

Probiotic Efficiency of *Spirulina platensis*-Stimulating Growth of Lactic Acid Bacteria

Dola Bhowmik, Jaishree Dubey and Sandeep Mehra

Department of Botany, Labortary of Phycology,
Dr.H.S. Gour University, Sagar, M.P., India

Abstract: Viability and metabolic activities are important characteristics of probiotics microorganisms. They give rise to therapeutic benefits as well as increase in physiological activity of cultured products. The stimulatory effect of *Spirulina platensis* was studied on three lactic acid bacteria. The addition of dry biomass of *S. platensis* at various concentrations of 1mg, 5 mg, 10 mg/ml promoted growth of *Lactobacillus acidophilus* up to 171.67% and 185.84% respectively at pH 6.2. The growth of other strains were also enhanced. Simultaneously the antibacterial activity of *S. platensis* was done against three gram negative and three gram positive bacteria. A maximum activity was shown against *Proteus vulgaris*. Other pathogenic bacterial growth was also inhibited. The results show the probiotic efficiency of *S. platensis* for lactic acid bacteria and also a potent antibacterial activity against human pathogenic bacteria.

Key words: Probiotic microorganisms • Lactic acid bacteria • *Spirulina platensis* • Antibacterial activity

INTRODUCTION

Probiotic microorganisms are 'live microbial food supplement which beneficially affect the host animal by improving its microbial balance [1]. To observe a positive health effect of their consumption, a minimum level of live microorganisms is required. This level, depending upon the strains used and the required health effect, it is usually between 10^8 and 10^{11} cfu/g [2]. Yoghurt and other fermented milk contribute to health with natural nutrients and enrich the intestinal flora with lactic acid bacteria (LAB). Therefore, assuming a daily consumption of fermented dairy products of 100g, they should contain between 10^6 cfu/g to 10^9 cfu/g of these live bacteria at the time of consumption. Some strains produce certain health promoting metabolites including proteins and fattyacids which are desirable from a nutritional and/ or physiological perspective. However it should be emphasized that the ingestion of probiotic organisms opens up the possibility that these health promoting metabolites may also be produced in vivo [3].

The probiotic effect of these microorganisms include prevention of constipation in elderly people [4], preventing diarrhea [5], stimulation of immune system [6], lactose intolerance (Kim and Gillard, 1983 missing), reduction in cholesterol levels in blood [7] and prevention of cancer [8]. Apart from these therapeutic benefits,

probiotics also offer protection against many opportunistic human pathogens [9-10].

Spirulina, a cyanobacterium is a photoautotrophic microorganism, widely distributed in nature and is consumed as human food supplement for centuries because of its best known nutritional value. It contains 78% proteins [11], vitamins [12], 4-7% lipids [13], minerals [14], carbohydrates [15] and some natural pigments [16]. Due to the presence of these phytonutrients, it has corrective properties against several diseases like cancer, hypertension, hypercholesterolemia, diabetes, anaemia etc. Recently Parada *et al.* [17] reported the growth promotion effect of LAB by *S. platensis*.

The purpose of our study was to evaluate the effect of *S. platensis* biomass on the growth of LAB and on certain human pathogenic bacteria.

MATERIALS AND METHODS

Probiotic Activity

Cyanobacterial Biomass: *Spirulina platensis* was obtained from CCUBGA, IARI, New Delhi and was cultured in CFTRI media and kept in room temperature near north facing window in natural light. After 15 days, the culture was filtered and washed with acid water to remove salts. The biomass was dried at 30°C and made into powder.

Lactic Acid Bacteria: The lactic acid bacteria- *Lactobacillus casei* MTCC1423, *Lactobacillus acidophilus* MTCC447 and *Streptococcus thermophilus* MTCC1938 were obtained from IMTECH, Chandigarh, India and were maintained in MRS broth medium.

Microbiological Analysis: Loopful cultures of lactic acid bacteria were inoculated in 100 ml MRS broth medium having pH 6.8 for *L. casei* and *S. thermophilus* while pH 6.2 for *L. acidophilus*.

S. platensis suspension at concentration of 1, 5 and 10 mg/ml were prepared in sterilized distilled water and added into MRS broth medium containing bacterial cultures. These were then incubated at 37°C for 5 hrs. and 10 hrs. Controls were also prepared without the addition of *S. platensis*.

For growth measurements, bacterial suspension (0.5 ml) was poured on previously prepared MRS agar media and incubated at 37°C in anaerobic jars for 48 hrs. All the experiments were performed in triplicates. Growth kinetics was established by counting colonies on MRS agar medium and then results were expressed as cfu/ml.

Antibacterial Activity

Bacterial Species: Three gram positive-*Staphylococcus aureus* MTCC96, *Bacillus subtilis* MTCC619 and *Bacillus pumulis* MTCC1456 and three gram negative bacteria-*Escherichia coli* MTCC443, *Pseudomonas aeruginosa* MTCC424 and *Proteus vulgaris* MTCC 426 were obtained from IMTECH, Chandigarh. Muller Hinton broth and agar medium was used to maintain

bacterial culture. For inoculum preparation, bacterial cell suspension which equilibrated their concentration to a 0.5 of Mc Farland standard turbidity scale (10^5 - 10^6 cfu/ml for bacteria) was used.

Antibacterial Assay: 0.1ml of bacterial suspension was seeded on Muller Hinton agar media. Wells of diameter of 5mm were made on these media plates and filled with 100µl of *S. platensis* having concentration of 1, 5 and 10 mg/ml. Standard drug gentamycin (50µl/ml) was used. Distilled water was taken as control.

The results were recorded as mean diameter of the zone of growth inhibition surrounding the well and compared with that of standard drug.

Statistical Analysis: Values are expressed as mean \pm S.D. The statistical analysis was performed using ANOVA followed by Dunnett's multiple comparison tests in order to compare more than two groups. All the data were processed with instat version 2.1 software.

RESULTS

Effect on Growth of LAB: Lactic acid bacteria growth promotion was evaluated when they were incubated with *S. platensis* dry biomass. Data in Table 1 showed that the increase in growth percentage was 45.63 % after 5 hrs. and 63.93% after 10 hrs. when 1mg/ml *S. platensis* was added with *L. casei*. In the same concentration growth was promoted in *L. acidophilus* was 42.92 % in 5 hrs. and 85.84 % in 10 hrs. *S. thermophilus* was promoted 27.32%

Table1: Growth kinetics of lactic acid bacteria (cfu/ml) added with *S. platensis*

Concentration of Spirulina (mg/ml)	<i>Lactobacillus casei</i>			<i>Lactobacillus acidophilus</i>			<i>Streptococcus thermophilus</i>		
	0 hr.	5 hr.	10 hr.	0 hr.	5 hr.	10 hr.	0 hr.	5 hr.	10 hr.
01	3.66 \pm 0.5	5.0 \pm 0.8	6.5 \pm 0.8	2.3 \pm 0.7	3.33 \pm 0.8	4.33 \pm 0.4	3.66 \pm 0.73	4.66 \pm 0.35	5.33 \pm 0.7
05	3.66 \pm 0.5	6.33 \pm 0.4 ^{ns}	7.66 \pm 0.7 ^{ns}	2.3 \pm 0.7	4.36 \pm 0.6 ^{ns}	5.33 \pm 0.3*	3.66 \pm 0.73	5.33 \pm 0.50 ^{ns}	7.0 \pm 0.8*
10	3.66 \pm 0.5	7.0 \pm 0.6 ^{ns}	9.0 \pm 0.6**	2.3 \pm 0.7	6.33 \pm 0.3**	6.66 \pm 0.25**	3.66 \pm 0.73	7.0 \pm 0.55**	8.33 \pm 0.8 ^{ns}
Control	3.66 \pm 0.5	4.66 \pm 0.25 ^{ns}	6.0 \pm 0.25 ^{ns}	2.3 \pm 0.3	3.0 \pm 0.2 ^{ns}	4.0 \pm 0.3 ^{ns}	3.66 \pm 0.4	4.0 \pm 0.25 ^{ns}	4.66 \pm 0.25 ^{ns}

Values are given as mean \pm S.D for triplicates

Growth of bacteria are compared with control group, ns p>0.05, * p<0.05, ** p<0.01

Table 2: Diameter of zone of inhibition (mm) of bacteria against *S. platensis*

Bacteria	Diameter of zone of inhibition (mm)			
	1 mg/ml	5 mg/ml	10 mg/ml	Standard drug (50 µg/ml)
<i>Escherichia coli</i>	3.23 \pm 0.15	6.0 \pm 0.20	7.95 \pm 0.20	18.8 \pm 0.10
<i>Pseudomonas aeruginosa</i>	3.13 \pm 0.18	5.8 \pm 0.20	7.63 \pm 0.33	18.6 \pm 0.15
<i>Proteus vulgaris</i>	3.56 \pm 0.20	6.4 \pm 0.21	10.33 \pm 0.36	22.1 \pm 0.15
<i>Staphylococcus aureus</i>	3.3 \pm 0.20	4.13 \pm 0.20	5.30 \pm 0.33	7.76 \pm 0.10
<i>Bacillus subtilis</i>	4.76 \pm 0.18	5.33 \pm 0.36	8.36 \pm 0.36	23.43 \pm 0.18
<i>Bacillus pumulis</i>	4.8 \pm 0.15	5.93 \pm 0.21	8.6 \pm 0.24	23.63 \pm 0.10

Values are given as mean \pm S.D for six replicates in each group.

and 45.63% after 5 hrs. and 10 hrs. of incubation respectively. Maximum growth was promoted at 10 mg/ml concentration of *S. platensis* up to 10 hrs. 145.90%, 171.67% and 185.84% growth was observed in *L. casei*, *L. acidophilus* and *S. thermophilus* respectively.

Effect on Pathogenic Bacteria: The antibacterial activity of *S. platensis* was seen on some pathogenic bacteria. Table 2 shows the diameter of zone of inhibition of *S. platensis* against six bacteria. The maximum activity was obtained at a concentration of 10 mg/ml. Among all bacteria, *P. vulgaris* was found to be more susceptible as the diameter of zone of inhibition was 10.33 mm and the bacteria which was least affected was *S. auerus* whose diameter of zone of inhibition was 5.3 mm at 10 mg/ml concentration of *S. platensis*.

DISCUSSION

Lactobacillus, *Streptococcus* and *Bifidobacteria* species have received attention as good probiotic organisms that can maintain balance between the beneficial and harmful pathogenic microorganisms in the gastrointestinal tract and also associated with health promoting effects. Our study reveals that the growth of lactic acid bacteria was enhanced in the presence of *Spirulina*. Similar results were also demonstrated by [18]. Among three lactic acid bacteria, *S. thermophilus* showed minimum growth at 10mg/ml concentration of *S. platensis*. Varga *et al.*, [19] also found that *S. thermophilus* were most numerous components and their count exceeded the value of 10^9 cfu/ml in the fermented milk at the beginning of storage time.

Spirulina biomass has stimulatory effect on the growth of coccus shaped starter bacteria. Parade *et al.*, found that addition of extra cellular products obtained from a late log phase culture of *S. platensis* promoted the growth of some lactic acid bacteria. On this account it was suggested that *S. platensis* could have a stimulatory effect on lactic acid bacteria by acting as probiotic substance [20]. The *bifidobacterial* count were 0.2 log cycles higher containing *Spirulina* biomass than did the control at the time of storage. One more reason for the survival of lactic acid bacteria in the *Spirulina* added culture was that *Spirulina* biomass had a stimulatory effect on the acid development. Thus pH of *Spirulina* supplemented product was lower and this reason favoured the growth of lactic acid bacteria.

Spirulina biomass also inhibited the growth of harmful pathogenic microorganisms. From our investigation, it could be concluded that *S. platensis* inhibited the growth of certain human opportunistic pathogens up to some extent. *P. vulgaris* was more susceptible bacteria found in our study. Ozdemir *et al.*, [21] have reported potent antibacterial activity of *S. platensis*. A maximum zone of inhibition was found in methanol extract at 8 mg/ml in *Staphylococcus epidermidis* while *Staphylococcus aureus* was the most resistant strain. In our study, the most resistant species was also the same bacteria.

Apart from *Spirulina* species, some other cyanobacteria were also reported to show antibacterial properties. Bloor and England [22] studied antimicrobial effects of *Nostoc*, Fish and Codd 1994) reported *Phormidium*, *Anabaena*, *Oscillatoria*, *Synechocystis* were studied by Kreitlow *et al.*, [23] while *Oscillatoria angustissima* and *Calothrix* extracts were studied by Issa [24]. They have also reported that the extracts of extracted in different solvents were effective against both gram negative and gram positive organisms. Zornitza *et al.*, [25] had shown that a broad spectrum antimicrobial antibiotic is produced by *Nostoc* sp. that inhibits the growth of bacteria, notably multiresistant *S. aureus* and *P. aeruginosa*. This is in agreement with our findings, since *S. platensis* exhibited similar effects on both types of organisms used in this study.

Antimicrobial activity shown by certain cyanobacteria is because they produce certain biologically active substance that are intracellular or extra cellular secondary metabolites having diverse biological activity. However, temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing antimicrobial agent production.

The results obtained showed a spectrum of antibacterial properties of *S. platensis* apart from increasing the growth of lactic acid bacteria. In addition to the above benefits, *Spirulina* biomass increases the essential amino acids and vitamin content. So the regular intake of *Spirulina* will not only improve the intestinal lactic acid bacteria but also inhibit the growth of harmful human pathogenic finally leading to the improved intestinal absorption. The abundance of bioactive components in *S. platensis* is of great importance from a nutritional point of view because it provides a new opportunity for the use of *Spirulina* as a perfect nutraceutical.

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