

Original Article

**Immunomodulatory effect of *S. thermophilus* :
An experimental study**

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Abstract

“The most promising development in the area of functional foods has been the use of probiotics & their role in human health and disease. Much of the research work in probiotics has been focused on the gastrointestinal tract. One of these putative effects is the alteration of immune function. Studies reveal that the probiotics have a positive effect on the gut immunity. But hardly any work has been carried out on the effect of probiotics on the systemic immunity. Moreover this effect varies with not only the genera and species of the probiotic but also with type of probiotic. It has been shown in the literature that only the live probiotics are capable of showing an effect. In the present study experiments were carried out to see the effect of probiotics *Streptococcus thermophilus* (*S. thermophilus*) both live and dead on systemic immune response. Results reveal that *S. thermophilus* feeding, both in dead and alive form, could modify both the humoral and cell mediated immune response, however, the effect was more pronounced with the live form.”

Keywords: Immune response (IR), Immunological tests, Probiotics, *S. thermophilus*

Introduction:

The idea of using microbes to promote a good health and to prevent disease is not new. The first study regarding beneficial effect of probiotics was carried out in the early 1900's (Metchnikoff, 1908) who reported the favorable effects of soured milk in human. Since then, microbial probiotics have gained an increasing interest and their use is now widely accepted due to their beneficial health effects (Salminen *et al.*, 1998) and their GRAS status. Probiotics have been used as growth promoters, for lactose intolerance, antitumor and anticholesterolaemic effects, heart disease, allergic reactions, cancer, diarrhea etc. (Suvarna and Boby, 2005; Christensen *et al.*, 2006). Several microorganisms, under the name of probiotic have been purposed and used in a wide range of clinical trials, ranging from diarrheal disease to cancer prevention (Fuller, 1994; Kaur *et al.*, 2001). Numerous studies in animals and to a lesser extent in humans have investigated the immunomodulatory effects of probiotics (Monika Roller *et al.*, 2007). However, much of the research work in probiotics has been focused on the gastrointestinal tract. Studies reveal that the probiotics have a positive effect on the gut immunity. Moreover this effect varies with the type of probiotic. On the other hand the literature reveals that many diseases are related to immune response of the host. Keeping in view the facts that

- (i) Probiotic can alter the gut immune response.
- (ii) The effect on the immune response by the probiotics varies with the type of probiotics.
- (iii) Need of most acceptable immunomodulators as alternative therapeutic agents.
- (iv) Food being the most potent and acceptable alternative therapeutic agent.

The present study was focused to find out experimentally the effect of probiotics *S. thermophilus* on systemic immune response because *S. thermophilus* (starter organism of yoghurt) has been least exploited for its immunomodulatory potential. The most exploited probiotics are those belonging to the genera *Lactobacillus* and *Bifidobacterium*.

Materials and methods

Ethical treatment of animals

All procedures (Animal handling, anesthesia, surgical interventions, and post-surgical care studies) using animals were approved by the institutional IACUC (Institutional Animal Care and Research Advisory Committee) Committee.

Experiment no.1:

Animals & groups

Swiss albino mice (weighing 20-25 gm, aged 4-6 weeks) were used for the experiments. After one week of acclimatization, the mice were divided into groups, each containing 10 animals. Control group was maintained on normal diet.

The mice were divided into 3 groups

Group- I: (n= 10) Normal diet fed

Group- II: (n= 10) Normal diet fed + live *S. thermophilus* (LST)

Group- III: (n= 10) Normal diet fed + dead *S. thermophilus* (DST)

Preparation of bacterial feed

S. thermophilus grown in broth were harvested by centrifuging the culture at 2600 rpm for 15 minutes at 4°C and washing with sterile normal saline. Cell count was done and the animals were fed @ 1×10^9 cells/mouse/day for 15 consecutive days.

Preparation of dead bacterial feed

Harvested *S. thermophilus* from the broth was washed with normal saline and proximate cell count was done. The suspension was heated for 45 min at 90 °C, cooled & viability was checked. A suspension consisting of 100% non-viability was given orally @ 10^9 killed cells/animal/day for 15 days.

Groups and feeding

Mice were divided into three groups, live *S. thermophilus* and dead *S. thermophilus* was administered orally in different groups daily for 15 days to group II and III respectively. Control group received only normal diet. All groups were immunized with BSA.

Follow up: The blood samples of the animals were collected on day 3rd, 7th and 11th of immunization and centrifuged to separate the serum for humoral immune response.

After completing the diet schedule the mice were anaesthetized and sacrificed and their spleen was taken for different cell mediated immunological tests like Nitroblue Tetrazolium Reduction (NBT), inducible Nitric Oxide Synthase (iNO'S), Phagocytic activity of leukocytes (Phagocytosis), Delayed Type of Hypersensitivity (DTH), Enzyme Linked Immunosorbent Assay (ELISA).

Immunological tests employed

Nitroblue Tetrazolium Reduction (NBT)

NBT reduction test, a measure of respiratory burst in the leucocytes was carried out by method given in the practical book by Hudson & Hay 1989. Briefly the splenocytes were incubated with NBT and extracting it in dioxan and taking the reading at 520 nm using dioxan as blank measured the formozone formed.

Inducible Nitric Oxide Synthase (iNO'S)

The iNO'S activity of leukocytes was assessed spectrophotometrically by using arginine by employing the method mentioned by Bhatia and Jha. 2000. Briefly the splenocytes were incubated with arginine at 37°C for 24 hours in CO₂ chamber. The citruline formation from arginine was read by taking O.D. at 540 nm using MEM + GRIESS reagent as (1:1) blank.

Phagocytic activity of leukocytes

The macrophages function was assessed by phagocytosis of microorganism using the method given by Raghuramulu, 1983. In short splenocytes and *E.coli* were incubated for 1 hour at 37°C followed by the suspension plated on the nutrient agar plate and checking the growth of bacteria, After 24 hour's colonies were counted on each plate. Only *E.coli*. Spread plate was taken as control.

% Phagocytosis = (Bacterial cell count in control- Bacterial cell count in test) × 100 Bacterial cell count in control.

Delayed Type of Hypersensitivity (DTH)

The delayed type of hypersensitivity a measure of the CMI was assessed by the footpad swelling method given by Hudson & Hay 1989. The swelling in foot pad was noticed after 24-48 hrs in both the foot pads after carrigenan induced in the right hind foot-pad and normal saline in the left hind foot- pad. The swelling in the foot was measured with micro vernier calliper at 0, 24, and 48 h after the challenge. The difference in paw thickness as compared to control was taken as a measure of DTH.

ELISA in various groups are given in Fig.5. The results show that the antibody titre of animals fed on probiotic was about 4 times higher in live fed *S. thermophilus* and 2 times higher in dead fed *S. thermophilus* as compared to the control group (Figs.1-5).*(given below the references)*

Enzyme Linked Immunosorbent Assay (ELISA)

Development of the anti BSA albumin antibodies was observed by ELISA test, following the method given in the practical manual of Hudson and Hay 1989 using Horse Raddish Peroxidase (HRP)

Discussion

The present study was carried out to evaluate the health effects of orally delivered probiotics (live and dead *S. thermophilus*) in swiss albino mice with major focus on its effect on immune response. Immune response was assessed by various methods as NBT reduction by splenocytes, Inducible Nitric Oxide Synthase expression, and phagocytosis, DTH and ELISA were the tests employed to assess the cell-mediated and humoral immune response of the animals, respectively. The results revealed that NBT, iNOS, bactericidal activity of splenocytes, DTH as well as the antibody titers were higher in live and dead *S. thermophilus* fed group as compared to control group fed on normal diet, which show potentiating effect of live and dead *S. thermophilus* on immune response.

Results

The NBT, iNOS, phagocytosis and DTH were higher in group fed on live as well as dead *S. thermophilus* as compared to the control group. The results are depicted in Fig. 1- 4. The anti-BSA antibody titers as determined by

The augmentation of the immune response by the live strain of probiotics has earlier been observed using one or the other immunological tests (Herich *et al.*, 1999). But in our studies we

observed that even the dead bacteria also could modulate the immune response. Bloksma *et al.*, 1979 showed that a viable strain of *Lactobacillus plantarum* applied intraperitoneally to mice stimulated the delayed type of hypersensitivity. In another study, intraperitoneally applied *Lactobacillus casei* activated macrophage (Phagocytic capacity and enzymatic activity) and natural killer cells (Kato *et al.*, 1984). Several studies have demonstrated the beneficial effects of lactic acid bacteria in boosting a nonspecific immune response. Probiotic bacteria have been shown to influence immune responses nonspecifically by enhancing phagocytosis of pathogens as well as modifying cytokine production. In one study, a strain of *L. acidophilus* isolated from human newborn was inoculated into germ-free and conventional mice, and phagocytosis of *E.coli* was assessed *in vivo* (Neumann *et al.*, 1998). The mono association of germ free mice with these lactic acid bacteria for 7 days improved macrophage phagocytosis capacity as demonstrated by the clearance of *E.coli* inoculated intravenously. Potential use of *L. casei* Shirota has been described as probiotic agent for stimulating immune responses, preventing enterobacterial infections (Matsuzaki, 1998). Besides cell mediated immunity the increase titer of antibody in our study reveal enhancement in the production of antibody by both the live and dead bacteria. This shows that *S. thermophilus* could modify

both the humoral and cell mediated immune response. Similar to our studies Saito *et al.*, 1983 observed that subcutaneous inoculation of *Lactobacillus casei* stimulated the production of specific antibodies against *Pseudomonas* antigens by increasing the circulating IgM antibodies.

It is concluded that the fortification of the live or dead *S. thermophilus* in diet may augment the immune response of the body. Our study is of special importance because the results that the dead bacteria could also modify the immune response means that the dead bacteria containing diet is absolutely safe and will not be changing the micro flora of the intestine and hence can be given to the immunosuppressed host too.

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Author's declaration

This is to certify that the article entitled "Immunomodulatory effect of *S. thermophilus* : An experimental study " has not been submitted elsewhere for publication.

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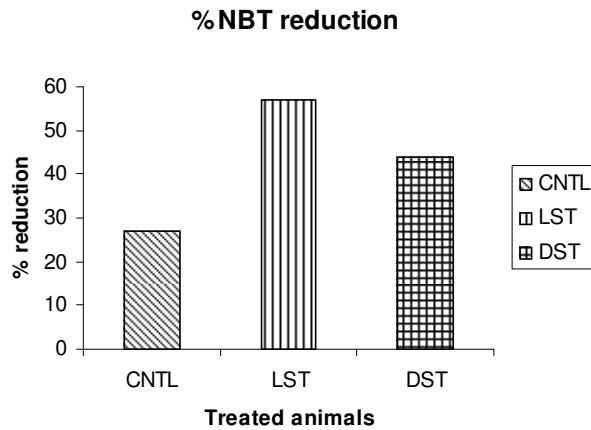


Fig 1: Effect of *L.S.thermophilus* (LST) and *D.S.thermophilus* (DST) on NBT reduction in Swiss albino mice

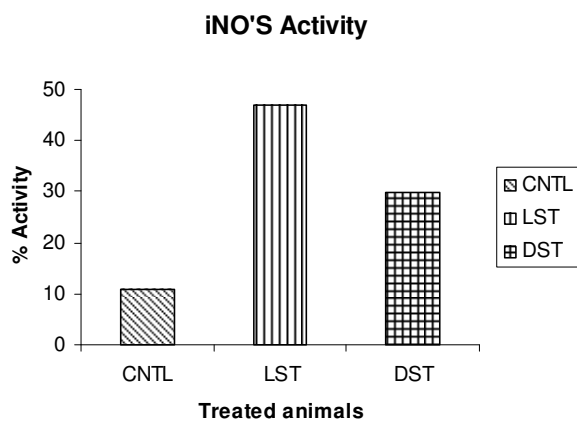


Fig 2: Effect of *L.S.thermophilus* (LST) and *D.S.thermophilus* (DST) on iNO'S activity in Swiss albino mice.

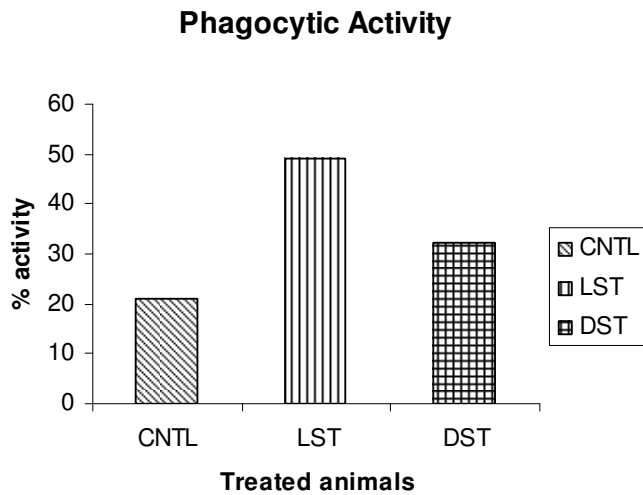


Fig 3: Effect of *L.S.thermophilus* (LST) and *D.S.thermophilus* (DST) on Phagocytic activity in Swiss albino mice.

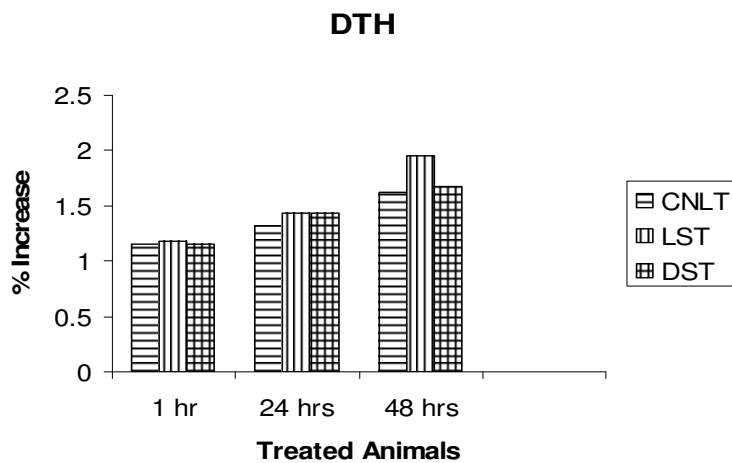


Fig 4: Effect of *L.S.thermophilus* (LST) and *D.S.thermophilus* (DST) on DTH in Swiss albino mice.

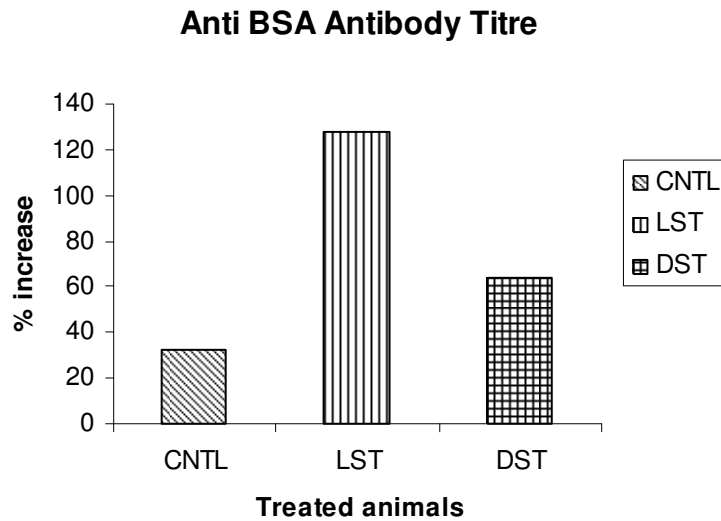


Fig 5: Effect of *L. S. thermophilus* (LST) and *D. S. thermophilus* (DST) on Anti BSA Antibody titre in Swiss albino mice.

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