosporus* that exhibits an important antimycobacterial activity. Furthermore, this study aimed to determine the nature of the substance responsible for this activity and we can use for the probiotic production.

Methodology

Soil samples were collected from rose nursery in Coimbatore area. The sample was collected and sealed in fresh polythene bag, to avoid contamination and it was brought to laboratory. The collected sample was used for further isolation and confirmation.

Isolation of Bacteria: One gram of soil sample was added in 100ml distilled water and kept in rotatory shaker for about 30 mins. Then the sample was serially diluted. From the diluted sample, 100µl of each from 10^{-4} to 10^{-7} tubes were taken and were spreaded over the nutrient agar plates and incubated at 37°C for 12- 24hrs.

Morphological Characterization

Bacterial isolates were inoculated on Nutrient media and incubated for 12- 24hrs at 37°C. The colonies were observed under a high-power magnifying lens and colony morphology was noted with respect to shape, size, color and texture.

Biochemical Characterization

After preliminary studies, the isolates which were found to be positive were selected for biochemical studies. Biochemical tests generally used are gelatin hydrolysis, starch hydrolysis, citrate utilization test, indole test, methyl red test and Voges-Proskauer test, **[20}**.The confirmed colonies were given to bioline lab, Coimbatore to identify genus and species name of the sample.

Antibiotic sensitivity assay

The isolated and confirmed colonies were cultivated under agitation on nutrient medium at 37°C for 24 hrs. The assay was performed against the different antibiotics such as ampicillin, vancomycin, rifampicin, chloramphenicol, tetracycline, gentamycin, erythromycin, penicillin. The activity was evaluated using an agar well assay on pre seeded with the isolated colonies. The antibiotic discs were placed on the pre seeded plates and incubated for 48 hrs at 37°C. The formation of clear zones around the discs clearly showed the sensitivity of bacteria against the respective antibiotic (The diameter of zone of inhibition was observed in mm). Each test was repeated 3 times.

Results and Discussion

Bacteria have been intensively studied in several underexplored environment, niche and extreme habitat in various parts of the world (including India) in the last few years. Yet, there is not sufficient report regarding isolation of *Bacillus laterosporus* from garden soil, Coimbatore (India). Therefore, an attempt has been made to isolate the bacterial species from this unexplored region in order to find novel species. Totally nine bacterial strains were isolated from the soil sample. Based on the gram staining and colony morphology, all the isolates were found to be positive and negative in gram staining and had different morphological structures. The biochemical properties of bacterial isolates were recorded (Table1). Among the nine isolates, isolate 2, 4, 8 showed gram positive rod shaped bacteria. Isolate 2 showed MR, VP, Indole and citrate utilization test showed negative and starch hydrolysis and Gelatin test showed positive. Isolate 4 showed negative results in MR, VP, Indole, starch hydrolysis and gelatin test and citrate utilization test showed positive. Isolate 8 showed negative results in MR, VP, Indole, citrate utilization test and gelatin test and starch hydrolysis showed positive (fig1, 2, 3 and table1). Finally isolate 2, 4, 8 selected for genus and species identification. Isolate 2 showed *Brevibacillus lactosporum* and isolates 4 & 8 showed unknown culture. Isolate 2 were selected for commercial antibiotic test.

The clear zone around the disc shows the sensitivity of the organism to the antibiotic. Growth of the microorganisms shows the resistance of the organism to the given antibiotics. The bacterium *Brevibacillus lacterosporus* shows resistance against penicillin antibiotics and for other antibiotics it showed clear zone of inhibition. The maximum zone of inhibition was seen in gentamycin antibiotic and least antibiotic resistance seen in penicillin antibiotic. Result showed in (table 2, fig.4). However, penicillin susceptibility may be a better indicator of in vivo effectiveness for β-lactamase producers such as *Bacillus* spp (WEBER, et al.1988). Microbial resistance to antimicrobial agents is due to either (i) intrinsic properties (natural phenotypic traits) or (ii) the acquisition of resistance genes through mobile genetic elements, such as plasmids and transposons, or the mutation of indigenous genes. In conclusion, antibiotic susceptibility of this *Brevibacillus lacterosporus* is of a very wide range and sensitive to most of the common antibiotics which makes it very safe for the use as a probiotic in human therapy for lactose intolerance.

Table 1 Morphological and Biochemical Characteristics of *Brevibacillus Lactosporum*

Samples (Isolates)	Staining	Morphology	MR	VP	Indole	Citrate Utilization	Gelatin Test	Starch Hydrolysis Test
1	-VE	Coccus	-	-	-	+	-	+
2	**+VE**	**Rod**	**-**	**-**	**-**	**-**	**+**	**+**
3	-VE	Coccus	-	-	-	-	+	+
4	+VE	Rod	-	-	-	+	-	-
5	-VE	Coccus	-	-	-	+	-	+
6	-VE	Coccus	+	-	-	+	-	-
7	-VE	Coccus	+	-	+	-	+	-
8	+VE	Rod	-	-	-	-	-	+
9	-VE	Coccus	-	-	-	-	-	-

-ve: negative, **+ve:** positive

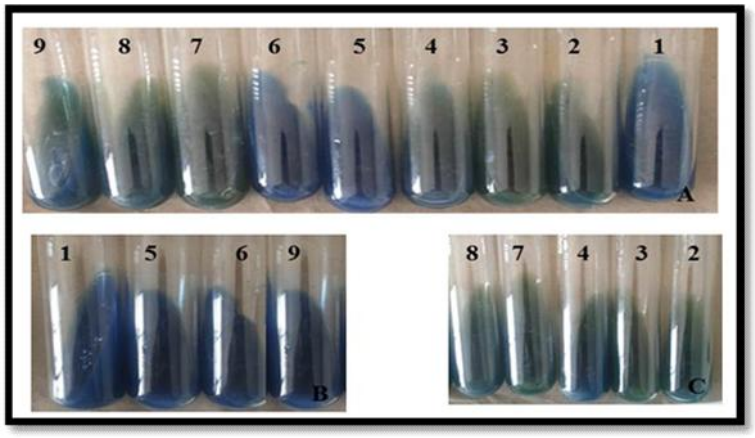
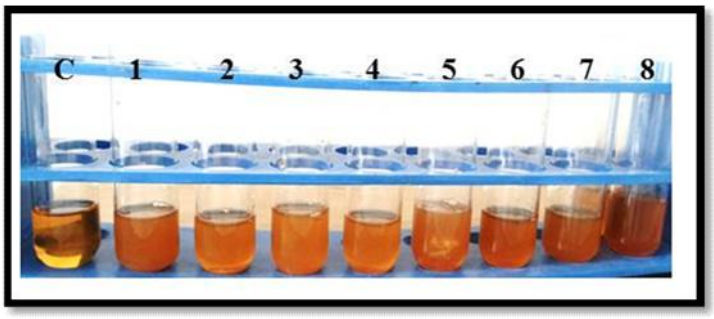


Fig. 1: Citrate Utilization Test

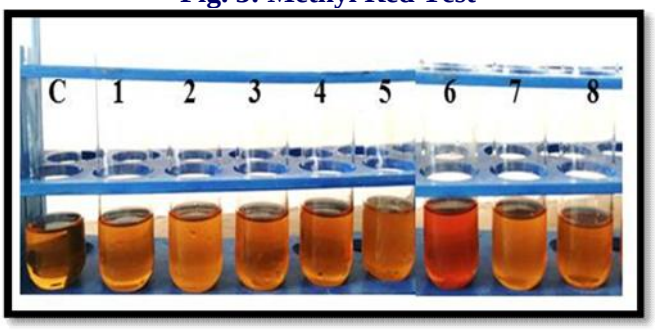
A-Isolate 1- 9, B-positive test (Green colour test tube changes into blue color after incubation), C- negative test (Green colour test tube dosen't changes into blue color after incubation)

Fig.2 : Voges Proskauer Test



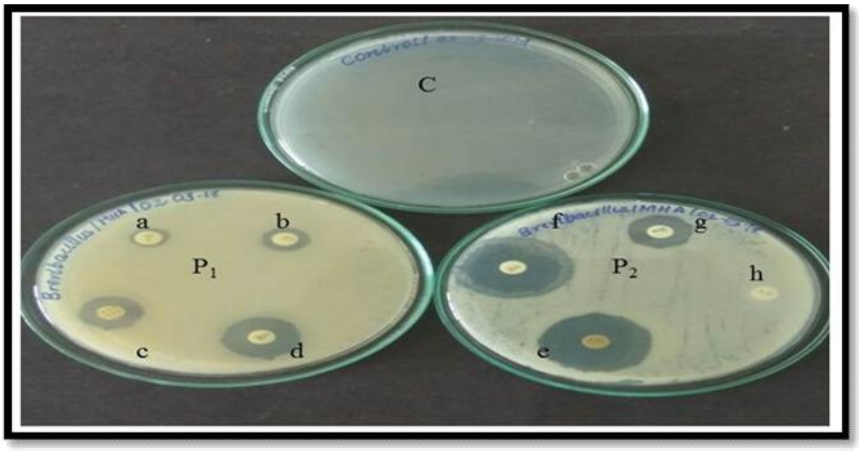
C-control; Isolates 1- 8

Fig. 3: Methyl Red Test



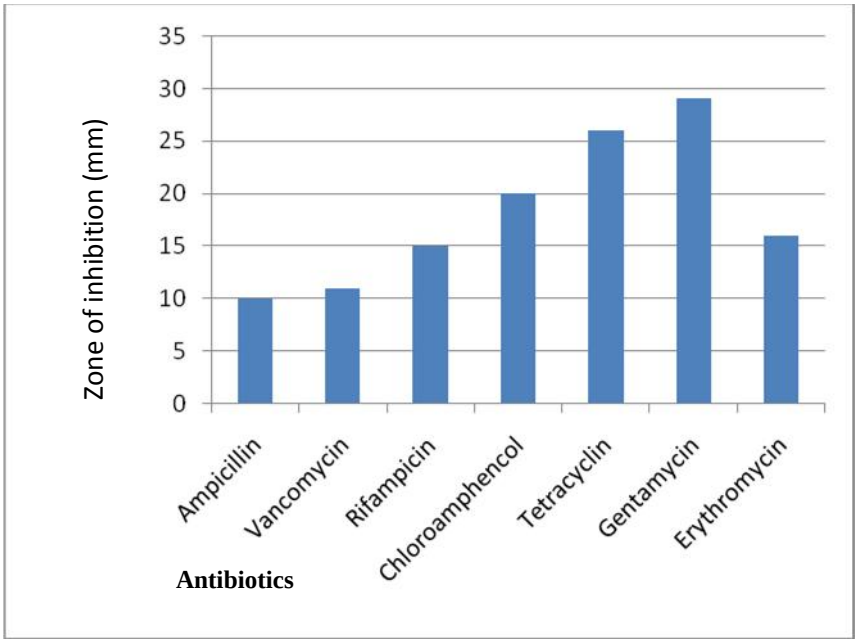
C-control; Isolates 1- 8

Fig. 4a: Antibacterial SusceptibilityTest For *Brevibacillus lacterosporus*



C-control; P1- Plate1; P2- Plate2;
a- Ampicillin; b-vancomycin; c-rifampicin; d- chloramphenicol; e- tetracycline; f- gentamycin; g- erythromycin; h- penicillin

B.



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