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Immunomodulatory effect of probiotic strain *Bacillus subtilis* MBTU PBBMI spores in Balb/C Mice

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***Abstract*— *In vitro* studies revealed the probiotic potential of MBTU PBBMI which was previously named as BM-3 (Anu *et al.*, 2012 [1,2]. The present study investigated the *in vivo* effects of 30 days consumption of MBTU PBBM1 on immune response in Balb/c mice . Serum IgA and IgG levels of spore fed mice were found to be significantly higher in comparison with the control group and was in a dose dependent manner.MBTU PBBMI spores in mice showed an increased significant response in antibody production against SRBC. MBTU PBBMI treatment in mice clearly indicates that it can stimulate spleen cell proliferation in response to Concanavalin A. Delayed type hypersensitivity studies revealed that MBTU PBBM1 can significantly enhance cell mediated immunity. Dose dependent studies revealed that 1x10^8 spores was an effective dose to stimulate both humoral and cell mediated immune response in balb/c mice**

***Index Terms*— *Bacillus subtilis* MBTU PBBMI , Spores, Probiotic,Immunomodulation**

I. INTRODUCTION

The gastrointestinal tracts of healthy animals, are colonized by complex microflora containing different species. A balance of these microorganisms in the gastrointestinal tract is important in maintaining immunity of the host. Immunomodulative capacity is considered to be an important mechanism to support probiosis. To evaluate probiotic effects on the immune system, it is very important to study the probiotic induced effects in animal models that mimic the physiological reality. *In vitro* probiotic studies identified MBTU PBBM1 isolated from milk, had strong probiotic properties such as acid, bile tolerance, non haemolysis, lecthinase negative, resistance in artificial gastric and intestinal fluid and antagonism to enteric pathogens such as *Salmonella typhi, Salmonella paratyphi A*, and *Vibrio cholerae*. [1,2]The present study investigated the *in vivo* effects of 30 days consumption of MBTU PBBM1 spores on immune response in Balb/c mice.

II. PREPARATION OF SAMPLE FOR *IN VIVO* STUDY

Preparation of spore sample

Spores of MBTU PBBM1 were cultured in Difco Sporulation medium for 48 hrs at 37^{o}C. Culture was centrifuged at 1500 g for 10 min at 4^{0}C and washed three times with PBS and resuspended in sterile PBS. Two dilutions of bacterial suspension giving a colony forming unit of 10 ^{8} spores and 10 ^{4} spores was prepared separately in 0.1ml PBS.

Experimental design for immunomodulatory studies

Mice were assigned into 3 groups, which included two experimental groups and one control group. Each group comprised of six mice. Among the three groups, group I received 10^8 spores, group II received 10^4 spores of MBTU PBBM1and group III the control group received 0.1 ml of sterile PBS alone. All experimental treatments were via intragastric gavage. Spore treatments lasted for 30 days and the 31st day mice of all groups were sacrificed for further laboratory analysis.

Humoral Immune Response

Serum collection

After the end of feeding period, and 8 hr of fasting, the animals of all groups were immediately exsanguinated by retro-orbital venous plexus puncture and sera were collected.

Enzyme linked immunoabsorbent assay for immunoglobulins.

Quantification of serum Immunoglobulins

Serum antibody levels of all groups under study were assayed according to the procedure on Quantitation Kit (Mouse Ig G ELISA kit catalog number: E-90G, Mouse Ig M ELISA kit catalog number: E-90M, Mouse Ig A ELISA kit catalog number: E-90A, Mouse Ig E ELISA kit catalog number: E-90E CRL Laboratories)

Haemagglutinin antibody titer

Preparation of SRBC

Fresh sheep blood was collected from the local slaughter house in vials under sterile conditions in sterile freshly prepared Alsevere's solution in a 1:1 proportion. Blood was kept in refrigerator and processed for the preparation of SRBCs batch, by centrifugation at 2000 rpm for 10 minutes and washing with PBS (10 mM sodium phosphate buffer containing 150 mM NaCl, pH – 7.4) 4-5 times and then suspending into buffered saline for further use and finally adjusting to a concentration of 1×10^8 cells/ml for immunization and challenge.

Assessment of antibody titer

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To determine the serum antibody response to SRBC, a direct hemagglutination technique was used according to Puri *et al.*, 1994 [3]The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer.

Cell mediated immune responses.

Delayed type Hypersensitivity

Followed by the spore treatment for 30 days, animals in all groups including control were immunized by intra peritoneal administration of 0.1 ml of $1x10^8$ SRBC/mouse.On the day 1 (24 hr after immunization), animals in all the groups were challenged by subcutaneous administration of 0.2 ml of 1% SRBC into the right hind foot pad ,while a 0.2 ml of PBS was administered in the left hind foot pad to serve as control. DTH response was measured at the 48 hr after the immunization and expressed as percent increase in foot pad swelling.(FPS). The inflammation percentage was calculated as per Manosroi *et al*. 2005[4]

Splenic lymphocyte Proliferation

Proliferation of spleen cells was measured by methyl thiazolyl tetrazolium (MTT) incorporation as described by Bujalance *et al.*, 2009.[5] Splenocytes from mice were isolated according to Shalini *et al., 2009*.[6]

Results

Serum Immunoglobulins levels

Serum IgA and IgG levels of spore fed mice were found to be significantly higher in comparison with the control group(P<0.05*)Increase in serum immunoglobulin Ig A and Ig G in spore treated groups was in a dose dependent manner (P< 0.05*). See Table:1.1. Comparison of serum immunoglobulins revealed that there is no marked variations in IgE and Ig M between spore treated groups and control (P> 0.05*).

Haemagglutinin antibody titer

Spore treated groups showed an increased response in antibodies against SRBC in a dose dependent manner. Haemagglutination of antibody titers against SRBC is given in Table 1

Name of Group	Serum Immunoglobulins µg/ml				Haemag-glutinin Titer Mean ±SD
	Ig G	Ig A	Ig M	Ig E	
Group I (received 10^8 spores)	820±120	680±114	189±2	1.12±.4	288±161*
Group II (received 10^4 spores)	640±164	595±108	188±1.8	1.19±.08	144±80.5*
Group III (Normal control)	620±88	525±74	186±1.6	1.24±.2	16±4.3*

Delayed type Hypersensitivity

Group I animals (which received 1 x 10^8spores) and Group II animals (which receives 1 x 10^4 spores showed significantly enhanced DTH ($p< 0.05$) than untreated controls group. Among the studied groups, group I animals showed significant increased DTH than the group II animals. The DTH responses of mice of various treated and untreated controls are shown in Figure I.I

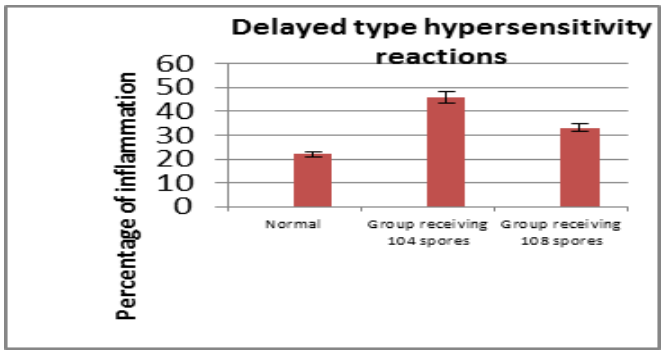

Figure I.I:Delayed type Hypersensitivity(DTH)

Splenic lymphocyte Proliferation.

Mice treated with MBTU PBBM1 spores showed significantly higher splenic lymphocyte proliferations than the untreated control group *(P*< 0.05). The responses in group I (which received 1 x 10^8spores) and group II (which receives 1 x 10^4spores) animals were found to be dose dependent. (See figure I.II).

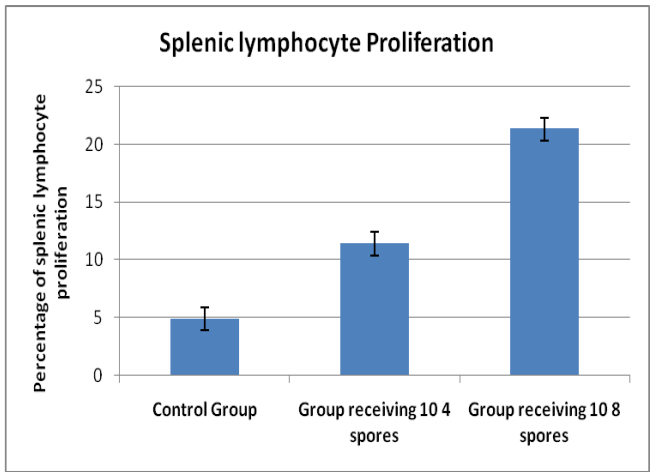

Figure I.II: Splenic lymphocyte Proliferation

III. DISCUSSION

Stimulation of the immune system, or immunomodulation, is considered an important mechanism to support probiosis. A number of studies in humans and animal models have provided strong evidence that oral administration of *Bacillus* spores stimulates the immune system.. After 30 days continuous ingestion of MBTU PBBM1 spores, a significant response in serum antibodies Ig G and Ig A clearly indicates the ability of the strain in inducing humoral response. The enhancement of antibody responsiveness to SRBC in mice, may be due to the enhanced responsiveness of macrophages

and B. lymphocyte subsets involved in the antibody synthesis (Benacerraf, 1978)[7]. Therefore, the augmentation of the antibody production response confirmed the enhancement of humoral immune response which was based on dose of spores consumed. DTH reactions in treated groups was found to be dose dependent and the group I animals showed increased DTH than the other groups. As expected, in the positive result, the increased percentage of inflammation in treated groups may be due to the proliferation of the T lymphocytes and thus results indicates that spore enhanced the cell-mediated immunologic function. MBTU PBBM1 spores treated mice, slightly induced the spleen cell proliferation in response to Conconavalin A in a dose dependent manner, Exact component involved in enhancing function of MBTU PBBM1 spores on lymphocytes proliferation has not yet understood. Indeed, increased T-cell proliferation may act as a wash-out mechanism for pathogenic microbial agents. Above results supports that MBTU PBBM1 spores have the potential to improve both the humoral and cellular immunity.

IV. CONCLUSION

Immunomodulatory studies on MBTU PBBM1 in Balb/c mice for 30 days proved that the strain in a dose of 10^8 spores has a significant role in improving both humoral and cell mediated immunity

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