

Antibacterial Action of *Bacillus coagulans* Against Diverse Pathogens and Its Relevance as Probiotics

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Abstract

Probiotics are living organisms that give benefits to the host organism by improving the intestinal microbial balance. Probiotics have various beneficial effects as it maintains a healthy gut, healthy vaginal flora and boost the immune system. There are different strains of lactic acid bacteria that are used as probiotics but *Bacillus coagulans* is a good bacterium that is used in the form of probiotic supplements. In the present study, *B. coagulans* was isolated from the probiotic medicine named Sporlac-DS using De Man, Rogosa and Sharpe agar medium at 37°C for 24 h. Isolated strain was monitored for its morphological, biochemical, and physiological examinations under in vitro conditions. It was monitored that the strain was gram-positive, rod-shaped bacteria, and displayed high milk curdling activity. Biochemical analysis proved that the strain was catalase-positive and fermented glucose and lactose. The antibacterial activity of the isolated strain was further confirmed when its inhibitory potential was observed against test organisms like *Pseudomonas*, *Staphylococcus*, and *Escherichia coli* by well diffusion assay. Clear zones of 8 mm, 10 mm, and 7 mm diameter, respectively, were observed when *Pseudomonas*, *Staphylococcus*, and *E. coli* were spread on the plate, respectively. The reason for this inhibition may be the diverse antagonistic mechanisms working in synchrony viz. lactic acid + bacteriocins. Hence, the study recommends the usage of Sporlac tablets due to their antimicrobial action against diverse pathogens and boost the immune system. Currently, Sporlac is taken during constipation, irritable bowel syndrome, diarrhea, gas, indigestion, and many other conditions, but there is also evidence that intake of Sporlac helps in treating bacterial vaginosis in females.

Keywords: Probiotics, Sporlac, *Bacillus coagulans*, antibacterial, *Escherichia coli*, *Pseudomonas*, *Staphylococcus*

INTRODUCTION

Unhealthy lifestyle choices and chronic stress can significantly impact women's health (Emokpae and Brown 2021) [1]. That may increase the risk of various health problems, including stress, cardiovascular issues, diabetes, fertility complications, irregular menstrual cycles, mental health disorders, changes in the normal microflora, etc. [2]. The abrupt change in normal microbiota may result in hormonal imbalances and weakened immune systems that may lead to susceptible infections like urinary tract infections (UTI), bacterial vaginosis, etc. Moreover, these behaviors and stress can reduce women's life expectancy by increasing the likelihood of chronic diseases. Women need to prioritize self-care by adopting healthy habits like regular exercise, a balanced diet, effective stress management techniques, consistent

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medical check-ups, and the addition of probiotics to the diet. Probiotics are live microbial supplements that when administered in adequate quantity help in improving gut microbiota and exert beneficial impacts on human health [3]. *E. coli*, *Pseudomonas*, and *Staphylococcus* are generally known to be part of normal microflora, but in immuno-compromised hosts, they can behave like opportunistic pathogens causing UTI and bacterial vaginosis in females [4]. UTI is one of the most common infections that affect women, mainly caused by *Escherichia coli* (69%), *Pseudomonas aeruginosa* (4%), *Staphylococcus aureus* (2%), and others [5]. Bacterial vaginosis is a condition in which there is an overgrowth of pathogenic bacteria that leads to infection in the vaginal regions which causes an imbalance in vaginal pH, making the region prone to the infection [6]. Here, the role of health-friendly bacteria or probiotics comes. These bacteria help to provide good immunity, maintain intestinal microbial balance, and provide resistance to the female genital tract against infection-causing pathogens [7]. There are diverse components including organic acid, bacteriocins, hydrogen peroxide, etc. which inhibit the growth of pathogenic bacteria by interfering with the pathogen's cell wall [7]. One such probiotic bacteria is *Bacillus coagulans* which is present in Sporlac tablets [8]. The main aim of this study was to isolate these bacteria, characterize them, monitor their antimicrobial activity against diverse pathogens, and suggest their relevance as a probiotic.

MATERIAL AND METHODS

Bacterial Isolation and Growth Conditions

Lactobacillus was isolated from the probiotic tablet Sporlac-DS (Sanzyme, India) by serial dilution technique and was grown on MRS agar media at 37°C for 24 hr. and CFU (colony-forming unit) was calculated.

Morphological Test

The morphological examination was done based on simple and gram staining [9]. To check the absence or presence of a capsule, Nigrosin staining was done and endospore staining was done using malachite green dye.

Biochemical Test

To check the biochemical features, a catalase test was done by adding freshly prepared hydrogen peroxide to the fresh bacterial culture on the glass slide. Isolate was also analyzed by oxidase test using the oxidase disc method. The glucose and lactose fermenting capacity of the isolate was checked using glucose and lactose fermenting medium [10].

Physiological Test

Milk curdling activity of the isolated bacteria was checked by introducing the fresh culture of the bacteria into the 10 ml of milk sample at room temperature for about 24 hr. in a test tube and one sample in a test tube was kept as a control without bacterial inoculation.

Antibacterial Assay

The antibacterial assay of the bacteria was checked against diverse test organisms including *Pseudomonas*, *Staphylococcus*, and *Escherichia coli* by a well-differential method [11]. These test organisms were provided by the Department of Life Sciences, JC Bose University of Science and Technology YMCA Faridabad. Briefly, *Lactobacillus* was grown on MRS broth at 37°C for 24 hr. and centrifuged at 4000 rpm for 25 minutes. The cell-free culture supernatant (CFCS) of *Lactobacillus* was then poured into the wells and the diverse test organisms were spread all over the MRS agar plate. The plate was incubated at 37°C for 24 hrs.

RESULTS AND DISCUSSION

A serially diluted sample of Sporlac-DS was allowed to grow on MRS media. creamish, color colonies of *Bacillus coagulans* SNZ 1969 were observed and their CFU was calculated which was around 1×10^8 CFU/ml (Figure 1). As per the findings, one tablet of Sporlac contains 120 million spores



Figure 1. Colony of *Bacillus coagulans* on MRS agar media.

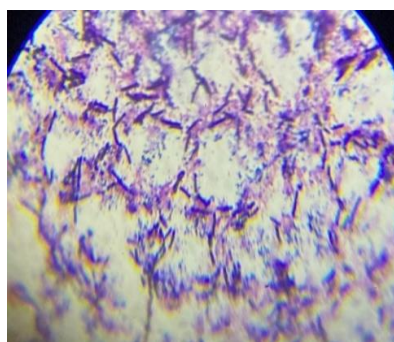


Figure 2. Gram-positive, rod-shaped bacteria.



Figure 3. Shows the catalase-positive test.

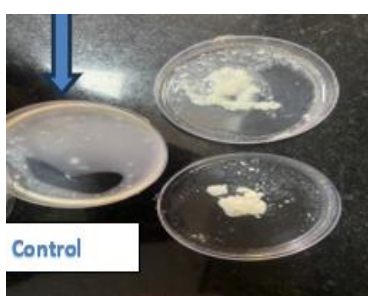


Figure 4. Milk curdling activity of the *Bacillus coagulans*.

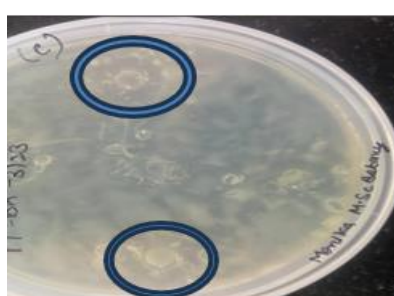


Figure 5. Zone of clearance near the well.

of *B. coagulans* SNZ 1969 (<https://www.sanzymebiologics.com>) [12]. The morphological features of *B. coagulans* were observed as gram-positive, rod-shaped (Figure 2), violet-colored bacteria, there was the presence of a thin fine capsule layer and an endospore. As per the findings, the results agree with the findings of the FDA where this strain was reported as gram-positive, catalase-positive, oxidase-positive, spore-forming, rod-shaped, slightly acidophilic, thermotolerant, aerobic to microaerophilic, highly resilient bacteria (<https://www.fda.gov/>) [13]. A similar study was also conducted by Ahmad et al. (2018) [14], in which the author monitored gram-positive, rod-shaped, capsulated features of bacteria. When a drop of hydrogen peroxide was added to the bacterial culture, there was the formation of bubbles which shows that the *B. coagulans* was catalase-positive (Figure 3), confirming it to be an aerobic organism. A similar finding was also monitored by Ahmad et al. (2018) [14]. In the physiological test, there was a positive milk curdling activity observed in comparison to the control tube suggesting it to be a lactic acid fermenting bacteria which even further confirmed its lactose fermenting capability.

The milk curdling activity of the bacteria indicates that the bacteria have a good fermenting activity (Figure 4) which can enhance the nutritional quality of food as well as maintain healthy gut health. Lactic acid causes coagulation and partial digestion of milk protein casein. Milk is changed into curd, yogurt, and cheese with the help of probiotic bacteria into probiotic products.

Antibacterial activity of the isolated strain was further confirmed when its inhibitory potential was observed against test organisms like *Pseudomonas*, *Staphylococcus*, and *E. coli*, with clear zones of 8 mm, 10 mm, and 7 mm diameter respectively (Figure 5). The reason for this inhibition may be the diverse antagonistic mechanisms working in synchrony viz lactic acid + bacteriocins. The acidic pH of lactic acid produced by the bacteria provides great resistance to the human gut from the growth of pathogenic bacteria and maintains an acidic pH to inhibit their further growth. The most common types of UTI are caused by *E. coli*, *Pseudomonas*, and *Staphylococcus* in females [4, 15, 16]. Nearly 11% of the females are associated with UTI and bacterial vaginosis [4]. To prevent these infections probiotic bacteria *B. coagulans* plays a very significant role. In the present study bacteria *B. coagulans* displayed

antibacterial activity against these test organisms which predominates in the vaginal and urogenital regions. As per the scientific findings *B. coagulans* play a significant role in improving the host digestive system, and immune system as well as preventing bacterial vaginosis [17]. Various types of components such as bacteriocins etc. are secreted through their metabolic activity by the bacteria which prevents the growth of pathogenic bacteria, provides various benefits to the host, and prevents infection.

The study was conducted in Indian premises on a group of 25 females between the age of 18–40 years. It was monitored that when these females were administered one tablet of Sporlac containing strains of *B. coagulans* SNZ 1969 in the vagina for a week, 64% showed complete symptomatic relief (<https://www.sanzymebiologics.com>) [12].

CONCLUSION

The study strongly recommends the usage of the probiotic tablet Sporlac for antagonizing the growth of harmful bacteria in the body, preventing vaginal infections, maintaining a healthy gut, and improving the immune system.

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