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Synbiotic effect of *Lactobacillus helveticus* M92 and prebiotics on the intestinal microflora and immune system of mice

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The synbiotic effect of the oral treatment of Swiss albino mice with milk-based diets supplemented with *Lactobacillus helveticus* M92 and various kinds of prebiotics was investigated. Survival, competition, adhesion and colonization, as well as, immunomodulating capability of *Lb. helveticus* M92, in synbiotic combination, in the gastrointestinal tract (GIT) of mice, were monitored. After the mice were fed with synbiotics, the lactic acid bacteria (LAB) counts in faeces were increased and reduction of enterobacteria and sulphite-reducing clostridia was observed. Similar results were obtained in homogenates of small and large intestine of mice on the 1st and 14th day, after feeding with synbiotics. After the mice were orally given viable *Lb. helveticus* M92 cells, alone or in combination with prebiotic, the concentration of faecal SIgA and total serum IgA antibodies from all immunized mice were higher compared with the control. The specific humoral immune response was not evoked after oral administration, therefore their synbiotic application is suitable. Among inulin, lactulose and raffinose, *Lb. helveticus* M92 in combination with inulin, has shown the best synbiotic effect on intestinal and faecal microflora and immune system of mice.

Keywords: *Lactobacillus helveticus*, intestinal microflora, probiotic, prebiotic, synbiotic, mice.

Abbreviations: GIT, gastrointestinal tract; LAB, lactic acid bacteria.

Currently great attention is dedicated to probiotics, prebiotics or their combined use as synbiotics, to improve human health in natural ways (Šušković et al. 2001). Intestinal microflora plays a prime role in health. Therefore, there is a growing interest in manipulating the composition of intestinal flora in order to achieve a more beneficial intestinal bacterial community (Šušković et al. 2001). Attempts have been made to increase the number of intestinal bifidobacteria and lactobacilli. The microbiological nutritional components, known as probiotics, are used to alter the composition of microflora in the colon. Thus, there is a great interest in dietary components known as prebiotics, which have a beneficial effect on human health through the selective stimulation of the growth and activity of beneficial bacteria, which already reside in the colon. It has been shown that prebiotics stimulate the growth of endogenous bifidobacteria, which in one week can predominate in human faeces. A combination of the probiotics and prebiotics properties suggests the concept of synbiotics, and makes these compounds suitable candidates for classification as functional dietary components that improve human health (Losada & Olleros, 2002).

Lactobacillus helveticus is an industrially important, thermophilic starter culture, mostly employed for cheese manufacture. In our laboratory, *Lb. helveticus* M92 was previously selected, based on *in vitro* selection criteria, as probiotic strain (Kos et al. 2000, 2003). *Lb. helveticus* M92 was identified and previously assigned as *Lactobacillus acidophilus* M92, but after the cluster analysis by DNA fingerprinting (FAFLP) the strain was re-identified as *Lb. helveticus* M92 (Frece, 2007). This strain has ability to survive simulated conditions in the gastrointestinal tract (GIT), is bile resistant, has antibacterial activity against some enteropathogenic and spore-forming bacteria, and as such is a potential candidate for probiotic (Šušković, 1996; Kos et al. 2000; Šušković et al. 2000). Furthermore, *in vitro* studies have shown that *Lb. helveticus* M92 assimilated cholesterol in the presence of bile, so it is postulated that this strain might help in lowering serum cholesterol *in vivo* (Kos, 2001). *Lb. helveticus* M92 adheres to porcine ileal epithelial cells *in vitro* (Kos et al. 2003). The aggregation

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and adhesion experiments performed on *Lb. helveticus* M92 suggested that these processes are mediated by proteinaceous components (S-layer) on the cell surface (Frece et al. 2005a; Frece, 2007). Preliminary results in the technological context have shown viability and activity of the selected strain at high population level during freeze-drying and storage at different temperatures (Kos et al. 2008). Moreover, *Lb. helveticus* M92 has a great potential as probiotic strain due to protective role of its S-layer proteins during transit through GIT and also during processing of culture for probiotic products (Frece et al. 2005a).

The aim of this study was to investigate, *in vivo*, synbiotic effect of probiotic strain *Lb. helveticus* M92 in combination with inulin, lactulose or raffinose, on the intestinal and faecal microflora and immune system of mice, in comparison with probiotic effect of *Lb. helveticus* M92 and prebiotic effect, respectively. After the monitoring of *in vitro* effects of variety of prebiotics on *Lb. helveticus* M92 growth, inulin, lactulose and raffinose best stimulated growth, hence were selected for further *in vivo* studies (Frece, 2007). In order to distinguish and monitor the survival and persistence of probiotic strain *Lb. helveticus* M92, between intestinal microflora, during the passage through GIT of mice, rifampicin-resistant variants of strain were used, and screened by RAPD (random amplified polymorphic DNA) method.

Methods and Materials

Bacteria

Lb. helveticus M92, from the culture collection of the Laboratory for antibiotic, enzyme, probiotic and starter culture technology, University of Zagreb was stored anaerobically in MRS medium containing 30% (v/v) glycerol at −70 °C.

Mice

Four month old female Swiss albino mice weighing from 22 to 24 g were used after a month quarantine period. Each experimental group consisted of 4 mice, housed in cage, kept in a controlled atmosphere (temperature 22±2 °C; humidity 55±2%) with a 12 h light/dark cycle. Mice had continual access to water and were fed ad libitum on skim milk powder (SMP)-based diet contained SMP (53%), corn oil (8%), vitamin (5%), minerals (5%), corn flour (28%), and cellulose (1%). All experimental procedures were carried out according to the standards set in the ''Guide for the Care and Use of Laboratory Animal's of the National Research Council'' (1996).

Rifampicin marking

Lb. helveticus M92 was cultured anaerobically in MRS-broth at 37 °C for 18 h. The cultured cells were plated on MRS media containing 100 μg/ml rifampicin (Sigma Chemical Co., St. Louis, MO, USA) and incubated for 2 d at 37 °C. The selected antibiotic-resistant strain was isolated and further used for monitoring survival and persistence of this strain in the GIT of mice.

Mouse feeding and faecal sampling

Rifampicin-resistant *Lb. helveticus* M92 cells were centrifuged at 10 000 ***g*** for 2 min, washed three times and resuspended in 10 g skim milk/l to final concentration of $1{\cdot}0\times10^{11}$ viable bacterial cells per ml. Mice were fed with 200 μl of this suspension without or with addition of 10 g inulin, lactulose or rafinose/l. Survival of *Lb. helveticus* M92, during transit through GIT, was determined in 1 g dry weight faecal samples, which were individually colleted on the 1st day of the mice feeding. Faecal samples were homogenized in 1 ml sterile 0·5% NaCl solution and serially diluted before plating in non-selective medium (Peptone yeast extract glucose agar, Biolife) and selective media: MRS-agar for LAB count and MRS-agar with rifampicin (100 μg/ml), violet red bile glucose agar (Biolife) for *Enterobacteriaceae* counts and Sulphite agar (Difco) for sulphite-reducing clostridia counts. The plates were incubated anaerobically at 37 °C for 48 h. Additionally, plates of total anaerobes and of clostridia were incubated in anaerobic jars (Oxoid Ltd, Hampshire, UK) by placing one activated Anaerocult A gas pack (Merck, Darmstadt, Germany) per jar. Lactic acid bacteria, *Enterobacteriaceae* and sulphite-reducing clostridia were identified on the basis of colony morphology, Gram staining, cell morphology and the catalase reaction.

In vivo adhesion test

Mice were fed orally with *Lb. helveticus* M92 with a daily dose of $2{\cdot}0\times10^{10}$ rifampicin-resistant cells, without and with addition of 10 g inulin, lactulose or raffinose/l, for 8 consecutive days, according to procedure described by Frece et al. (2005b). The control group was fed with 200 μl sterile 0·5% NaCl solution. On the d1 and d14, after the above described 8 day feeding procedure was ended, adhesion ability of examined probiotic strain and its synergic effect in combination with different prebiotics were determined in homogenates of small and large intestine of Swiss albino mice. Tissue samples were preformed from intestines from mice sacrificed by ether inhalation.

Immunization

On d4, 8, 10, 14, 17 and 21 after the first immunization the blood samples were collected by bleeding of the tail vein with heparinized capillaries into the tubes, allowed to cloth at room temperature for 1 h and left overnight at 4 °C. Tubes were centrifuged at 3000 ***g*** for 20 min. The sera samples were kept at −20 °C until use.

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Table 1. Comparison of the bacterial count in faeces of mice not fed (control) and fed by probiotic strain *Lb. helveticus* M92, prebiotics (inulin, lactulose, raffinose) and synbiotics (*Lb. helveticus* M92 in combination with inulin, lactulose or raffinose). Total aerobic (A) and anaerobic bacteria (B) on Peptone yeast extract glucose agar; total lactic acid bacteria (C) and rifampicin-resistant lactic acid bacteria (D) on MRS-agar; *Enterobacteriaceae* (E) on Violet red bile glucose agar; sulphite-reducing clostridia (F) on Sulfite agar

Values are mean ± standard deviations of results from three separate experiments

Growth media	log cfu/g faeces							
	control	M92	inulin	M92+inulin	lactulose	M92+lactulose	raffinose	M92+raffinose
A	$9{\cdot}68\pm0{\cdot}14^{a}$	$9{\cdot}23\pm0{\cdot}11^{a}$	$8{\cdot}74\pm0{\cdot}16^{b}$	$8{\cdot}68\pm0{\cdot}16^{b}$	$8{\cdot}65\pm0{\cdot}14^{b}$	$8{\cdot}71\pm0{\cdot}14^{b}$	$8{\cdot}85\pm0{\cdot}21^{b}$	$8{\cdot}89\pm0{\cdot}16^{b}$
B	$9{\cdot}81\pm0{\cdot}19^{a}$	$9{\cdot}69\pm0{\cdot}16^{b}$	$9{\cdot}76\pm0{\cdot}14^{b}$	$9{\cdot}81\pm0{\cdot}12^{a}$	$9{\cdot}71\pm0{\cdot}23^{b}$	$9{\cdot}84\pm0{\cdot}21^{a}$	$9{\cdot}63\pm0{\cdot}25^{b}$	$9{\cdot}65\pm0{\cdot}15^{b}$
C	$7{\cdot}95\pm0{\cdot}29^{c}$	$10{\cdot}25\pm0{\cdot}14^{b}$	$10{\cdot}73\pm0{\cdot}14^{b}$	$11{\cdot}20\pm0{\cdot}12^{a}$	$10{\cdot}45\pm0{\cdot}18^{b}$	$10{\cdot}85\pm0{\cdot}18^{b}$	$10{\cdot}50\pm0{\cdot}22^{b}$	$10{\cdot}78\pm0{\cdot}12^{b}$
D	$1{\cdot}17\pm0{\cdot}21^{c}$	$3{\cdot}15\pm0{\cdot}22^{b}$	—[d]	$4{\cdot}42\pm0{\cdot}14^{a}$	—[d]	$4{\cdot}10\pm0{\cdot}21^{a}$	—[d]	$3{\cdot}97\pm0{\cdot}11^{a}$
E	$5{\cdot}51\pm0{\cdot}36^{a}$	$4{\cdot}89\pm0{\cdot}17^{b}$	$4{\cdot}35\pm0{\cdot}21^{bc}$	$3{\cdot}95\pm0{\cdot}21^{c}$	$4{\cdot}41\pm0{\cdot}13^{b}$	$4{\cdot}12\pm0{\cdot}13^{c}$	$4{\cdot}58\pm0{\cdot}15^{b}$	$4{\cdot}25\pm0{\cdot}15^{c}$
F	$2{\cdot}89\pm0{\cdot}19^{a}$	—[c]	$1{\cdot}12\pm0{\cdot}11^{b}$	—[c]	$1{\cdot}15\pm0{\cdot}18^{b}$	—[c]	$1{\cdot}11\pm0{\cdot}21^{b}$	—[c]

(—) colonies are not detected

[a,b,c,d] Values in the same row having a different letters differ significantly ($P<0{\cdot}05$)

On d3, 5, 7, 9, 11 and 13 after first immunization the faecal samples were collected and stored immediately at −20 °C until use. For the determination of the faecal secretory SIgA antibody by ELISA method, the frozen faecal samples were defrosted on ice. Suspensions were prepared by adding 1 g faeces to 9 ml phosphate-buffered saline (PBS) and homogenizing for 10 min using a stomacher. The homogenates were stored at −80 °C until further processing.

Serum and faecal antibody determination (ELISA method)

The total antibody sera titres and faecal SIgA antibodies were determined in polystyrene microtiter plates (NUNC) (Frece et al. 2005b).

Reaction conditions for RAPD (random amplified polymorphic DNA)

Each reaction was performed in a volume of 50 µl with the following components: 10–100 ng purified chromosomal DNA (chromosomal DNA were isolated by Ulrich & Hughes (2001); 50 pmol oligonucleotide ISS1rev (5′-GG-ATCCAAGACA-ACGTTTCAAA-3′) (Veyrat et al. 1999); 50 pmol of each dNTP; 1·5 mmol l^{-1} $MgCl_2$; 5 µl 10 × Taq buffer and 0·5 U Taq polymerase (Boehringer GmbH, Mannheim, Germany). The reaction set-up was as performed by Veyrat et al. (1999).

Statistical methods

A randomized complete block design which incorporated the 8 treatments (control, *Lb. helveticus* M92, prebiotics: inulin, lactulose, raffinose and synbiotics, *Lb. helveticus* M92 in combination with inulin, lactulose or raffinose) and 15 treatments (control, *Lb. helveticus* M92 1 or 14 day (M92 1d, M92 14d), inulin 1 or 14 day (I 1d, I 14d), lactulose 1 or 14 day (L 1d, L 14d), raffinose 1 or 14 day (R 1d, R 14d), *Lb. helveticus* M92+inulin 1 or 14 day (M92+I1d, M92+I14d), *Lb. helveticus* M92+lactulose 1 or 14 day (M92+L1d, M92+L14d), *Lb. helveticus* M92+raffinose 1 or 14 day (M92+R1d, M92+R14d) and three block trials was used for analysis of the response variables. Analysis of variance of the randomized complete block design was carried out using a general linear model of SAS (1995) where the effect of treatment and replicates were estimated for all response variables. Duncan's multiple comparison test was used as a guide for pair comparisons of the treatment means. Differences between treatments that are described subsequently as being significant were determined at least $P<0{\cdot}05$.

Results

Survival of probiotic in combination with prebiotics during the transit through GIT of mice

The number of LAB on MRS and MRS with rifampicin, obtained from faeces of mice after introducing synbiotic, and probiotic or prebiotics alone, was increased compared with control, approximately ∼3·2, ∼2·0 and ∼2·7 log units, respectively (Table 1). Furthermore, synbiotic preparations and probiotic or prebiotics alone, positively influenced the faecal microflora by decreasing the number of enterobacteria by ∼1·6, ∼0·6 and ∼1·2 log cfu/g respectively, and totally reducing the number of sulphite-reducing clostridia, with exception of prebiotics, which decreased the number of clostridia by ∼1·8 log units (Table 1). Synbiotic with *Lb. helveticus* M92 and inulin has shown the best synergic effect on faecal microflora of mice (Table 1).

Probiotic adhesion and influence of synbiotics on intestinal microflora of mice

In vivo adhesion of rifampicin-resistant cells of probiotic strains was monitored by determination of microflora in

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Table 2. Comparison of the bacterial counts in large intestine of mice on days 1 and 14 after feeding with probiotic strain *Lb. helveticus* M92, prebiotics (inulin, lactulose or raffinose) and with synbiotics (*Lb. helveticus* M92 in combination with inuline, lactulose and rafinose). Total aerobic (A) and anaerobic bacteria (B) on Peptone yeast extract glucose agar; total lactic acid bacteria (C) and rifampicin-resistant lactic acid bacteria (D) on MRS-agar; *Enterobacteriaceae* (E) on Violet red bile glucose agar; sulphite-reducing clostridia (F) on Sulfite agar, Control (con.), *Lb. helveticus* M92 1 or 14 day (M92 1d, M92 14d), Inulin 1 or 14 day o(I 1d, I 14d), Lactulose 1 or 14 day (L 1d, L 14d), Raffinose 1 or 14 day (R 1d, R 14d), *Lb. helveticus* M92+Inulin 1 or 14 day (M92+I1d, M92+I14d), *Lb. helveticus* M92+Lactulose 1 or 14 day (M92+L1d, M92+L14d), *Lb. helveticus* M92+Raffinose 1 or 14 day (M92+R1d, M92+R14d)

Values are means ± standard deviations of results from three separate experiments

Growth media	log cfu/g homogenates														
	Con.	M92 1d	I 1d	L 1d	R 1d	M92 14d	I 14d	L 14d	R 14d	M92+I1d	M92+L1d	M92+R1d	M92+I14d	M92+L14d	M92+R14d
A	8·56±0·14^{b}	8·61±0·27^{b}	9·12±0·22^{a}	8·96±0·42^{a}	8·67±0·15^{b}	8·43±0·11^{b}	8·98±0·14^{a}	8·76±0·34^{b}	8·34±0·27^{b}	9·12±0·43^{a}	8·96±0·21^{a}	8·67±0·15^{b}	8·98±0·11^{a}	8·76±0·22^{b}	8·34±0·33^{b}
B	8·67±0·29^{b}	8·83±0·53^{a}	9·31±0·18^{a}	9·43±0·19^{a}	9·28±0·14^{a}	8·65±0·22^{b}	8·98±0·12^{a}	9·14±0·26^{a}	8·83±0·14^{a}	9·31±0·24^{a}	9·43±0·32^{a}	9·28±0·17^{a}	8·98±0·48^{a}	9·14±0·33^{a}	8·8±0·15^{a}
C	7·56±0·17^{d}	8·98±0·76^{b}	9·68±0·26^{b}	9·54±0·14^{b}	9·34±0·31^{b}	8·68±0·14^{c}	9·38±0·13^{b}	9·17±0·19^{b}	9·07±0·17^{b}	10·45±0·57^{a}	9·89±0·43^{a}	9·69±0·19^{b}	9·65±0·34^{b}	9·35±0·47^{b}	9·12±0·14^{c}
D	7·56±0·23^{a}	3·35±0·14^{c}	—^{d}	—^{d}	—^{d}	3·12±0·16^{c}	—^{d}	—^{d}	—^{d}	4·43±0·62^{b}	3·86±0·14^{b}	3·67±0·25^{b}	3·67±0·56^{b}	3·34±0·61^{c}	3·12±0·24^{c}
E	7·56±0·11^{a}	3·24±0·23^{b}	2·15±0·13^{c}	2·38±0·31^{c}	2·46±0·17^{c}	3·32±0·42^{b}	2·21±0·14^{c}	2·43±0·23^{c}	2·6±0·74^{b}	1·92±0·12^{c}	2·16±0·45^{c}	2·34±0·27^{c}	1·97±0·21^{c}	2·3±0·15^{c}	2·46±0·23^{c}
F	7·56±0·15^{a}	—^{d}	0·79±0·15^{c}	0·97±0·18^{c}	8·67±0·14^{b}	—^{d}	0·83±0·25^{c}	1·12±0·14^{c}	0·92±0·54^{c}	—^{d}	—^{d}	—^{d}	—^{d}	—^{d}	—^{d}

(—) colonies are not detected

a,b,c,d Values in the same row having a different letters differ significantly ($P<0·05$)

homogenates of large intestine of mice. *Lb. helveticus* M92 in synbiotic combination with inulin has shown the best synergic effect on intestinal microflora of mice (Table 2). The LAB counts were most effectively increased, while enterobacteria and sulphite-reducing clostridia counts were decreased after the mice were fed with synbiotics (Table 2). 8 colonies from MRS-rifampicin agar (samples of large intestine of mice fed with probiotic strain *Lb. helveticus* M92) were randomly screened and identified by random amplified polymorphic DNA (RAPD). RAPD patterns identical with the pattern of probiotic strain *Lb. helveticus* M92 indicated that those colonies belonged to the same strain *Lb. helveticus* M92 (Fig. 1).

First day after oral administration of synbiotics, and *Lb. helveticus* M92 or prebiotics alone, the number of LAB in large intestine of mice was increased in comparison with the control group by ~4·0, ~1·8 and ~2·5 log cfu/g, respectively (Table 2). The higher number of LAB in large intestine was also detected 14 d after oral administration of synbiotics compared with the counts in control mice (Table 2). When mice were fed with synbiotics the number of enterobacteria was decreased compared with the control group 1 d as well as 14 d after feeding by ~2·0 log units.

Furthermore, application of probiotic strain *Lb. helveticus* M92 and synbiotics totally reduced the number of sulphite-reducing clostridia in large intestine of mice (Table 2). Similar results were obtained in small intestine of mice after probiotic and synbiotics administration (Frece, 2007).

Modulation of mice immune system

Oral administration of mice with viable cells of *Lb. helveticus* M92 alone or in combination with applied prebiotics stimulated the immune response in mice (Fig. 2). The levels of faecal and total serum IgA antibodies, from all groups of mice, were higher in comparison with control groups. The highest level of serum and faecal IgA antibodies were observed after oral administration of mice with *Lb. helveticus* M92 in combination with inulin (Fig. 2).

Discussion

The concept of synbiotics has recently been proposed to characterize health-enhancing foods and supplements used as functional food ingredients in humans (Mountzouris et al. 2006). Prebiotics are known for their ability to stimulate the growth of beneficial intestinal bacteria, bifidobacteria and lactobacilli, *in vitro* and *in vivo*. Although there is a great interest in the physiological and pharmacological effects of dietary fibres, only a few studies are available concerning the influence of prebiotics on the intestinal immune system (Manhart et al. 2003). *Lb. helveticus* M92 fulfils *in vitro* selection criteria for probiotic strains and exerts inhibitory activity against a wide range

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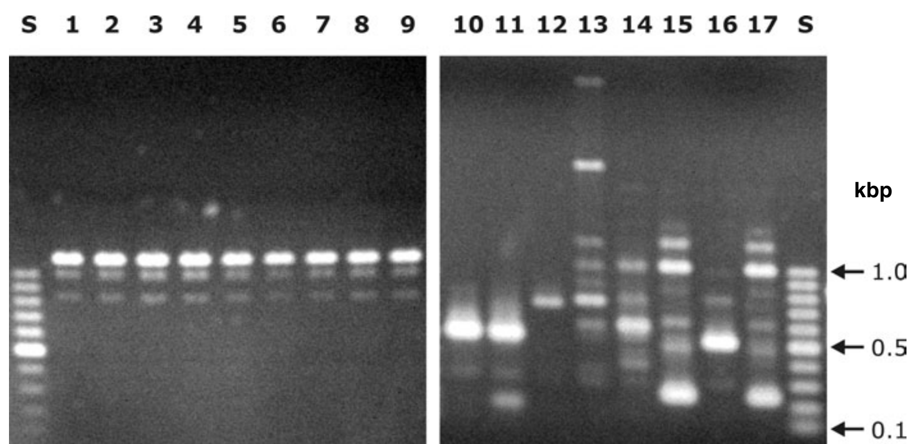

Fig. 1. RAPD-PCR patterns obtained with *Lactobacillus*-specific primers. S, 1000 bp DNA ladder, lane 1, *Lb. helveticus* M92 (standard), lanes 2–9, isolates from large intestine from rifampicin MRS-agar (mice fed with *Lb. helveticus* M92), lanes 10–17, isolates from large intestine from MRS-agar (mice which were not fed with probiotic strain *Lb. helveticus* M92 – control).

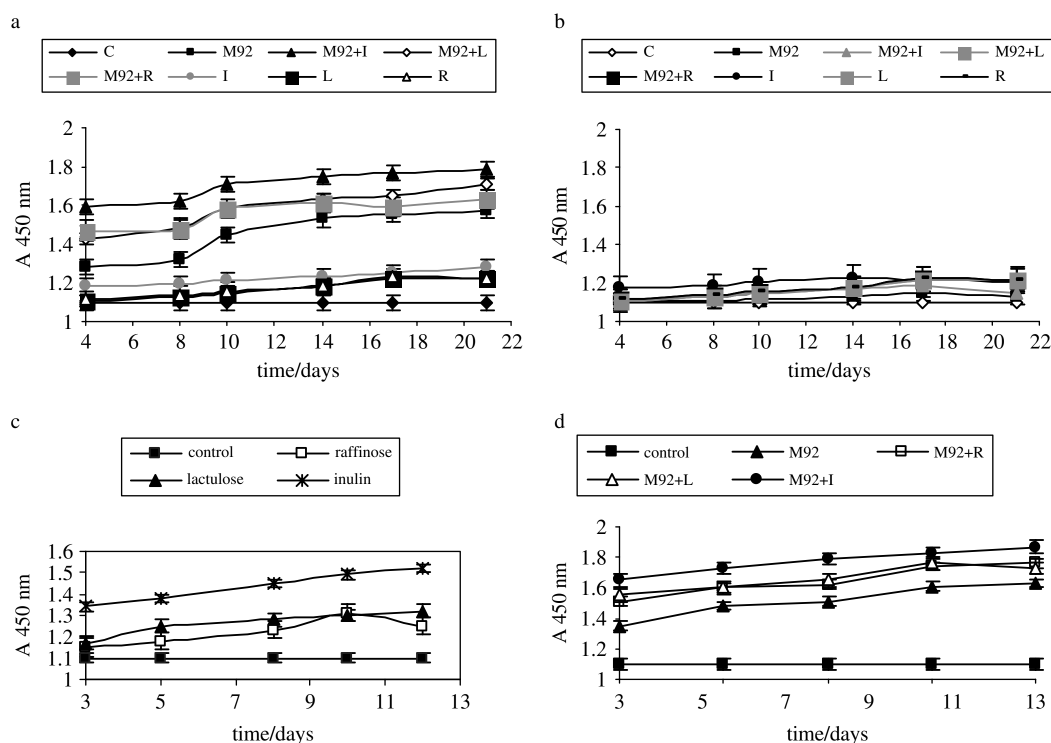

Fig. 2. Determination of total (a) and specific (b) IgA antibodies in sera diluted 1:100 and faecal SIgA antibodies in faecal samples by ELISA method after oral administration of mice with: (c) prebiotics: inulin, lactulose and raffinose, and (d) with *Lb. helveticus* M92 alone or in combination with prebiotics: inulin (M92+I), lactulose (M92+L) and raffinose (M92+R). Error bars represent standard deviations of the mean values.

of bacteria including some pathogens (Kos et al. 2000; Šušković et al. 2000; Frece et al. 2005a, b).

Therefore, *in vivo* adhesion of *Lb. helveticus* M92 in Swiss albino mice was studied. The increased number of LAB and decreased number of enterobacteria have shown that the bacterial composition in faeces and intestine of mice was altered by applied synbiotics. The increased number of LAB in large intestine was detected even 14 d after *Lb. helveticus* M92 administration, and the number of enterobacteria and sulphite-reducing clostridia in large intestine of mice was decreased. These results could be a consequence of lactic acid and bacteriocins production proved for examined probiotic strain. Namely, their antibacterial activities were confirmed against some

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enteropathogenic bacteria *in vitro* (Šušković, 1996; Kos, 2001).

Results of RAPD analysis confirmed the capability of *Lb. helveticus* M92 to survive transit through GIT of mouse and to interact and compete with other microorganisms within the gut environment. The results of other authors have also shown that administration of certain strains of LAB can decrease the numbers of faecal *Escherichia coli*, anaerobic coci and sulphite-reducing clostridia (Lund et al. 2002; Marquina et al. 2002), but probiotic strain *Lb. helveticus* M92 has shown very strong inhibition against clostridia. Besides the antibacterial activity, the advantage of *Lb. helveticus* M92 strain is that it contains surface (S-layer) proteins responsible for survival of this strain in the GIT of mice and adhesion to intestinal epithelial cells. Namely, *Lb. helveticus* M92 S-layer proteins have been proved to be resistant to pepsin and pancreatic juice. In addition, the adhesion of *Lb. helveticus* M92 cells was higher when S-layer protein was not removed, compared with *Lb. helveticus* M92 cells if S-layer protein was removed by 5M-LiCl (Frece et al. 2005a).

The number of probiotic bacteria detected 14 d after the administration of probiotic strains was lower than 1 d after administration of these strains. Therefore, it appears likely that administration at regular intervals is necessary for maintenance of high probiotic level.

The possible competitive exclusion mechanisms of probiotic action include not only direct attack of probiotic cells by production of antibacterial substances and competition for nutrients and receptors on the gut enterocytes, but also stimulation of the non-specific immune system. SIgA plays a key role in the gastrointestinal defence mechanism against dietary and microbial antigens. Therefore, the effect of probiotic strain *Lb. helveticus* M92 itself, or in combination with prebiotics, on the faecal SIgA and total serum IgA levels in mice was investigated. The levels of faecal and total serum IgA antibodies from all groups were higher compared with control groups after oral administration of mice with viable probiotic cells, prebiotics or synbiotics. The increase of intestinal IgA antibody represents an important result, since SIgA is the predominant mucosal antibody and plays an important role in protection against intestinal pathogens (Shu & Gill, 2001). There is accumulating evidence that the intestinal SIgA production is highly influenced by the intestinal microflora (Zierikzee et al. 2006). Moreau & Baforiau-Routhiau (2000) have shown that bifidobacteria from the infant's intestine, in particular, are important for the synthesis of SIgA against viral enteropathogens. Therefore, the authors suggested that prebiotics, which promote growth of bifidobacteria in the intestine, could be instruments in stimulating endogenous SIgA production and hence promote resistance in infants. Several studies reported that supplementation of food with prebiotics or probiotics or their combination can increase SIgA response to viruses and bacteria (Hosono et al. 2003; Zierikzee et al. 2006). Furthermore, *Lb. helveticus* M92, prebiotics and synbiotics did not evoke the specific humoral immune response after oral application and are as such suitable for probiotic, prebiotic and synbiotic application.

Our findings indicated that *Lb. helveticus* M92 in combination with inulin, lactulose or raffinose is an immunomodulator, because the application of these synbiotics stimulated the mucosal and total humoral immune response. Applied probiotic strain have shown ability to survive and adhere in the intestinal tract of mouse and positively influenced the intestinal microflora of the host. Confirmed synbiotic properties of *Lb. helveticus* M92 are of great importance for its application in fermented foods as functional starter culture and fermented dairy product (*Lb. helveticus* M92 in mixture with inulin). However, further investigations will be carried out to determine the influence of food matrix and applied processing technology on the functionality of this probiotic strain.

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