CHAPTER IV

RESULTS

1. Isolation of *Lactobacillus* Isolates

Bacteria were isolated from 64 healthy human volunteers and selected for Genus *Lactobacillus* by presumptive tests including Gram stain, catalase test and vancomycin susceptibility testing. Five hundred and ten *Lactobacillus* isolates were obtained. They are all gram-positive, catalase-negative and vancomycin resistant. Cell morphology in each isolate varied from long and slender rods, straight rods to bent rods, sometimes shot rods to coccobacilli; arranged in single, in pairs, or short chain formation. Some isolates exhibit bipolar staining or internal granulations. The most frequently found colonies varied from small to medium colonies (1-2 mm) with white, circular, smooth and convex colonial morphologies. Most isolates grew well under anaerobic conditions. Most *Lactobacillus* isolates were obligate anaerobes, while some isolates were facultative anaerobes.

2. Antagonistic Activities of *Lactobacillus* Isolates Against Gastrointestinal Pathogens by Agar Well Diffusion Method

In this study, 510 *Lactobacillus* isolates were tested for antagonistic activity against 10 gastrointestinal pathogens including *E. coli* ATCC 25922, *S.* Typhimurium ATCC 13311, *Sh. flexneri* DMST 4423, *V. cholerae non* O1 DMST 2873, EHEC O157:H7 DMST 12743, ETEC DMST 20970, EPEC DMST 20972, EIEC DMST 20971, *C. jejuni* and *C. difficile*. An agar well diffusion method was used to determine the inhibitory effect of *Lactobacillus* isolates. In preliminary studies, each *Lactobacillus* isolate was cultivated in MRS broth and supernatants were collected by centrifugation. A non-neutralized supernatant (pH 4.0) of each isolate was tested for antimicrobial activity against gastrointestinal pathogens. Most of the isolates showed strong inhibitory activities against all target strains. However, after excluding acidic factors by neutralization of culture supernatants, no such inhibitory reactions were observed for any of the cultures (data not shown). *Lactobacillus* species produce lactic acid that generates an acidic environment that may affect the growth of surrounding bacteria [88]. Therefore, the inhibitory actions of most *Lactobacillus* isolates were due to acid production rather than the production of bacteriocin-like metabolites [173]. Low glucose MRS medium containing 0.2% glucose (modified MRS: MMRS) was then used to restrict the extent of acid production [4]. All *Lactobacillus* isolates were grown in MMRS and supernatants were collected and neutralized with NaOH to raise their pH equal to MMRS (pH 6.6-6.8) culture media. Non-neutralized and neutralized supernatants were tested against indicator strains. The results indicated that 4 *Lactobacillus* isolates designated as SB42-6, BJ48-5, RT49-5 and RT49-7, displayed antagonistic activities towards *V. cholerae* non O1

DMST 2873 only, but these isolates demonstrated no effect on other pathogens as shown in Table 4. Most *Lactobacilllus* isolates also had no effect on 10 target strains. The non-neutralized supernatants of SB42-6, BJ48-5, RT49-5 and RT49-7 strains showed moderate inhibitory activities with clear zones of 15±0.58 mm, 16±0.26 mm,16±0.26 mm and 15±0.32 mm against *V. cholerae* non O1 DMST 2873, respectively (Figure 1A, Table 5). Whereas, the neutralized supernatants of SB42-6, BJ48-5, RT49-5 and RT49-7 strains showed weak inhibitory activities with clear zones of 12±0.41 mm, 14±0.26 mm, 14±0.32 mm and 13±0.32 mm against *Vibrio cholerae* non O1 DMST 2873, respectively (Figure 1B, Table 5). The MMRS bacterial media control as in the middle well of plate (Figure 1) showed no inhibitory effect on any of the pathogenic strains tested. These four strains were then selected for further investigations.

Table 4. The antagonistic effects of neutralized supernatants of SB42-6, BJ48-5, RT49-5, RT49-7 *Lactobacillus* strains on 10 target strains by using agar well diffusion assay. Modified MRS (MMRS) was used as bacterial media control.

Lactobacillus Strain	*E. coli*	*S.. typhimurium*	*Sh.. flexneri*	*Vibrio Cholerae*	EHEC	ETEC	EPEC	EIEC	*Cam. jejuni*	*C. difficile*
SB42-6	-	-	-	12±0.41	-	-	-	-	-	-
BJ48-5	-	-	-	14±0.26	-	-	-	-	-	-
RT49-5	-	-	-	14±0.32	-	-	-	-	-	-
RT49-7	-	-	-	13±0.32	-	-	-	-	-	-

-, No inhibition zone; *E*, *Escherichia*; *S.*, *Salmonella*; *Sh.*, *Shigella*; *Cam*, *Campylobacter*; *C*, *Clostridium*

Reported values are the diameters of inhibition zone in millimeters (mm)

The minimum well diameter was 10 mm

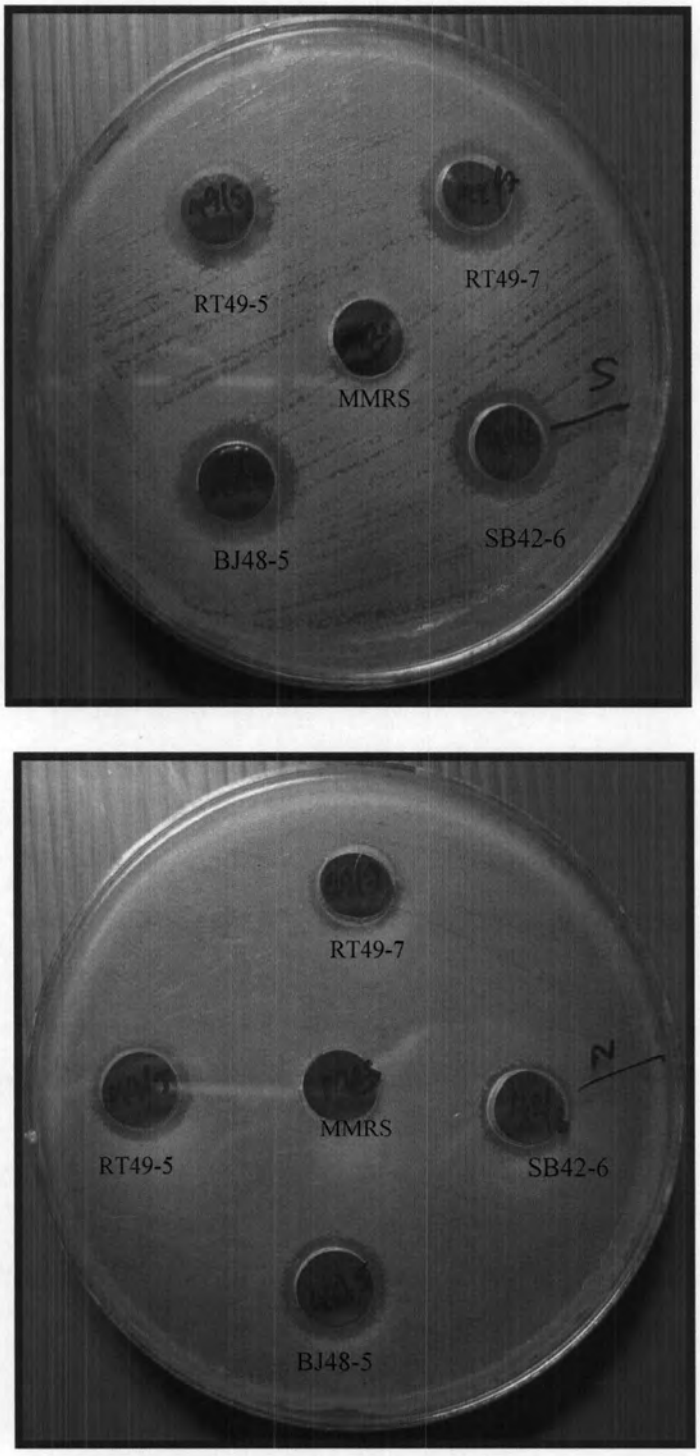


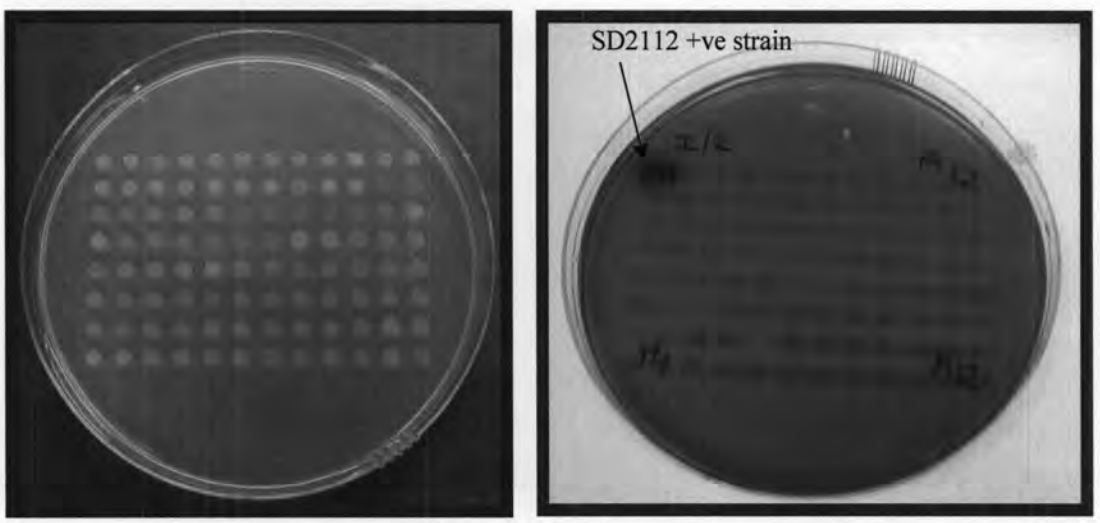
Figure 1. Antibacterial activities of SB42-6, BJ48-5, RT49-5, RT49-7 *Lactobacillus* strains against *V. cholerae* non O1 DMST 2873 using agar well diffusion assay. MMRS, bacterial media control;
S, Non-neutralized supernatants (A);
N, Neutralized supernatants (B);
A minimum well diameter was 10 mm

Table 5. The antibacterial activities of SB42-6, BJ48-5, RT49-5, RT49-7 *Lactobacillus* strains against *V. cholerae* non O1 DMST 2873 by using agar well diffusion assay. MMRS, bacterial media control; S, Non-neutralized supernatants; N, Neutralized supernatants; A well diameter was 10 mm; p-value <0.0001 when compared to MMRS bacterial media control

Lactobacillus strain	Inhibition zones of non-neutralized supernatants (S) (mm)	p-value	Inhibition zones of neutralized supernatants (N) (mm)	p-value
SB42-6	15±0.58	<0.0001	12±0.41	<0.0001
BJ48-5	16±0.26	<0.0001	14±0.26	<0.0001
RT49-5	16±0.26	<0.0001	14±0.32	<0.0001
RT49-7	15±0.32	<0.0001	13±0.32	<0.0001

3. Reuterin Detection of *Lactobacillus* Isolates

Four hundred and thirty-seven *Lactobacillus* isolates were tested for reuterin production by using a spot overlay method described previously [158]. This method is performed in a 15 x 90 mm plate and use in small volume of detecting solution. To simplify this method, we modified it by cultivating *Lactobacillus* isolates in 96-well plates and transferring to a 20 x 140 mm large plate containing BHI with 20 mM glucose and assayed in a large volume of indicator solution. As shown in Figure 2, the results indicated that none of all 437 *Lactobacillus* isolates were capable of producing reuterin when compared to *L. reuteri* SD2112, while the positive control (reuterin-producing strain SD2112) displayed reddish brown coloration around its spot.

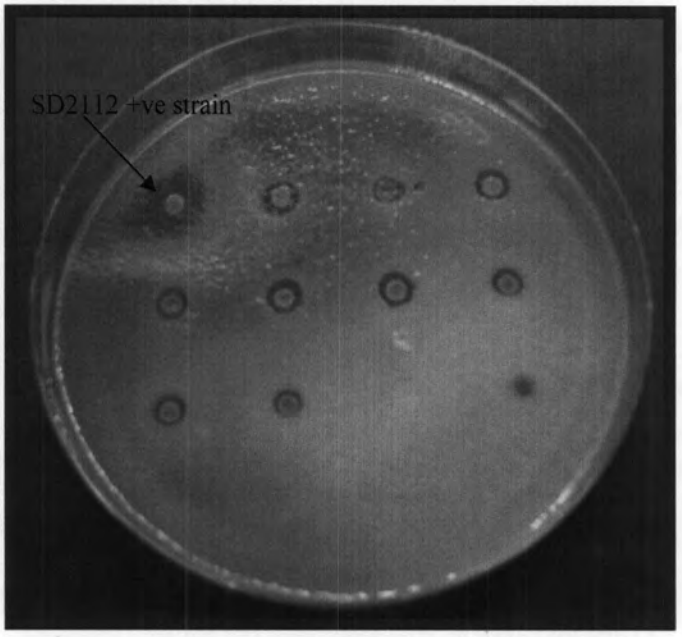


A. Spots of *Lactobacillus* B. Reuterin detection

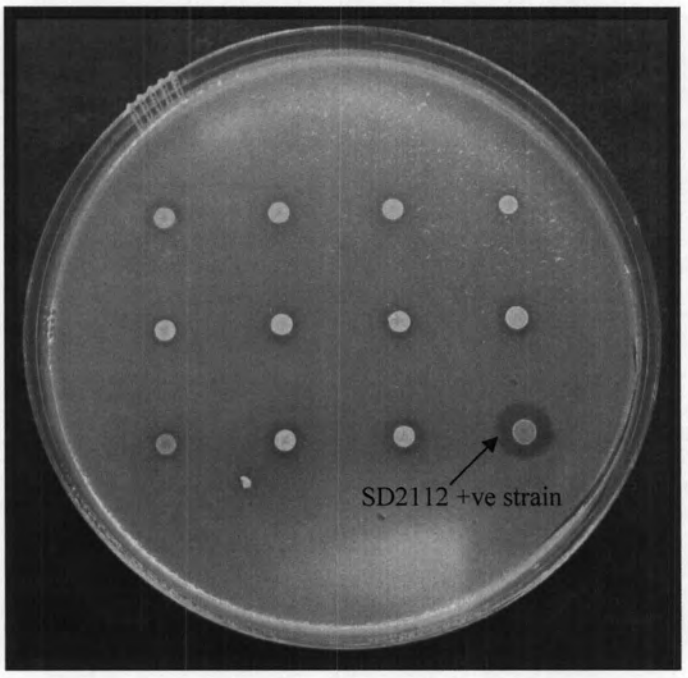
Figure 2. Reuterin screening of *Lactobacillus* isolates using a spot overlay method; SD2112, reuterin producing strain (positive control)

4. Antagonistic Activity of *Lactobacillus* Isolates Against Gastrointestinal Pathogens by Agar Spot Method

An agar spot method was used to assay the inhibitory activity of *Lactobacillus* isolates which could not be detected by agar well diffusion assay or reuterin detection. This method allows for the direct determination of antimicrobial substances which are secreted directly to the surrounding environment by *Lactobacillus* spots. *Lactobacillus* isolates were spotted and grown on BHI agar supplement with 20 mM glucose and subsequently overlaid with soft agar containing glycerol and *Vibrio cholerae.* A clear zone of inhibition ≥ 1 mm around a spot as demonstrated in Figure 3 was scored as positive. Strains which showed inhibitory activity were repeated by spotted (2 µl) separately onto surface of media. The results revealed that weak inhibitory activity (6-8 mm) to medium inhibitory activity (>8-11 mm) of 144 from 437 *Lactobacillus* isolates were observed when overlaid with *V. cholerae* (Tables 6, 7). All 144 inhibitory strains were chosen for further investigation of antagonistic activity against *Salmonella enterica.* As shown in Table 6 and Table 7, these results demonstrated that 32 of 144 strains also showed weak inhibitory activities against *Salmonella enterica* with the agar spot assay. No inhibitory effects of MRS on any of the target strains tested were observed. The antimicrobial activity of four strains including SB42-6, BJ48-5, RT49-5 and RT49-7 (as described above) which displayed inhibitory activity in agar well diffusion assay also inhibited *V. cholerae* and *S. enterica* in the agar spot assay (Tables 6, 7).



A. *Vibrio cholerae*



B. *Salmonella enterica*

Figure 3. Representative results of antibacterial activities of *Lactobacillus* isolates against *V. cholerae* and *S. enterica* using the agar spot method. SD2112 (ATCC 55730), positive control

Table 6. Antagonistic activities of *Lactobacilus* isolates toward *V. cholerae* and *S. enterica*

Lactobacillus isolates	Indicator strains		*Lactobacillus* isolates	Indicator strains	
	V. cholerae	*Sal. enterica*		*V. cholerae*	*Sal. enterica*
PS6-1	++	+	NS19-21	+	-
PS6-2	++	-	NS19-22	+	-
PS6-3	+	-	NS19-23	+	-
PS6-4	+	+	HW21-1	+	-
PS6-5	+	+	HW21-2	+	-
PS6-6	++	+	HW21-3	+	-
KN9-1	++	+	NS22-2	+	-
KN9-5	++	+	NS22-7	+	-
JC10-1	+	-	NS22-15	+	-
JC10-2	+	-	NS22-19	+	-
JC10-3	+	-	NS22-21	+	-
JC10-4	+	+	PJ23-1	++	-
JC10-6	+	-	AP24-1	++	-
WA12-10	+	-	AP24-2	++	-
WA12-14	+	+	AP24-8	+	-
WA12-16	+	-	PW27-1	++	-
WA12-21	+	-	PW27-3	++	-
TA14-2	++	-	GP29-1	+	-
TA14-3	+	-	GP29-4	+	-
TA14-4	+	-	GP29-7	+	-
TA14-5	+	-	AP33-5	++	-
TA14-9	++	-	NS34-1	+	-
TA14-12	+	-	NS34-2	+	-
TA14-18	+	+	NS34-3	+	-
TA14-19	++	-	KK35-1	+	-
SS15-6	+	-	KK35-3	+	-
SS15-9	+	-	KK35-4	+	-
SS15-11	+	+	KK35-5	+	-
SS15-17	+	-	KK35-6	++	-
SS15-18	++	-	KK35-7	++	-
SS15-20	+	-	KK35-8	++	+
NS16-3	+	+	KK35-9	++	-
NS16-17	+	+	KS36-4	+	-
NS16-18	+	-	WP37-1	+	-
ST17-1	++	-	WP37-3	++	-
NS19-1	++	-	WP37-4	+	-
NS19-16	+	+	WP37-9	+	-
NS19-20	+	-	WP37-11	+	+

Lactobacillus spot diameter = 5 mm; -, no inhibition; +, 6-8 mm of inhibition; ++, >8-11 mm of inhibition; +++, >11 mm of inhibition

Table 7. Antagonistic activities of *Lactobacilus* isolates toward *V. cholerae* and *S. enterica* (continue)

Lactobacillus isolates	Indicator strains		*Lactobacillus* isolates	Indicator strains	
	V. cholerae	*Sal. enterica*		*V. cholerae*	*Sal. enterica*
WP37-13	+	-	WK47-6	+	-
WP37-14	++	-	WK47-7	+	-
WP37-15	++	+	WK47-8	+	-
WP37-16	++	+	WK47-9	+	-
WP37-17	++	-	WK47-10	+	-
WP37-18	++	-	WK47-11	+	-
WP37-19	++	-	WK47-12	+	-
WP37-21	+	-	WK47-13	+	-
WP37-24	+	-	WK47-14	+	-
WP37-25	+	-	BJ48-3	+	-
WP37-26	+	-	BJ48-4	+	-
WP37-27	++	-	BJ48-5	++	+
WP37-28	+	-	BJ48-7	+	-
WM38-4	+	-	BJ48-8	+	+
WM38-5	+	-	BJ48-9	+	-
WM38-7	++	-	BJ48-11	+	-
WM38-8	+	-	BJ48-12	+	-
WM38-9	+	-	BJ48-14	+	-
AB39-1	+	-	BJ48-15	+	+
AB39-2	+	-	BJ48-16	+	-
AK40-8	+	-	RT49-2	+	+
AK40-10	+	-	RT49-5	++	+
AK40-11	+	-	RT49-6	+	-
AK40-14	+	+	RT49-7	++	+
AK40-15	+	-	RT49-8	+	+
AK40-17	+	-	RT49-9	+	+
SB42-2	+	-	RT49-10	+	+
SB42-5	+	+	RT49-11	+	+
SB42-6	++	+	RT49-13	+	-
SB42-7	+	-	RT49-14	+	+
SB42-10	+	-	RT49-15	+	-
SB42-11	+	+	RT4919	+	-
SB42-14	+	-	SD50-2	+	-
SB42-15	+	-	SD50-7	+	-

Lactobacillus spot diameter = 5 mm; -: no inhibition; +: 6-8 mm of inhibition; ++ : >8-11 mm of inhibition; +++ : >11 mm of inhibition

5. Immunomodulatory Effects of *Lactobacillus* Isolates on TNF-α Production in LPS-activated THP-1 Monocytic Cells

A total of forty-six *Lactobacillus* isolates were randomly selected from the ones obtained from each volunteer and recovered from -80°C to determine the modulation of TNF-α protein production in LPS-activated THP-1 human monocytic cells. *Lactobacillus* isolates were cultivated in MRS broth for 24 hr and then *Lactobacillus* conditioned media (LCM) were prepared as described in Material and Methods (Chapter III). Bioassays were performed by THP-1 cells incubated with *Lactobacillus* conditioned media and activated with lipopolysaccharide (LPS). TNF-α secretion in culture supernatants were collected and measured by using cytokine-specific sandwich quantitative ELISA and cytokine concentration were quantified from standard curve and expressed as pg/ml of culture medium as shown in Figure 4. Percentage of TNF-α inhibition and cell viability were calculated by the formula as follows.

$$\% \text{ TNF-}\alpha \text{ inhibition} = \frac{\text{Observed}}{\text{Baseline}} - 1$$

Observed = secreted TNF-α of experiment (pg/ml)

Baseline = secreted TNF-α of MRS bacterial media control (pg/ml)

$$\% \text{ Cell viability} = \frac{\text{Dead cells}}{\text{Total cells}} - 1$$

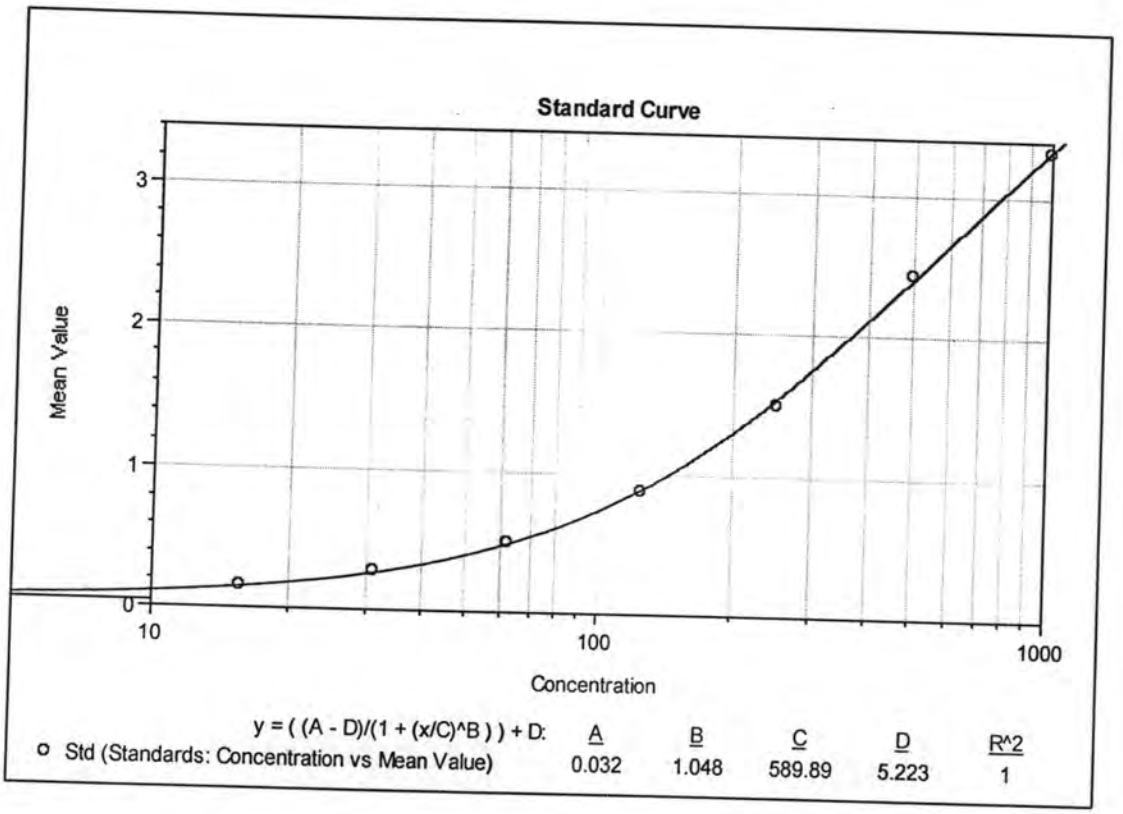


Figure 4. Standard curve of TNF-α protein determination at the concentration of 15.625, 31.5, 62.5, 125, 250, 500, and 1,000 pg/ml; $R^2 = 1$

Forty-six tested isolates exhibited immunomodulatory activities as indicated in TNF-α level as shown in Tables 8-12 and Figures 5-9. These tables and figures showed the levels of TNF-α secretion by THP-1 monocytic cells incubated with *Lactobacillus* conditioned media (LCM) of each isolate in the presence or absence of lipopolysaccharide (LPS). Percentage of TNF-α inhibition was also displayed in the tables. MM4-1A, TNF-α inhibitory strain used as positive control, suppressed TNF-α production to the lowest level. In contrast, SD2112, non-TNF-α inhibitory strain used as negative control showed weak TNF-α inhibition. MRS bacterial media control showed no effect on TNF-α production. LPS acted as TNF-α activator and led to

increasing of TNF-α production as shown in right hand compared to no LPS on left hand of each graph. In the presence of *Lactobacillus*-conditioned media, TNF-α production was suppressed in varying degrees among each of the *Lactobacillus* isolates. In addition, *Lactobacillus* conditioned media alone (without LPS) did not activate TNF-α production in most isolates (Figures 5-9). Interestingly, without LPS, TH14 exhibited TNF-α stimulatory activity as shown in Table 12 and Figure 9.

The data as shown in Table 13 and Figure 10 demonstrated the immunomodulatory effects of 46 isolates on TNF-α production in LPS-activated THP-1 monocytic cells in percentage of TNF-α inhibition. The inhibitory activities varied in each isolate from 8-65% inhibition. Twelve isolates: TH9, TH12, TH15, TH16, TH17, TH36, TH56, TH57, TH59, TH60, TH63 and TH64 displayed 8-20% TNF-α inhibition. Seventeen isolates: TH6, TH14, TH21, TH22, TH23, TH24, TH27, TH32, TH34, TH35, TH37, TH40, TH41, TH44, TH50, TH54 and TH62, displayed 21-30% TNF-α inhibition. Seven isolates: TH19, TH29, TH33, TH46, TH51, TH52 and TH61 displayed 31-40% TNF-α inhibition. Three isolates: TH38, TH45 and TH47, displayed 41-50% TNF-α inhibition and six isolates: TH39, TH42, TH43, TH48, TH49 and TH58, displayed >50% TNF-α inhibition. Interestingly, TH58 exhibited the most potent TNF-α inhibition by 65%.

From the results described above, several isolates which displayed ≥ 25% TNF-α inhibition and TH14 which showed TNF-α stimulatory activity as shown in Figure 9 were selected to confirm immunomodulatory effects using the same conditioned of bioassay in three times with triplicate. The data demonstrated that 12 isolates: TH24, TH27, TH33, TH39, TH43, TH45, TH47, TH48, TH49, TH58, TH61 and TH62, significantly inhibited TNF-α production in LPS-activated THP-1

monocytic cells when compared to MRS bacterial media control as depicted in Table 14 and Figure 11. *Lactobacillus* conditioned media alone of these 12 isolates did not stimulate TNF-α production in THP-1 monocytic cells. These data were similar to the ones displayed in Tables 8-12 and Figures 5-9. The TNF-α inhibitory activities varied among isolates. *Lactobacillus* conditioned media of isolates TH24, TH33, TH39, TH43 and TH45 inhibited TNF-α production by 32-35% ($p<0.05$). Isolates TH27, TH47, TH48 and TH49 inhibited TNF-α production by 37-39% ($p<0.01$), whereas isolates TH61 and TH62 inhibited TNF-α production 42% and 45% respectively ($p<0.01$), when compared to MRS bacterial media control. The one with strongest inhibitory activity was isolate TH58 which inhibited TNF-α production by 68% ($p<0.001$) when compared to MRS bacterial media control.

In order to define the optimal condition of TNF-α production by *Lactobacillus*, conditioned media of TH58 were prepared by cultivation in MRS 48 hr compared to 24 hr. As demonstrated in Table 15 and Figure 12, it was found that *Lactobacillus* conditioned media of TH58 prepared from 48 hr cultivation in MRS was able to inhibit TNF- α production by 82%. Whereas the one collected from 24 hr cultivation showed 70% TNF-α inhibition.

In summary, it was found that there were three types of *Lactobacillus* in modulation of TNF-α production. As shown in Table 16 and Figure 13, the results demonstrated that *Lactobacillus* isolates exhibited difference properties; TH14, TNF-α stimulatory strain, was able to stimulate TNF-α production with and without LPS. While TH58, TNF-α inhibitory strain, did not stimulate TNF-α production by itself and exhibited TNF-α inhibitory activity in LPS-activated THP-1 cells, whereas TH64, non-TNF-α stimulatory and non-TNF-α inhibitory strain, did not-stimulate

TNF-α production by itself and did not inhibit TNF-α production in LPS-activated THP-1 cells. These three different strains and all TNF-α inhibitory strains were chosen for further studies in phenotypic and genotypic characterization. In this study, suppression of TNF-α production did not appear to be associated with any cytotoxic effects to the cells as determined by Trypan Blue dye exclusion assay.

Table 8. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; SD, standard deviation

LCM	TNF-α (pg/ml)	SD	LCM+LPS	TNF-α (pg/ml)	SD	% Inhibition
MRS	2.158	0.77	MRS	1107	18.523	
MM4-1A	10.809	1.525	MM4-1A	177	4.031	84
SD2112	123.47	1.896	SD2112	968	35.867	13
TH10	180.19	16.453	TH10	940	55.962	15
TH12	196.211	5.649	TH12	987	49.349	11
TH16	4.733	0.59	TH16	942	22.585	15
TH17	5.165	1.248	TH17	1022	40.843	8
TH19	176.728	8.998	TH19	717	116.56	35
TH21	127.332	10.183	TH21	812	8.46	27
TH23	3.059	0.565	TH23	850	27.165	23
TH29	92.945	16.998	TH29	767	19.697	31
TH32	3.36	1.011	TH32	838	30.971	24
TH33	49.908	5.499	TH33	733	7.341	34

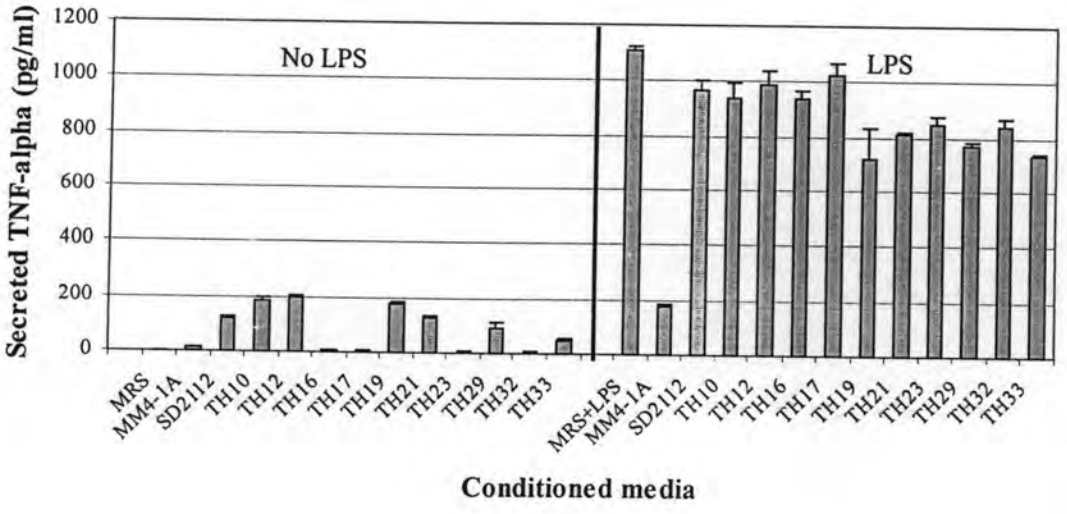


Figure 5. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; LPS, lipopolysaccharide; error bars indicate standard deviations; n=3

Table 9. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; SD, standard deviation

LCM	TNF-α (pg/ml)	SD	LCM+LPS	TNF-α (pg/ml)	SD	% Inhibition
MRS	0	0	MRS	940	45.986	
MM4-1A	8.017	2.523	MM4-1A	161	2.204	83
SD2112	117.976	15.462	SD2112	807	20.155	14
TH34	3.38	1.089	TH34	681	27.778	28
TH35	0	0	TH35	707	19.687	25
TH36	132.201	17.156	TH36	808	64.845	14
TH37	1.682	1.441	TH37	707	40.231	25
TH38	272.349	1.014	TH38	545	35.356	42
TH39	0	0	TH39	433	34.254	53
TH40	102.745	13.281	TH40	654	23.824	30
TH41	73.804	7.57	TH41	662	27.896	30
TH42	1.742	0.989	TH42	418	29.016	56
TH43	1.752	0	TH43	408	13.56	57

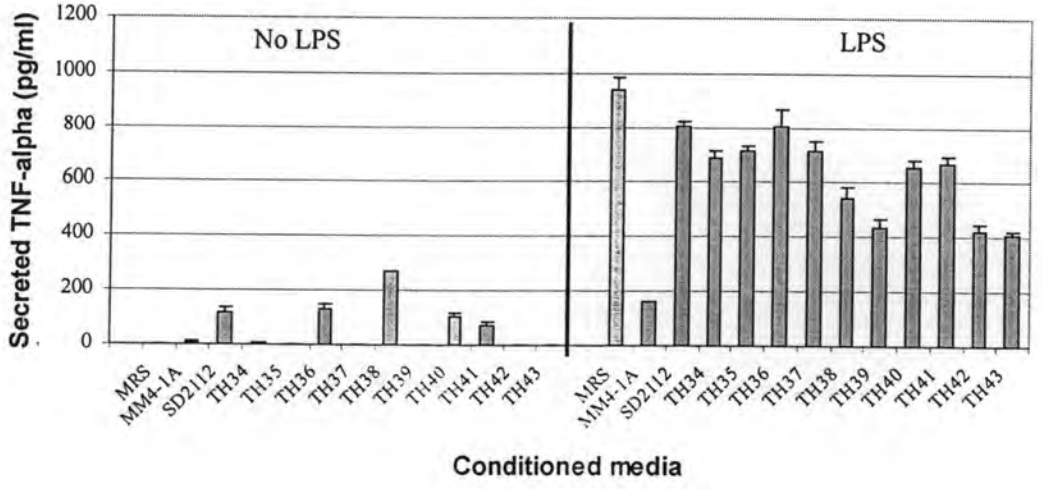


Figure 6. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; LPS, lipopolysaccharide; error bars indicate standard deviations; n=3

Table 10. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; SD, standard deviation

LCM	TNF-alpha (pg/ml)	SD	LCM+LPS	TNF-alpha (pg/ml)	SD	% Inhibition
MRS	0	0	MRS	894	32.404	
MM4-1A	15.132	2.139	MM4-1A	168	4.982	81
SD2112	145.976	13.6	SD2112	689	47.871	23
TH44	0	0	TH44	661	48.246	26
TH45	10.778	1.121	TH45	485	39.093	45
TH46	2.665	0.921	TH46	570	59.911	36
TH47	5.86	1.739	TH47	457	21.48	49
TH48	7.928	0.788	TH48	417	11.042	53
TH49	4.918	1.39	TH49	409	29.02	54
TH50	18.613	1.731	TH50	707	29.008	21
TH51	146.748	1.135	TH51	591	17.675	34
TH52	163.832	8.428	TH52	569	30.585	36
TH54	0.363	0	TH54	646	23.878	28

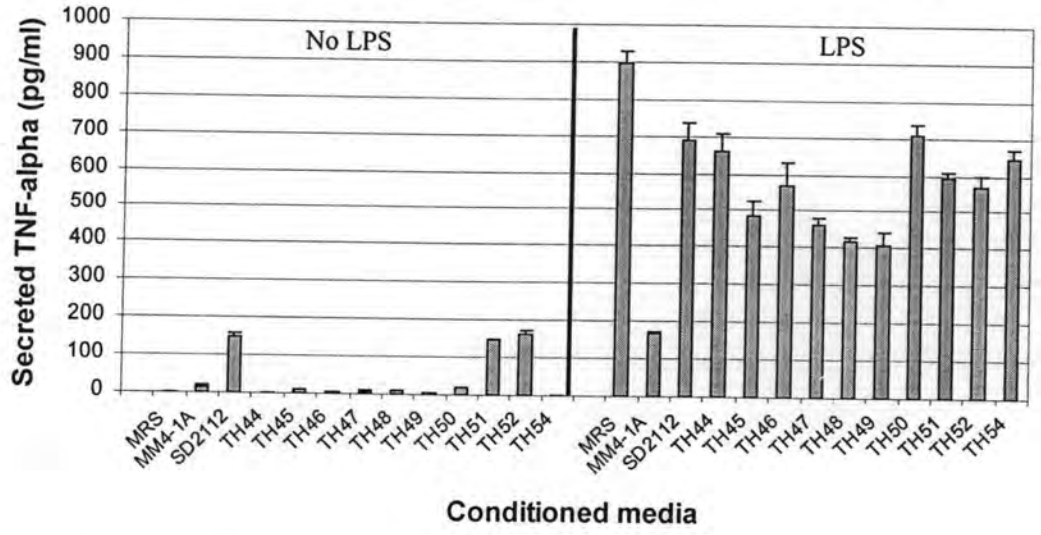


Figure 7. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; LPS, lipopolysaccharide; error bars indicate standard deviations; n=3

Table 11. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; SD, standard deviation

LCM	TNF-alpha (pg/ml)	SD	LCM+LPS	TNF-alpha (pg/ml)	SD	% Inhibition
MRS	4.365	2.844	MRS	1892	94.566	
MM4-1A	21.337	1.99	MM4-1A	293	40.653	85
SD2112	289.734	63.073	SD2112	1627	108.913	14
TH6	15.963	4.472	TH6	1324	105.52	30
TH56	282.198	31.362	TH56	1517	129.311	20
TH57	46.43	10.882	TH57	1684	166.802	11
TH58	4.313	0.626	TH58	666	60.532	65
TH59	63.756	10.269	TH59	1626	126.761	14
TH60	278.919	64.26	TH60	1709	77.005	10
TH61	31.222	2.138	TH61	1238	222.777	35
TH62	31.087	0.483	TH62	1318	201.045	30
TH63	170.441	11.232	TH63	1636	171.71	14
TH64	1.602	0.226	TH64	1630	331.001	14

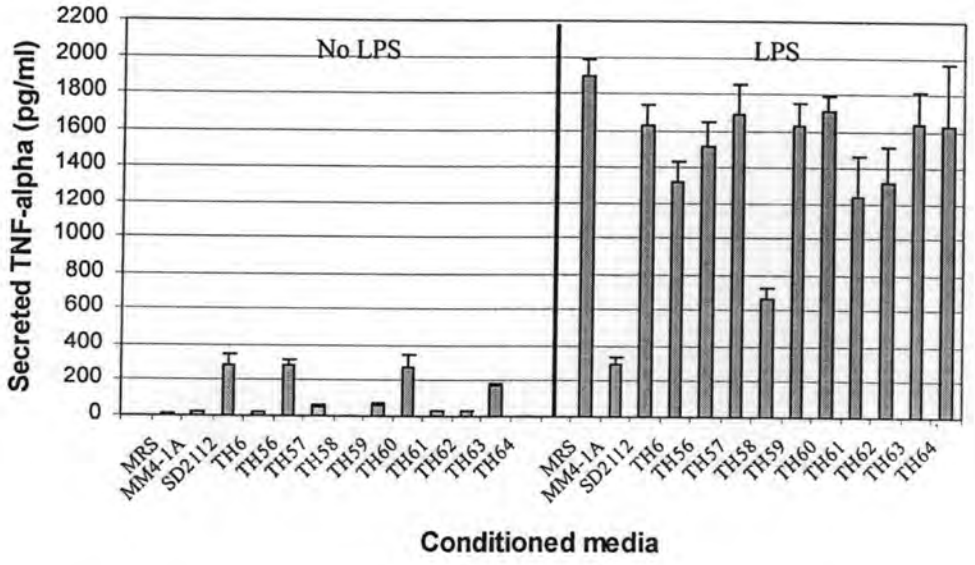


Figure 8. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; LPS, lipopolysaccharide; error bars indicate standard deviations; n=3

Table 12. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; SD, standard deviation

LCM	TNF-alpha (pg/ml)	SD	LCM+LPS	TNF-alpha (pg/ml)	SD	% Inhibition
MRS	0	0	MRS	1841	78.039	4
MM4-1A	23.557	6.878	MM4-1A	310	34.236	83
SD2112	274.26	36.148	SD2112	1515	86.527	18
TH9	33.897	19.1	TH9	1533	63.112	1
TH14	520.763	42.7	TH14	1453	60.95	21
TH15	260.577	21.685	TH15	1639	38.327	11
TH22	209.802	19.703	TH22	1366	48.364	26
TH24	2.527	1.042	TH24	1307	36.71	29
TH27	8.245	6.276	TH27	1290	19.355	30

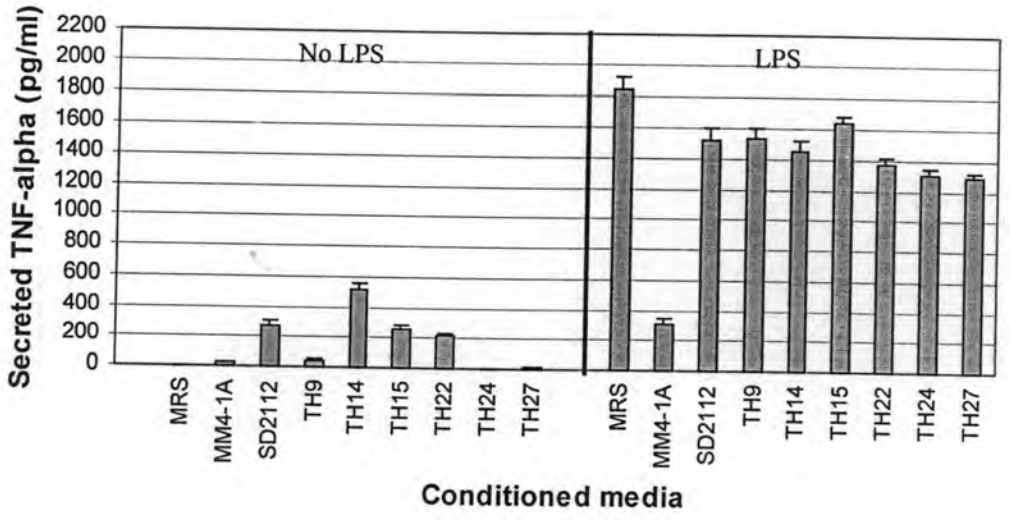


Figure 9. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; LPS, lipopolysaccharide; error bars indicate standard deviations; n=3

Table 13. Summary of immunomodulatory effects of 46 *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain

LCM+LPS	TNF-α inhibition (%)	LCM+LPS	TNF-α inhibition (%)
MRS	4	TH39	53
MM4-1A	80	TH40	30
SD2112	14	TH41	30
TH6	30	TH42	56
TH9	16	TH43	57
TH10	15	TH44	26
TH12	11	TH45	45
TH14	21	TH46	36
TH15	11	TH47	49
TH16	15	TH48	53
TH17	8	TH49	54
TH19	35	TH50	21
TH21	27	TH51	34
TH22	26	TH52	36
TH23	23	TH54	28
TH24	29	TH56	20
TH27	30	TH57	11
TH29	31	TH58	65
TH32	24	TH59	14
TH33	34	TH60	10
TH34	28	TH61	35
TH35	25	TH62	30
TH36	14	TH63	14
TH37	25	TH64	14
TH38	42		

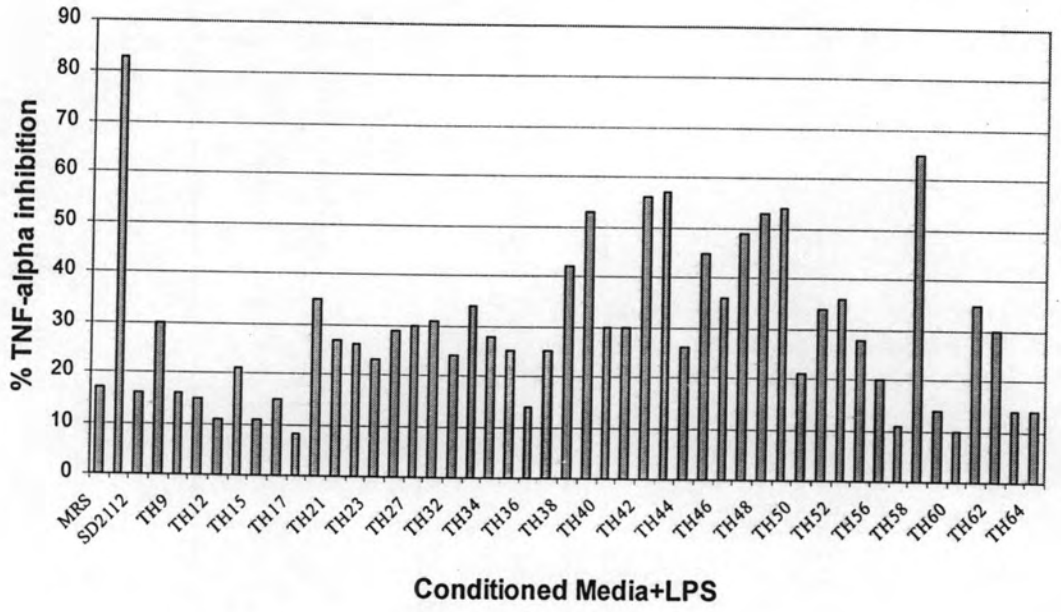


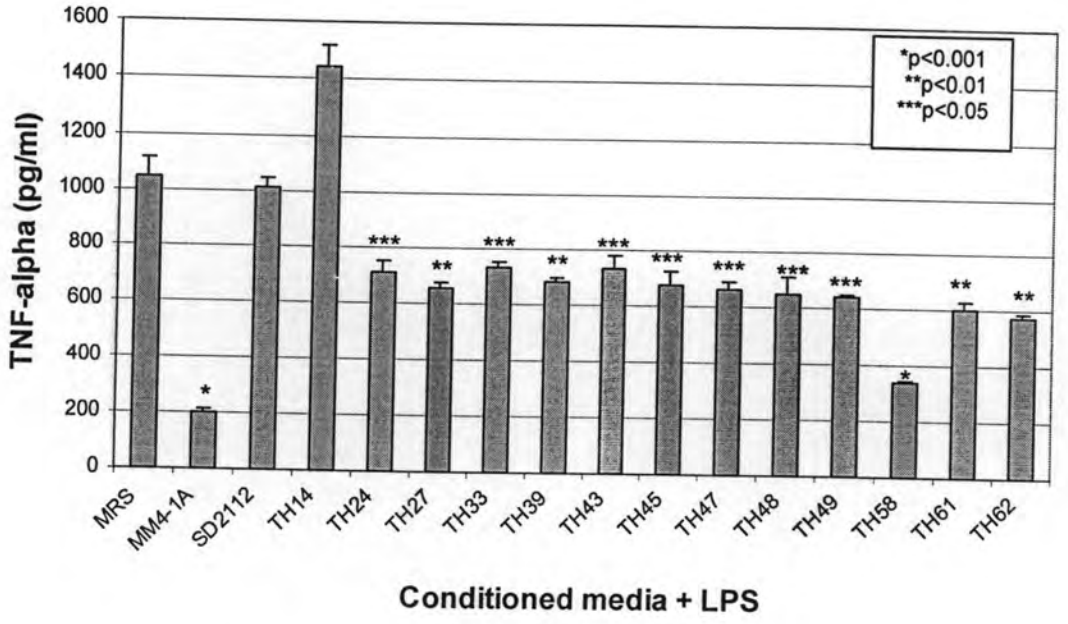
Figure 10. Summary of immunomodulatory effects of 46 *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain

Table 14. Immunomodulatory effects of selected *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF- α inhibitory strain; SD, standard deviation

LCM+LPS	TNF-α (pg/ml)	SD	% Inhibition	p-value
MRS	1048	64.809	-33	
MM4-1A	197	14.404	-81	<0.001
SD2112	1009	40.136	-4	N.S
TH14	1443	77.208	66	N.S
TH24	710	43.372	-32	<0.05
TH27	655	22.735	-38	<0.01
TH33	734	19.435	-30	<0.05
TH39	684	15.193	-35	<0.01
TH43	731	48.98	-30	<0.05
TH45	680	42.661	-35	<0.05
TH47	665	27.051	-37	<0.01
TH48	647	67.088	-38	<0.01
TH49	640	11.882	-39	<0.01
TH58	332	7.18	-68	<0.001
TH61	603	27.428	-42	<0.01
TH62	574	13.215	-45	<0.01

N.S, Not significant when compared to MRS bacterial media control

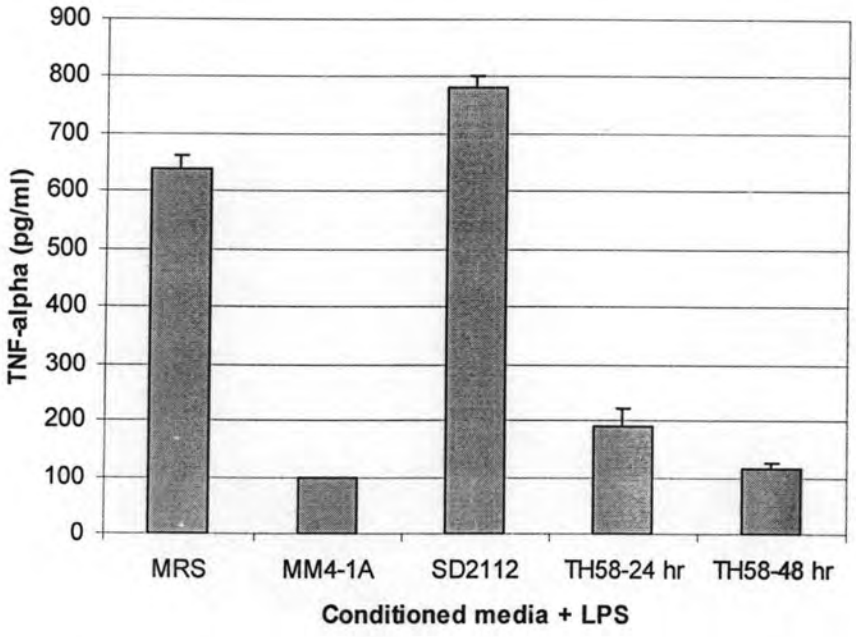
- , inhibited; +, activated



Figures 11. Immunomodulatory effects of *Lactobacillus* isolates on TNF-α production in LPS-activated THP-1 monocytic cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; TH14, TNF-α stimulatory stain; n=9 ; Asterisks denote significantly different from MRS bacterial media control * ($p<0.001$); ** ($p<0.01$); *** ($p<0.05$); error bars indicated standard deviations.

Table 15. TNF-α inhibitory activity of TH58 grown in MRS bacterial media for 24 hr and 48 hr. LPS, lipopolysaccharide; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibiory stain; SD, standard deviation; -, inhibited; +, activated

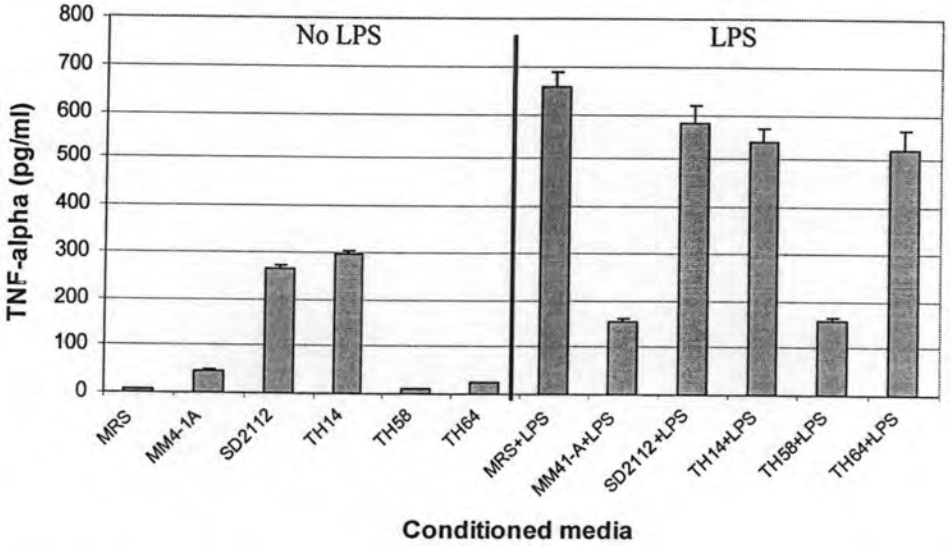
LCM+LPS	TNF-α (pg/ml)	SD	% Inhibition
MRS	640	22.395	
MM4-1A	99	0.816	-85
SD211	781	19.41	22
TH58-24 hr	190	29.327	-70
TH58-48 hr	114	13.062	-82



Figures 12. Inhibitory effect of TH58 strain on TNF-α production in LPS-activated THP-1 monocytic cells. LPS, lipopolysaccharide; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; MRS, bacterial media control; n=9; Asterisks denote significantly different from MRS media control * ($p<0.001$); error bars indicated standard deviations.

Table 16. Summary of TNF-α inhibitory properties of immunomodulatory strains. LPS, lipopolysaccharide; MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibiory stain; SD, standard deviation

LCM	TNF-α	SD	% Inhibition
MRS	8	0.336	
MM4-1A	45	2.14	
SD2112	264	8.866	
TH14	295	8.056	
TH58	9	0.215	
TH64	24	1.716	
MRS+LPS	656	30.96	
MM41-A+LPS	152	8.503	-77
SD2112+LPS	582	37.114	-11
TH14+LPS	539	30.062	-18
TH58+LPS	158	6.619	-76
TH64+LPS	523	43.517	-20



Figures 13. Summary of immunomodulatory properties of TH14, TH58, TH64 in LPS-activated THP-1 cells. MRS, bacterial media control; MM4-1A, positive control of TNF-α inhibitory strain, SD2112: negative control of non-TNF-α inhibitory strain; error bars indicated standard deviations; n=6.

6. Effect of Selected *Lactobacillus* Strains on Nuclear Factor kappa B (NF-κB) Activation

In this study, TH58 and TH14, the most potent TNF-α inhibitory activity and the immunostimulatory strains, respectively were chosen for further investigation to test the effect on NF-κB activation in LPS-activated THP-1 human monocytic cells. THP-1 cells were incubated with LCM and treated with or without LPS for 30 min. NF-κB transcription factor proteins were extracted from nuclei of THP-1 cells, and nuclear protein quantities were determined by BCA protein assay as described in Materials and Methods (Chapter III). Protein contents were evaluated using standard curve as shown in Figure 14. NF-κB p65 ELISA was used to evaluate NF-κB activation and displayed as OD_{450} values. As displayed in Table 17 and Figure 15, TH58, TNF-α inhibitory strain had no effect on relative the amounts of active NF-κB in nuclei of LPS-activated THP-1 cells. In addition, in absence of LPS, TH58 did not activate NF-κB. In contrast, TH14, a TNF-α stimulatory strain, induced NF-κB activation in absence of LPS ($p<0.001$). None of other strains significantly suppressed NF-κB activation in LPS-activated THP-1 human monocytic cells.

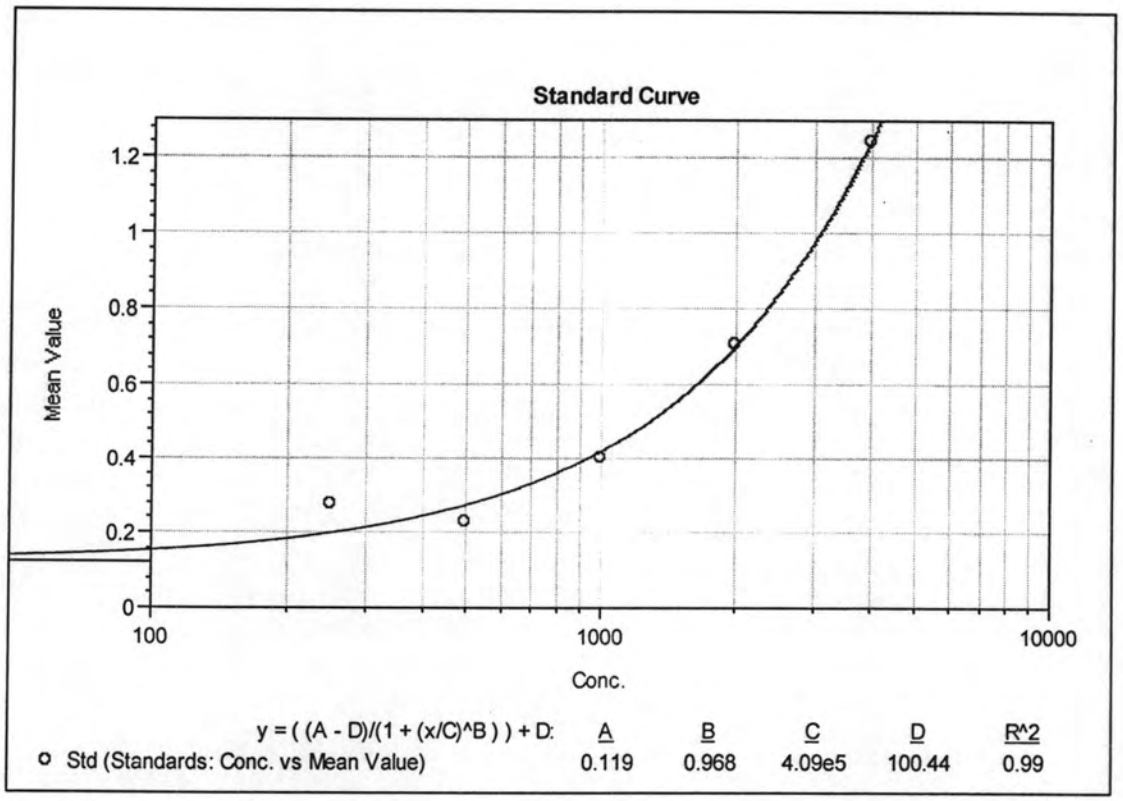
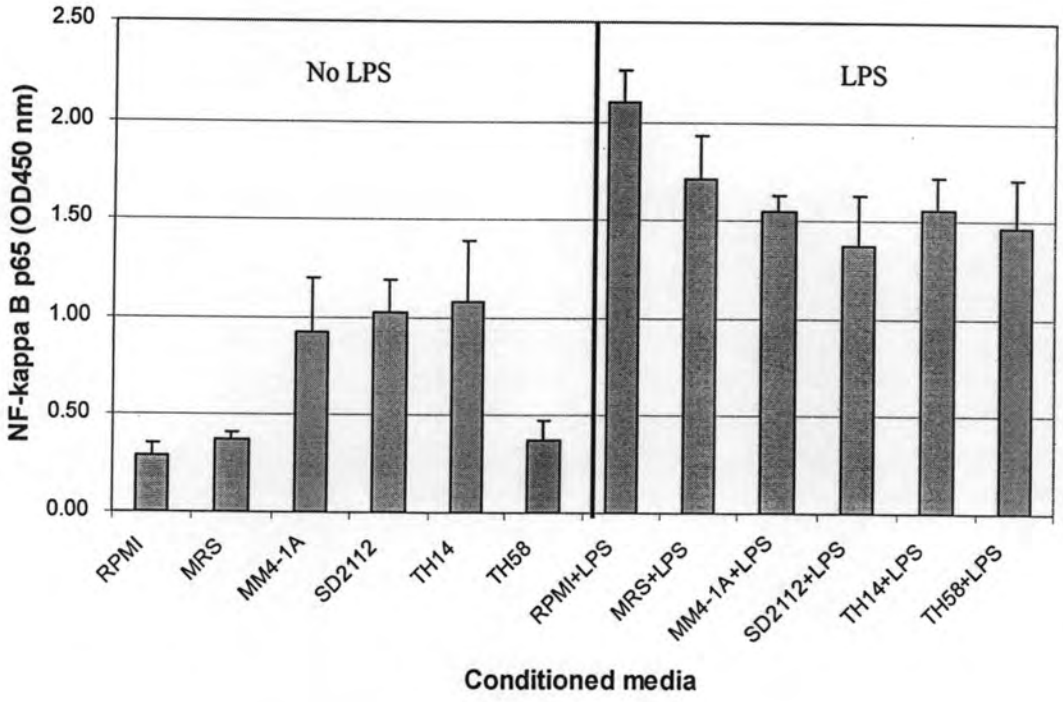


Figure 14. Standard curve of nuclear protein determination by BCA protein assay at the concentration of 250, 500, 1,000, 2,000, and 4,000 μg/ml; $R^2 = 0.99$

Table 17. Effects of selected *Lactobacillus* strains on NF-κB activation in LPS- activated THP-1 monocytic cells. RPMI, cell culture media; MRS, bacterial media control; LPS, lipopolysaccharide; MM4-1A, positive control of TNF-α inhibitory stain; SD2112, negative control of non-TNF-α inhibiory stain; SD, standard deviation.

Conditioned media	Mean (OD)	SD	p value
RPMI	0.282	0.071	
MRS	0.365	0.041	
MM41-A	0.923	0.280	
SD2112	1.028	0.165	
TH14	1.083	0.313	0.001
TH58	0.371	0.098	
RPMI+LPS	2.106	0.158	
MRS+LPS	1.718	0.215	
MM41-A+LPS	1.551	0.080	0.1
SD2112+LPS	1.375	0.258	0.06
TH14+LPS	1.556	0.167	0.165
TH58+LPS	1.468	0.246	0.069
positive	0.443	0.004	
negative	0.143	0.024	

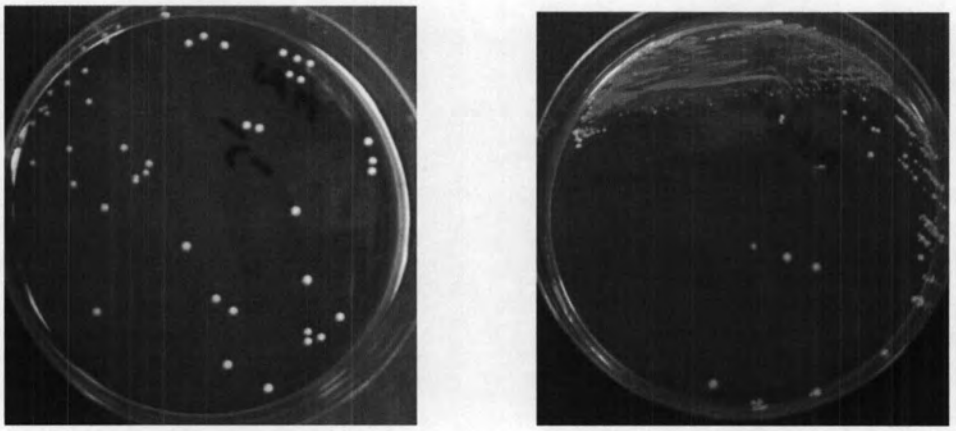


Figures 15. Effects of selected *Lactobacillus* strains to suppress NF-κB activation in LPS-activated THP-1 monocytic cells. RPMI, cell culture media; MRS, bacterial media control; LPS, lipopolysaccharide MM4-1A, positive control of TNF-α inhibitory strain; SD2112, negative control of non-TNF-α inhibitory strain; TH14, TNF-α stimulatory stain; n=6; error bars indicated standard deviations.

7. Phenotypic characteristics of TH58 strain

7.1 Morphology of TH58 strain

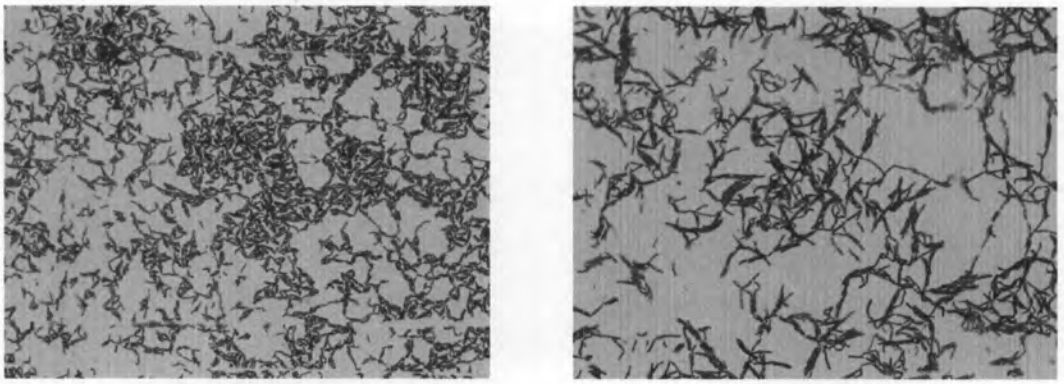
In Figure 16 demonstrated colony characteristics on MRS agar of the TH58 strain and TH14 strains. Colony morphologies of TH58 (Figure 16 A) and TH14 (Figure 16 B) were characterized by 1-2 mm diameters, white color, smooth margins, round and convex contours.



A. TH58 strain B. TH14 strain

Figure 16. Colony morphologies of TH58 and TH14 strains

Gram staining was performed to observed microscopic morphology of TH58, TNF-α inhibitory strain and TH14, TNF-α stimulatory strain. TH58 was a gram-positive regular rod, arrange as single cells or in pairs (Figure 17 A). TH14 was a gram-positive rod arrange as single cells, pairs or short chains of cells (Figure 17 B).



A. TH58 strain B. TH14 strain

Figure 17. Gram stain morphologies of TH58 and TH14 strains.

7.2 Growth characteristics of *Lactobacillus* strain TH58

As shown in Figure 18, lag phase of TH58 was 6 hr and grew rapidly into log phase during 6-28 hr. Stationary phase of TH58 was evident at 28-40 hr and a decline phase follow after 40 hrs. The generation time of TH58 was 5.45 hr.

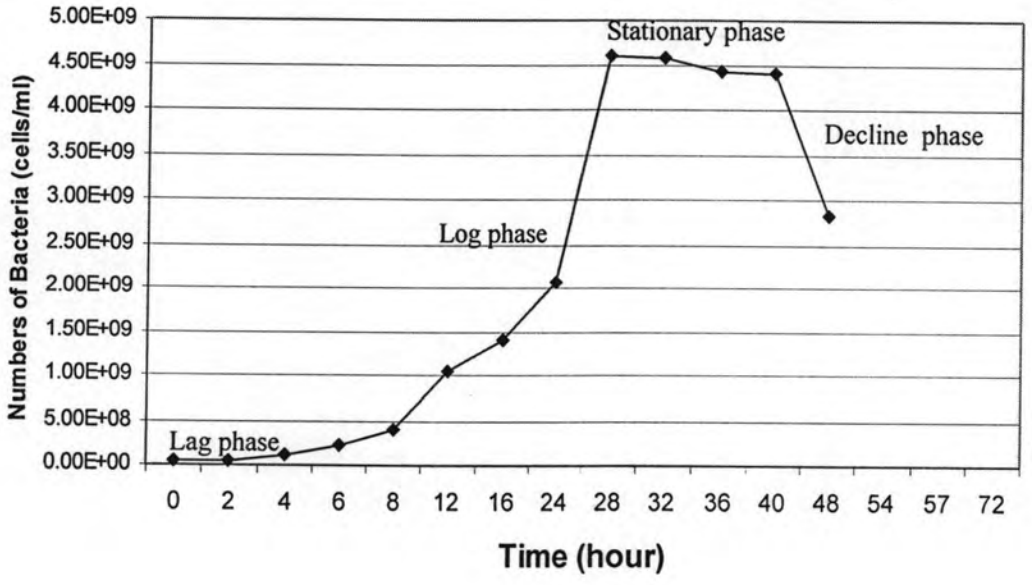


Figure 18. Growth curve of TH58 in MRS broth

7.3 Acid, bile and aerotolerance of TH58 strain

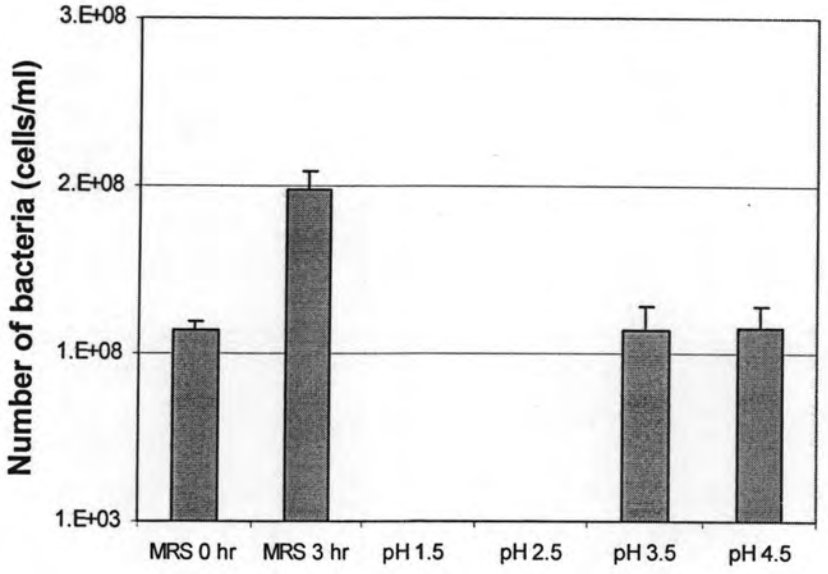
7.3.1 Acid tolerance

Acid tolerance test for TH58 anti-TNF-α inhibitory strain was performed by inoculated into MRS media at pH level 1.5, 2.5, 3.5 and 4.5. The result of acid tolerance (survival at various pH values) showed that viability of TH58 was changed after incubation for 3 hr at pH 2.5, 3.5, 4.5. The viable counts of pH 2.5 decreased about 3 log values when compared to MRS control, but showed more acid tolerance in the pH 3.5, 4.5 which showed no log difference as report in Table 18 and Figure 19. No growth occurred after incubated at pH 1.5 for 3 hr.

Table 18. Survival of TH58 strain after incubated at various pH values

pH value	Number of bacteria (cells/ml)	SD
MRS 0 hr	1.13E+08	5.8E+05
MRS 3 hr	1.98E+08	1.1E+07
pH 1.5	0.00E+00	0.0E+00
pH 2.5	1.22E+05	4.5E+03
pH 3.5	1.14E+08	1.3E+07
pH 4.5	1.15E+08	1.3E+07

A



B

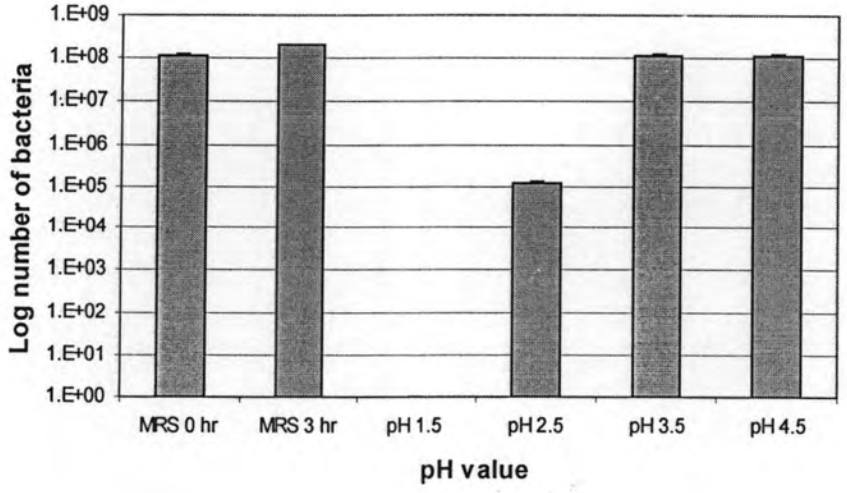


Figure 19. Survival of TH58 strain after incubation at various pH values.

A: Indicated in cell number

B: Indicated in log number

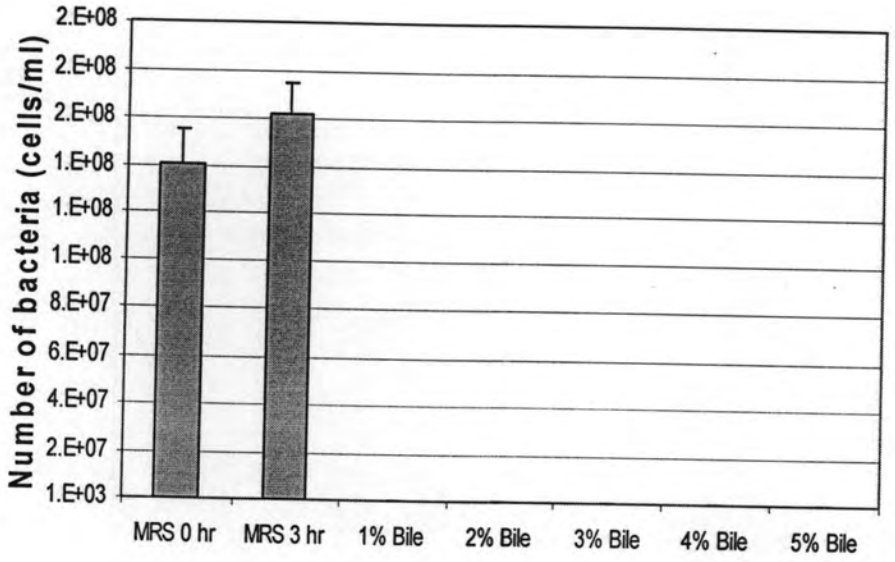
7.3.2 Bile tolerance

In this study, TH58 was cultivated in MRS broth with or without various concentrations of bovine bile as shown in Table 19 and Figure 20. After 3 hr incubation, viability of TH58 was decreased by about 3.5 log in 1% and 2% bovine bile when compared to MRS bacterial media control. Whereas, TH58 incubated in 3%, 4%, 5% bovine bile, the viable counts were decreased by about 4.5 log differences when compared to MRS bacterial media control.

Table 19. Survival of TH58 strain after incubated in various concentration of bile

% Bile	Number of bacteria (cells/ml)	SD
MRS 0 hr	1.40E+08	1.50E+07
MRS 3 hr	1.62E+08	1.27E+07
1% Bile	5.57E+04	1.54E+03
2% Bile	2.90E+04	5.13E+02
3% Bile	5.77E+03	8.00E+01
4% Bile	3.34E+03	1.15E+02
5% Bile	2.01E+03	5.50E+01

A



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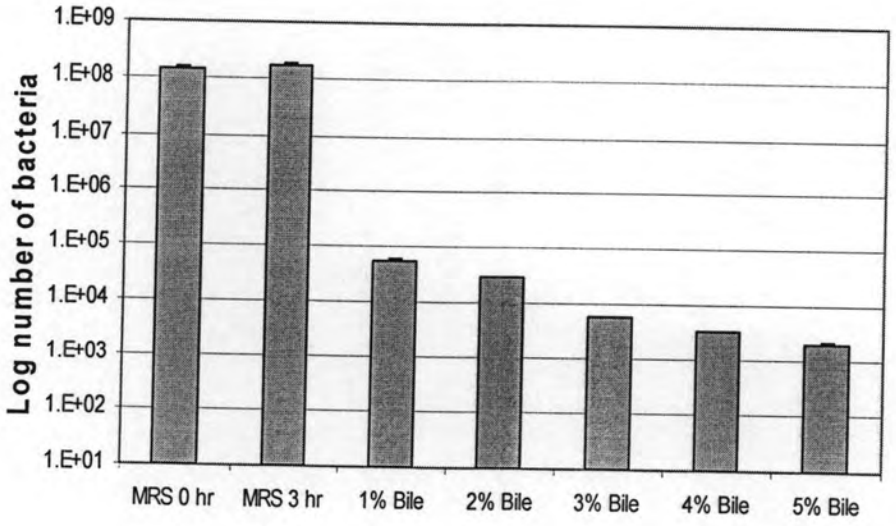


Figure 20. Bile tolerance of TH58

A: Indicated in cell number

B: Indicated in log number

7.3.3 Aerotolerance test

TH58 was cultivated in MRS and incubated in both of aerobic and anaerobic conditions. In Table 20 and Figure 21 demonstrated TH58 was able to survive in aerobic conditions about 0.5 log less than in anaerobic condition.

Table 20. Aerotolerance test of TH58

Incubation condition	Mean number of bacteria (cell/ml)	SD
Aerobic	1.45E+09	3.68E+08
Anaerobic	5.30E+09	9.90E+08

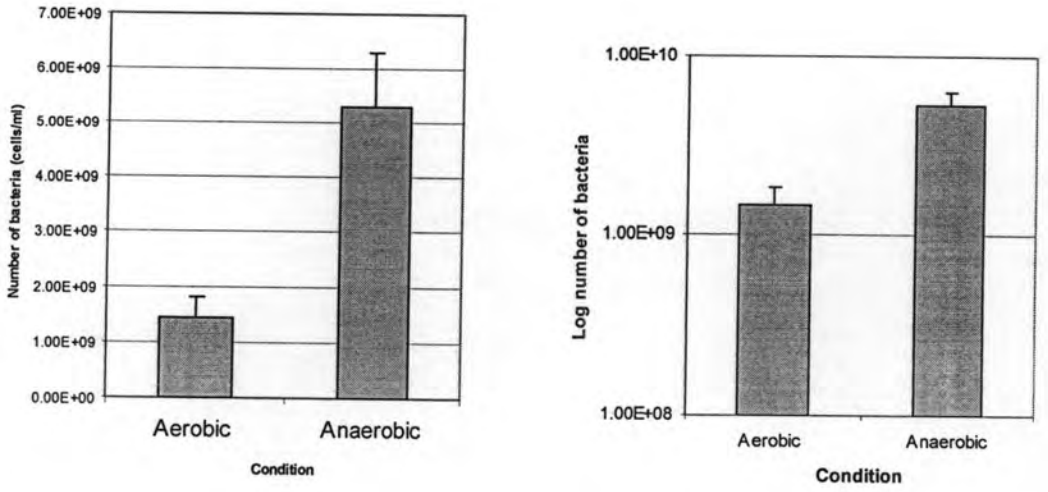


Figure 21. Aerotolerance of TH58 in MRS broth in aerobic and anaerobic conditions

A: Indicated in cell number

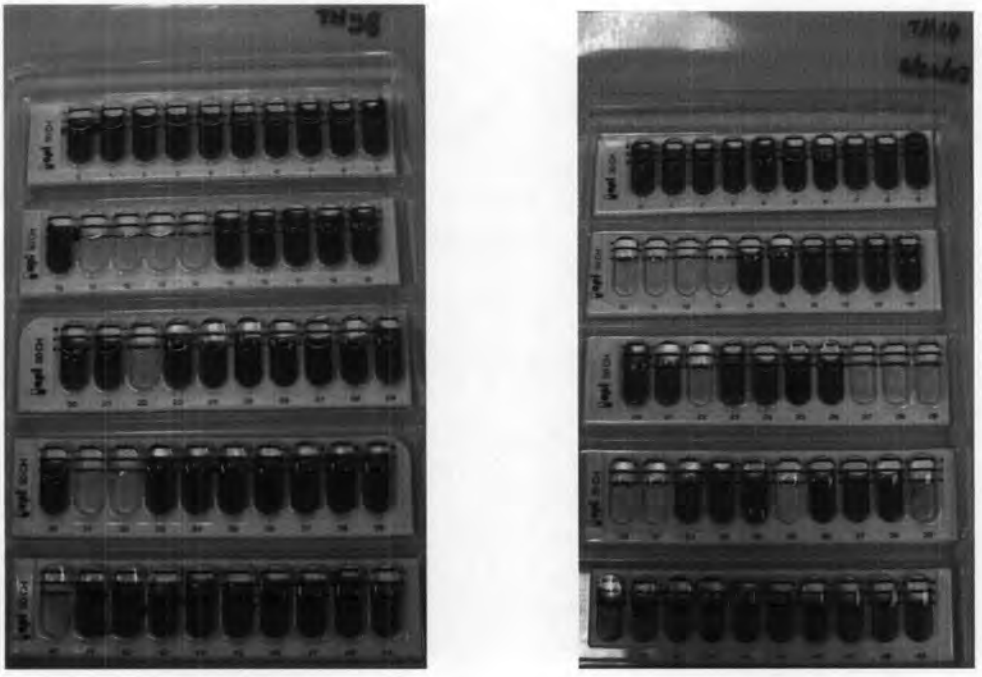
B: Indicated in log number

8. Phenotypic Characteristics of Selected *Lactobacillus* isolates by Carbohydrate Fermentation Profile (API 50 CHL)

Selected *Lactobacillus* strains including anti-pathogenic strains, TNF-α inhibitory strains, TNF-α stimulatory strain, non-TNF-α inhibitory and non-TNF-α stimulatory strain were characterized by API 50 CHL as shown in Tables 21-38 and Figure 22. The carbohydrate fermentation patterns were used to determine the species of selected *Lactobacillus* isolates and analyzed by API database, API 50 CHL V5.1 at https://apiweb.biomerieux.com/servlet/Identify. Four anti-pathogenic strains SB42-6, BJ48-5, RT49-5 and RT49-7 obtained by agar well diffusion assay were able to utilize L-arabinose, ribose, galactose, glucose, fructose, mannose, mannitol, sorbital, methyl-D-mannoside, N-acethyl-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, gentiobiose, D-turanose and gluconate as shown in Tables 21-24. These four strains were showed 99.9% identity to *L. plantarum* and 0.1 % identity to *L. pentosus*.

For the immunomodulatory strains, 8 strains of TH24, TH27, TH39, TH45, TH47, TH48, TH49 and TH61 were able to utilize almost carbohydrates similar to 4 anti-pathogenic strains as described above but different in some sugars as indicated in Tables 26, 27, 29, 31-34, 36. They were also identified as *L. plantarum* in varying identity of 99%, 53%, 53%, 99%, 92%, 99.9%, 99.9% and 91% respectively. The lowest identity of each strain was identified as *L. pentosus*. TH33 and TH58 strains showed different carbohydrate utilization patterns from strains as described above. TH33 was identitical to *L. salivarius* with 99.9% (Table 28). TH58, as shown carbohydrate utilization patterns in Figure 22A showed low identity to *Pediococcus damnosus*, *L. acidophilus*, *Weissella viridescens*, *L. delbrueckii* spp. lactis,

L. delbrueckii spp *delbrueckii* with 22%, 21%, 18%, 13%, 13% identity respectively (Table 35). TH43 as demonstrated in Table 30 was identified as *L. paracasei* spp. *paracasei* or *L. plantarum* with 61% and 37% identity respectively. TH62 showed some carbohydrate utilization patterns different from *L. plantarum* group and identified as *L. brevis* or *L. plantarum* with 90% and 3% similarity respectively (Table 37). TH14, TNF-α stimulatory strain as shown carbohydrate utilization patterns by API 50 CHL in Figure 22B was identified as *L. lactis* or *L. acidophilus* with 98% and 1% identity respectively (Table 25). TH64, non- TNF-α inhibitory and non-TNF-α stimulatory strain, was identified as *L. brevis*, *P. pentosacceus*, *L. lactis* spp *lactis* and *W. confusa* with 62%, 17%, 15% and 5% identity respectively (Table 38).



A. TH58 strain B. TH14 strain

Figure 22. Carbohydrate utilization patterns of TH58, TNF-α inhibitory strain and TH14, TNF-α stimulatory strain by API 50 CHL

Table 21. Carbohydrate fermentation of SB42-6 *Lactobacillus* strain*

Test	Carbohydrate	SB42-6	Test	Carbohydrate	SB42-6
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	B-methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9 % identity to *L. plantarum* and 0.1 % identity to

L. pentosus

Table 22. Carbohydrate fermentation of BJ48-5 *Lactobacillus* strain*

Test	Carbohydrate	BJ48-5	Test	Carbohydrate	BJ48-5
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9 % identity to *L. plantarum* and 0.1 % identity to

L. pentosus

Table 23. Carbohydrate fermentation of RT49-5 *Lactobacillus* strain*

Test	Carbohydrate	RT49-5	Test	Carbohydrate	RT49-5
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9 % identity to *L. plantarum* and 0.1 % identity to

L. pentosus

Table 24. Carbohydrate fermentation of RT49-7 *Lactobacillus* strain*

Test	Carbohydrate	RT49-7	Test	Carbohydrate	RT49-7
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9 % identity to *L. plantarum* and 0.1 % identity to

L. pentosus

Table 25. Carbohydrate fermentation of TH14 *Lactobacillus* strain*

Test	Carbohydrate	TH14	Test	Carbohydrate	TH14
1	Glycerol	-	26	Salicin	-
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	-	29	Lactose	+
5	Ribose	-	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	-
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	-
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	-	43	D-fucose	-
19	Sorbital	-	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	-
23	Amygdalin	-	48	2-keto-gluconate	-
24	Arbutin	-	49	5-keto-gluconate	-
25	Esculin	-			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 98 % identity to *Leuconostoc lactis* (*Leu. lactis*) and 1 % identity to *L. acidophilus*

Table 26. Carbohydrate fermentation of TH24 *Lactobacillus* strain*

Test	Carbohydrate	TH24	Test	Carbohydrate	TH24
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	+
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99% identity to *L. plantarum* and 1 % identity to *L. pentosus*

Table 27. Carbohydrate fermentation of TH27 *Lactobacillus* strain*

Test	Carbohydrate	TH27	Test	Carbohydrate	TH27
1	Glycerol	+	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	+
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	+	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 53% identity to *L. plantarum* and 47% identity to *L. pentosus*

Table 28. Carbohydrate fermentation of TH33 *Lactobacillus* strain*

Test	Carbohydrate	TH33	Test	Carbohydrate	TH33
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	-
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	-	29	Lactose	+
5	Ribose	-	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	-
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	-
23	Amygdalin	-	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	-			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9% identity to *L. salivarius*

Table 29. Carbohydrate fermentation of TH39 *Lactobacillus* strain*

Test	Carbohydrate	TH39	Test	Carbohydrate	TH39
1	Glycerol	+	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	+
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	+	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 53% identity to *L. plantarum* and 47% identity to *L. pentosus*

Table 30. Carbohydrate fermentation of TH43 *Lactobacillus* strain*

Test	Carbohydrate	TH43	Test	Carbohydrate	TH43
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	-	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	-
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	+	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	-
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	-
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 61% identity to *L. p*aracasei spp *paracasei* and 37% identity to *L. plantarum*

Table 31. Carbohydrate fermentation of TH45 *Lactobacillus* strain*

Test	Carbohydrate	TH45	Test	Carbohydrate	TH45
1	Glycerol	+	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	-	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	+	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	-
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99% identity to *L. plantarum* and 0.4% identity to *L. pentosus*

Table 32. Carbohydrate fermentation of TH47 *Lactobacillus* strain*

Test	Carbohydrate	TH47	Test	Carbohydrate	TH47
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 92% identity to *L. plantarum* and 8% identity to *L. pentosus*

Table 33. Carbohydrate fermentation of TH48 *Lactobacillus* strain*

Test	Carbohydrate	TH48	Test	Carbohydrate	TH48
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9% identity to *L. plantarum* and 0.1% identity to *L. pentosus*

Table 34. Carbohydrate fermentation of TH49 *Lactobacillus* strain*

Test	Carbohydrate	TH49	Test	Carbohydrate	TH49
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	+	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 99.9% identity to *L. plantarum* and 0.1% identity to *L. pentosus*

Table 35. Carbohydrate fermentation of TH58 *Lactobacillus* strain*

Test	Carbohydrate	TH58	Test	Carbohydrate	TH58
1	Glycerol	-	26	Salicin	-
2	Erythritol	-	27	Cellobiose	-
3	D-arabinose	-	28	Maltose	-
4	L-arabinose	-	29	Lactose	-
5	Ribose	-	30	Melibiose	-
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	-	35	Raffinose	-
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	+	39	Gentiobiose	-
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	-	43	D-fucose	-
19	Sorbital	-	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	-
23	Amygdalin	-	48	2-keto-gluconate	-
24	Arbutin	-	49	5-keto-gluconate	-
25	Esculin	-			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 22% identity to *Pediococcus damnosus,* 21% identity to *L. acidophilus*, 18% identity to *Weissella viridescens*, 13% identity to *L. delbrueckii* ssp *lactis*, 13% identity to *L. delbrueckii* ssp *delbrueckii*

Table 36. Carbohydrate fermentation of TH61 *Lactobacillus* strain*

Test	Carbohydrate	TH61	Test	Carbohydrate	TH61
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	+
9	β – methyl-D-xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 91% identity to *L. plantarum*, 8% identity to *L. brevis* and 0.4% identity to *L. pentosus*

Table 37. Carbohydrate fermentation of TH62 *Lactobacillus* strain*

Test	Carbohydrate	TH62	Test	Carbohydrate	TH62
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	-	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	-	31	Sucrose	+
7	L-xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inuline	+
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	-
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	+
15	Rhamnose	-	40	D-turanose	+
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	+	43	D-fucose	-
19	Sorbital	+	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	+
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 90% identity to *L. brevis* and 3% identity to *L. pentosus*

Table 38. Carbohydrate fermentation of TH64 *Lactobacillus* strain*

Test	Carbohydrate	TH64	Test	Carbohydrate	TH64
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-arabinose	-	28	Maltose	+
4	L-arabinose	+	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-xylose	+	31	Sucrose	+
7	L-xylose	-	32	Trehalose	-
8	Adonitol	-	33	Inuline	-
9	β – methyl-D-xyloside	-	34	Melezitose	-
10	Galactose	+	35	Raffinose	+
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentiobiose	-
15	Rhamnose	-	40	D-turanose	-
16	Dulcitol	-	41	D-lyxose	-
17	Inositol	-	42	D-tagatose	-
18	Mannitol	-	43	D-fucose	-
19	Sorbital	-	44	L-fucose	-
20	Methyl-D-mannoside	-	45	D-arabitol	-
21	Methyl-D-glucoside	-	46	L-arabitol	-
22	N-acethyl-glucosamine	+	47	Gluconate	-
23	Amygdalin	+	48	2-keto-gluconate	-
24	Arbutin	+	49	5-keto-gluconate	-
25	Esculin	+			

+, acid production; -, no acid production

* The patterns were analyzed by API 50 CHL V 5.1 at

https://apiweb.biomerieux.com/servlet/Identify

API database indicated 62% identity to *L. brevis*, 17% identity to *Pediococcus pentosacceus*, 15% entity to *L. lactis* ssp *lactis* and 5% dentity to *Weissella confusa*

9. Genotypic Characteristics of Selected *Lactobacillus* strains

9.1 Genotypic identification based on 16S rRNA gene dideoxy DNA sequencing and DNA pyrosequencing

Selected *Lactobacillus* stains including 4 anti-pathogenic strains and 14 immunomodulatory strains characterized by 16S rRNA gene dideoxy DNA sequencing. Genomic DNA of these strains were extracted and 16S rRNA genes were amplified. Purified 16S rRNA gene products were sequenced with the same forward and reverse primer. The bases sequences displayed as N at the beginning and terminal of sequences were excluded and then analyzed by using the sequence match program at the RDP II. The highest similarity value closely related to 100% was used for species identification. The sequence of 16S rRNA genes and 90-100% closet match organism of each *Lactobacillus* was displayed in Tables 39-52. These tables demonstrated forward and reverse nucleotide sequences of 16S rRNA genes and identity of closet match organism.

For immunomodulatory strains, 10 strains of TH24, TH27, TH39, TH43, TH45, TH47, TH48, TH49, TH61 and TH62 were identified as *L. plantarum*, *L. pentosus* and *L. paraplantarum* with 98-100% identity both of forward and reverse sequences as shown in Tables 40, 41, 43-48, 50, 51 respectively. Both forward and reverse sequences of TH14 were identified as *L. ruminis* with 97% and 98% identity respectively (Table 39). The TH33 as shown in Table 42, both forward and reverse sequences were classified as *L. salivarius* with 98% and 99% identity respectively. TH58, the most potent TNF-α inhibitory strain was identified as *L. saerimneri* with 99% and 97% identity of forward and reverse sequences respectively (Table 49).

Forward sequences of TH64 as demonstrated in Table 52, was identified as *W. confusa* with 94% identity whereas, reverse sequences was identified as *W. cibaria* and *W. confusa* with 100% and 98% identity respectively.

Selected *Lactobacillus* strains as described above also chosen to characterize by pyrosequencing in the V1 and V3 variable regions of 16S rRNA gene. Approximately 20-45 bases of the V1 and V3 sequences were analyzed at RDP II. The highest similarity closed to 100% was used to identify (Tables 53-58).

Four anti-pathogenic strains SB42-6, BJ48-5, RT49-5 and RT9-7 as demonstrated in Table 53 were identified as *L. plantarum* and *L. pentosus* with 100% identity in V1 sequences.

For immunomodulatory strains, 10 strains of TH24, TH27, TH39, TH43, TH45, TH47, TH48, TH49, TH61 and TH62 were identified as *L. plantarum*, *L. pentosus* and *L. paraplantarum* with 100% identity both V1 and V3 sequences of all strains as shown in Tables 54-58, excepted TH47 displayed 81% and 100% identity of V1 and V3 sequences respectively. TH14 was identified as *L. ruminis* with 100% identity both V1 and V3 sequences (Table 54). TH33 strain was identified as *L. salivarius* with 100% and 81% of V1 and V3 sequences respectively (Table 55). The V1 sequence of TH58 strain was identified as *L. saerimneri* with 100% identity, whereas V3 sequence was identified as *L. saerimneri* and *L. aviaries* with 100% identities (Table 57). The V1 sequences of TH64 was showed 100% identity to *W. cibaria*, *W. confusa* and *W. viridescens*, whereas V3 sequences was displayed 100% identity to *Anaerofustis stercorihominis, A. contaminans, A. voinovskiensis, Facklamia sourekii, Vagococcus salmoninarum, W. thailandensis, W. confusa, W. hellenica* and *W. cibaria* (Table 58).

In this study, phenotypic and genotypic characteristics were used to identify selected *Lactobacillus* strains. The highest identity closely related to 100% was used for species identification. As showed in Table 59, anti-pathogenic strains belonged to *L. plantarum* or *L. plantarum* group [33] including *L. plantarum, L. pentosus* and *L. paraplantarum* based on API 50 CHL, 16S rRNA gene dideoxy DNA sequencing and DNA pyrosequencing.

The immunomodulatory strains, 8 strains named TH24, TH27, TH39, TH45, TH47, TH48, TH49 and TH61 were identified as *L. plantarum* by API (Table 60). As the same results obtained by 16S rRNA gene dideoxy sequencing and pyrosequencing, these strains were identified as *L. plantarum* or *L. plantarum* group. TH33 strain was identified as the same species of *L. salivarius* by API, 16S rRNA gene sequencing and pyrosequencing. TH43 was identified as *L. paracasei* ssp. *paracasei* by API, while by 16S rRNA gene dideoxy sequencing and pyrosequencing was identified as *L. plantarum* or *L. plantarum* group. TH58 was identified as *Pediococcus damnosus* or *L. acidophilus* by API, while it was identified as *L. saerimneri* by 16S rRNA gene dideoxy sequencing and pyrosequencing. TH62 was identified as *L. brevis* by API, while by 16S rRNA gene sequencing and pyrosequencing was identified as *L. plantarum* or *L. plantarum* group.

TH14, immunostimulatory strain was identified as *L. lactis* by API, while by 16S rRNA gene dideoxy sequencing and pyrosequencing, it was identified as *L. ruminis*. TH64, non-anti-inflammatory and non-immunostimulatory strain was identified as *L. brevis* by API, while by 16S rRNA gene dideoxy sequencing and pyrosequencing was identified as *W. cibaria*.

Table 39. Genotypic identification of TH14 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH14 Forward	AGTGGCGAACGGGTGAGTAACACGTAGGCAACCTGCCC AAAAGAGGGGGATAACACTTGGAAACAGGTGCTAATAC CGCATAACCATGAACACCGCATGATGTTCATGTAAAAG ACGGCTTTTGCTGTCACTTTTGGATGGGCCTGCGGCGT ATTAACTTGTTGGTGGGGTAACGGCCTACCAAGGTGAT GATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTG GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA GTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG CAACGCCGCGTGAATGAAGAAGGCCTTCGGGTCGTAAA ATTCTGTTGTCAGAGAAGAACGTGCGTGAGAGTAACTG TTCACGTATTGACGGTATCTGACCAGAAAGCCACGGCT AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCG AGCGTTGTCCGGATTTATTGGGCGTAAAGGGAACGCAG GCGGTCTTTTAAGTCTGATGTGAAAGCCTTCGGCTTAA CCGAAGTAGTGCATTGGAAACTGGAAGACTTGAGTGCA GAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGAAATG CGTAGATATATGGAAGAACACCAGTGGCGAAAGCGGCT CTCTGGTCTGTAACTGACGCTGANGTTCGAAAGCGTGG GTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGATGAGTGCTAAGTGNTGGAGGGTTTCCCGCCC TTCANTGCTGCAGCTAANGCATTAA	*Lactobacillus ruminis*	97 %
TH14 Reverse	CCCCAATCATCTGTCCCACCTTAGGCGGCTGGCTCCAA AAGGTTACCCCACCGACTTTGGGTGTTACAAACTCTCA TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTA TTCACCGCGACATGCTGATTCGCGATTACTAGCGATTC CGACTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAAC TGAGAACGGCTTTAAGAGATTAGCTTGCCCTCGCGAGT TAGCGACTCGTTGTACCGTCCATTGTAGCACGTGTGTA GCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCC CCACCTTCCTCCGGTTTGTCACCGGCAGTCTCGCCAGA GTGCCCAACTTAATGATGGCAACTGACAATAAGGGTTG CGCTCGTTGCGGGACTTAACCCAACATCTCACGACACG AGCTGACGACAACCATGCACCACCTGTCATTCTGTCCC CGAAGGGAACGTTCCATCTCTGGAATTGTCAGAAGATG TCAAGACCTGGTAAGGNTCTTCGCGTTGCTTCGAATTA AACCACATGCTCCACCGCTTGNGCGGGCCCCCG	*Lactobacillus ruminis*	98 %

Table 40. Genotypic identification of TH24 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH24 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTG CATCATGATTNNNNATNNNAGTGAGTGGCGAACTGGT GAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGAT AACACCTGGAAACAGATGCTAATACCGCATAACAACT TGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGC TATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGA TGGTGGGGTAACGGCTCACCATGGCAATGATACGTAG CCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAG ACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCT GTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG CGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGG CGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAA CCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGC AGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA TGCGTAGATATATGGAAGAACACCAGTGGCGAANGCG GCTGTCTGGTCTGTAACTGACGCTGANGCTCGAAAGT ATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCA TACCGTAAACGATGAATGCTAAGTGTTGGANGGTTTC CGCCCTTCAGTGCTGCAGCTAACGCNTTAAGCATTCC GCCTGGGGAGTANNGNCGCNAGGNTGANCTCNAAGNA NTTGACGGGGGNCCGCACAAGCGGTGNANCNTGTGNT TTNANTCNAANCTNCNCGANNAA	*Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum*	100 % 99 % 99 %
TH24 Reverse	ACTTCNCCCTAATCATCTGTCCCACCTTAGGCGGCTG GTTCCTAAAAGGTTNCCCCACCGACTTTGGGTGTTAC AAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCC CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATT ACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCC TACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCT TACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATT GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGAT GATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC CGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGCA ACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAAC CCAACATCTCACGACACGAGCTGACGACAACCATGCA CCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATC TCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGGT TCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACC GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAG CCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGC GTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACA CTTAGCATTCATCGTTTACGGTATGGACTACCAGGGT ATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCA GCGTCAGTTACAGACCANACAGCCGCCTTCGCCACTG GTGTTCTTCCATATATCTACGCATTTCACCGCTACAC ATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTNC CAGTTTCCGATGCACTTCTTCGGNTGAGCCGAANNNT TTNNNATCNNANTTAAAAAACCGCCTGCGCTCGCTTT ACGCCCAATAAANCC	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 41. Genotypic identification of TH27 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH27 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTG CATCATGATTNNNANNNNNGTGAGTGGCGAACTGGTG AGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAACAACTT GGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCT ATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGAT GGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGA CACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAA TCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCC GCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTG TTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAG GTATTGACGGTATTTAACCAGAAAGCCACGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC GTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGC GGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAAC CGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCNTGTGTAGCGGTGAAAT GCGTANATATATGGAAGAACACCAGTGGCGAANNNGG CTGTCTGGTCTGTAACTGACGCTGANGCTCGAAAGTA TGGGTAGCAAACANGATTAGATACCCTGGTAGTCCAT ACCGTAAACNATGAATGCTAAGTGTTGGAGGGTTTCC GCCNTNNNGTGCTGCAGCTAACGCATTAANCNTTCCN CCNGGGGAGNACNNCCGCAAGGCTGAAACTCNNNNAN TTGANGGGGGCCCGCACAANCCGNNGGANNNTGNGGT TTAATTCGAA	*Lactobacillus pentosus* *Lactobacillus plantarum* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 99 % 99 % 95 %
TH27 Reverse	ACTTCNCCCTAATCNTCTGTCCCACCTTAGGCGGCTG GTTCCTAAAAGGTNNNCCNNACCGACTTTGGGTGTTA CAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGC CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGAT TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGC CTACAATCCGAACTGAGAATGGCTTTAAGAGATTAGC TTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCAT TGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGA TGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCA CCGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGC AACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAA CCCAACATCTCACGACACGAGCTGACGACAACCATGC ACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAAT CTCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGG TTCTTCGCGTAGCTTCNAATTAAACCACATGCTCCAC CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCA GCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATG CGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAAC ACTTANCATTCATCGTTTACGGTATGGACTACCAGGG TATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTC ANCGTCAGNTACAGANCANACAGCCGCCTTCNCCACT GGNGNTCNTNNNNATATCTACNCATTTCACCGCTACA CANGGAGTTCNNTGTCCNTCTTCTGNNNTCAAGTTCC CANTTTCCNATGCACTTCTTNNGNTGAGCCNAAAGGC TTNNNNTCANANTTAAAAAACCGCCTGNNNTCNNNTT ANNCCNATNAANCCGGNANAANGCTNG	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	99 % 99 % 99%

Table 42. Genotypic identification of TH33 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH33 Forward	AGTCGAACGAAACTTTCTTACACCGAATGCTTGCATTCACCG TNNNGAAGTTGAGTGGCGGACGGGTGAGTAACACGTGGGTAA CCTGCCTAAAAGAAGGGGATAACACTTGGAAACAGGTGCTAA TACCGTATATCTCTAAGGATCGCATGATCCTTAGATGAAAGA TGGTTCTGCTATCGCTTTTAGATGGACCCGCGGCGTATTAAC TAGTTGGTGGGGTAACGGCCTACCAAGGTGATGATACGTAGC CGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGG CCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAA TGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAG GTCTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACACGAG TGAGAGTAACTGTTCATTCGATGACGGTATCTAACCAGCAAG TCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGAACGCAG GCGGTCTTTTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGG AGTAGTGCATTGGAAACTGGAAGACTTGAGTGCAGAAGANGA GAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATG GAAGAACACCAGTGGCGAAAGCGGCTCTCTGGTCTGTAACTG ACGCTGANGTTCGAAAGCGTGGGTAGCAAACAGGATTAGATA CCCTGGTAGTCCACGCCGTAAACGATGAATGCTNNGNGTTGG AGGGNTTCCGCCCTTCAGTGCCGCAGCTAACGCAATAAGCAT TCCGCCTGGGGAGTACGACCGCANGGNTGAAACTCNAANGAN TTGANGGGGGCCCGCA	*Lactobacillus salivarius*	98 %
TH33 Reverse	ACTTCNCCCCNATCATCTGTCCCACCTTAGACGGCTGGCTCC TTGCGGTTACCCCACCGGCTTTGGGTGTTACAAACTCTCATG GTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC GCGACATGCTGATTCGCGATTACTAGCGATTCCGACTTCATG TAGGCGAGTTGCAGCCTACAATCCGAACTGAGAACGGCTTTA AGAGATTAGCTAAACCTCGCGGTCTCGCGACTCGTTGTACCG TCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGA TGACTTGACGTCGTCCCCACCTTCCTCCGGTTTGTCACCGGC AGTCTCGCCAGAGTGCCCAACTTAATGCTGGCAACTGACAAC AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA CACGAGCTGACGACAGCCATGCACCACCTGTCACTTTGTCCC CGAAGGGAAAGCCTAATCTCTTAGGTGGTCAAAGGATGTCAA GACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACAT GCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTT CAACCTTGCGGTCGTACTCCCCAGGCGGAATGCTTATTGCGT TAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACACCTAGCA TTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGT TTGCTACCCACGCTTTCNAACCTCAGCGTCAGTTACAGACCA GAGAGCCNCTTTCGCCACTGGTGTTCTTCCATATATCTACGC ATTTCANCGCTACACATGNNNTTCCACTCTCCTCTTCTGCAC TCAAGTCTTCCAGTTTCCAATGNACTACNNCNGTTAAGCCGA ANNNTTTNACNTCNNACTNAAANACCGCCTGCGTTCCCNTTA	*Lactobacillus salivarius*	99 %

Table 43. Genotypic identification of TH39 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences 16S rRNA gene	Match organism	Identity
TH39 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTG CATCATGATTTACATTTGAGTGAGTGGCGAACTGGTG AGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAACAACTT GGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCT ATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGAT GGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGA CACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAA TCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCC GCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTG TTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAG GTATTGACGGTATTTAACCAGAAAGCCACGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC GTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGC GGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAAC CGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAAT GCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGG CTGTCTGGTCTGTAACTGACGCTGANGCTCGAAAGTA TGGGTAGCAAACANGATTAGATACCCTGGTAGTCCAT ANCGTAAACGATGAATGCTAAGTGTTGGAGGGNTTCC GCCCTTCAGTGCTGCAGCTNACGCATTAAGCATTCCG CCTGGGGAGTACNGGCCGCANGGCTGAAACTCAANNN ANTNANNGGGGCCCGCACAAGCGGTGGAGCATGNNGN TTAATTCNAAGCTACGCNAANAAC	*Lactobacillus pentosus* *Lactobacillus plantarum* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 99 % 98 % 94 %
TH39 Reverse	ACTTCNCCCTAATCATCTGTCCCACCTTAGGCGGCTG GTTCCTAAAAGGNNNNCCCNACCGACTTTGGGTGTTA CAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGC CCGGGAACGTATTCACCGCGGCATGCTGATCCGCGAT TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGC CTACAATCCGAACTGAGAATGGCTTTAAGAGATTAGC TTACTCTCGCGAGTTCGCAACTCGTTGTACCATCCAT TGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGA TGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCA CCGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGC AACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAA CCCAACATCTCACGACACGAGCTGACGACAACCATGC ACCACCTGTATCCATGTCCCCGAAGGGAACGTCTAAT CTCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGG TTCTTCGCGTAGCTTCGAATTAAACCACATGCTCCAC CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCA GCCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATG CGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAAC ACTTAGCATTCATCGTTTACGGTATGGACTACCAGGG TATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTC AGCGTCAGTTACAGACCAGANAGCCGCCTTCGCCACT GGTGNTCTTCCATATATCTACGCATTTCACCGCTACA CATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTC CCAGTTTCCGATGCACTTCTTCGGTTGAGCCNANNNN TTTCACATCANANTTAAAAAACCGCCTGCGCTCGCTT TACGCCCAATAA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 44. Genotypic identification of TH43 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH43 Forward	TGCNAGTCGAACGAANNTCTGGTATTGATTGGTGCTT GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGT GAGTAACACGTGGGAAACCTGCCCNGAAGCGGGGGAT AACACCTGGAAACAGATGCTAATACCGCATAACAACT TGGACCGCATGGTCCGAGNTTGAAAGATGGCTTCGGC TATCACTTNTGGATGGTCCCGCGGCGTATTAGCTAGA TGGTGAGGTAACGGCTCACCATGGCAATGATACGTAG CCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAG ACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCT GTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG CGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGG CGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAA CCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGC AGAAGAGGACAGTGGAACTCCNTGTGTAGCGGTGAAA TGCGTAGATATATGGAAGAACACCAGTGGCGAANGCG GCTGTCTGGTCTGTAACTGACNCTGANGCTCGAAAGT ATGGGTAGCAAACAGGANTAGATACCCTGGTAGTCCA TACCGTAAACGATGAATGNTAAGTGTTGGANGGTTTC CGCNCTTCANTGCTGCAGCTAACGCATTAANCATTCC GCCTGGGGAGTA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	99 % 99 % 98 % 93 %
TH43 Reverse	ACTTCNCCCTAATCATCTGTCCCACCTTAGGCGGCTG GTTCCTAAAAGGTTACCCCACCGACTTTGGGTGTTAC AAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCC CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATT ACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCC TACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCT TACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATT GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGAT GATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC CGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGCA ACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAAC CCAACATCTCACGACACGAGCTGACGACAACCATGCA CCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATC TCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGGT TCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACC GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAG CCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGC GTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACA CTTAGCATTCATCGTTTACGGTATGGACTACCAGGGT ATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCA GCGTCAGTTACAGACCAGACAGCCGCCTTCGCCACTG GTGTTCTTCCATATATCTACGCATTTCACCGCTACAC ATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCC CAGTTTCCNATGCACTTCTTCNGNTGAGCCNANNNTT TCACATCANANTTAAAAANCGCCTGNNCTCGCTTTAC NNCCNATAAAT	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 45. Genotypic identification of TH45 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH45 Forward	TGCAAGTCGAACGAANNTCTGGTATTGATTGGTGC TTGCATCATGATTNNCATTNNAGTGAGTGGCGAAC TGGTGAGTAACACGTGGGAAACCTGCCCNNAAGNG GGGGATAACACCTGGAAACAGATGCTAATACCGCA TAACAACTTGGACCGCATGGTCCGAGNTTGAAAGA TGGCTTCGGCTATCACTTNTGGATGGTCCCGCGGC GTATTAGCTAGATGGTGAGGTAACGGCTCACCATG GCAATGATACGTAGCCGACCTGAGAGGGTAATCGG CCACATTGGGACTGAGACACGGCCCAAACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG AAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAA GGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG AACATATCTGAGAGTAACTGTTCAGGTATTGACGG TATTTAACCAGAAAGCCACGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTT TTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCG AAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAA ATGCGTAGATATATGGAAGAACACCAGTGGCGAAN GCGGCTGTCTGGTCTGTAACTGACGCTGANGCTCG AAAGTATGGGTAGCAAACAGGATTAGATACCCTGG TAGTCCATACCGTAAACGATGAATGCTAAGTGNTG GAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCA TTAAGCATTCNNCCTGGGGAGTANNGCCGC	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	99 % 99 % 99 %
TH45 Reverse	CNACTTCNCCCTAATCNTCTGTCCCACCTTAGGCG GCTGGTTCCTAAAAGGNNNNCCCNACCGACTTTGG GTGTTACAAACTCTCATGGTGTGACGGGCGGTGTG TACAAGGCCCGGGAACGTATTCACCGCGGCATGCT GATCCGCGATTACTAGCGATTCCGACTTCATGTAG GCGAGTTGCAGCCTACAATCCGAACTGAGAATGGC TTTAAGAGATTAGCTTACTCTCGCGAGTTCGCAAC TCGTTGTACCATCCATTGTAGCACGTGTGTAGCCC AGGTCATAAGGGGCATGATGATTTGACGTCATCCC CACCTTCCTCCGGTTTGTCACCGGCAGTCTCACCA GAGTGCCCAACTTAATGCTGGCAACTGATAATAAG GGTTGCGCTCGTTGCGGGACTTAACCCAACATCTC ACGACACGAGCTGACGACAACCATGCACCACCTGT ATCCATGTCCCCGAAGGGAACGTCTAATCTCTTAG ATTTGCATAGTATGTCAAGACCTGGTAAGGTTCTT CGCGTAGCTTCGAATTAAACCACATGCTCCACCGC TTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAG CCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAAT GCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCC AACACTTAGCATTCATCGTTTACGGTATGGACTAC CAGGGTATCTAATCCTGTTTGCTACCCATACTTTC GAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCC TTCGCCACTGGTGTTCTTCCATATATCTACGCANT TCACCGCTACNCATGNAGTTCCACTGTCCTCTTCT GCACTCAAGTTTCCCAGTTTCCGATGCACTTCTTC NGTTGAGCCNANNNTTTCACATCNNANTTAAAAAN CGCCTGCGCTCGCTTTACGNCCAATAAATCCGGAC AANGCTTGCCNNCTAC	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 46. Genotypic identification of TH47 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH47 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTG CATCATGATTNNNATTNGAGTGAGTGGCGAACTGGTG AGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAACAACTT GGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCT ATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGAT GGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGA CACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAA TCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCC GCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTG TTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAG GTATTGACGGTATTTAACCAGAAAGCCACGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC GTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGC GGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAAC CGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAAT GCGTAGATATATGGAAGAACACCAGTGGCGAANGNGG CTGTCTGGTCTGTAACTGACGCTGANNNTCGAAAGTA TGGGTAGCAAACAGGANTAGATACCCTGGTAGTCCAT ACCGTAAACGATGAATGCTAAGTGNTGGAGGGTTTCC GCCCTTCANTGCTGCAGCTAANNCATTAAGCATTCCG CCTGGGGAGTANNNCNNCNNGGCTGAAACTCAAAGNA NNNGANNGGGGGCCCGCNNNANCGGTGNANCATGNNG NTTNATTCNAANCTACNCNNNAANCTTA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 99 % 98 %
TH47 Reverse	CCCTAATCATCTGTCCCACCTTAGGCGGCTGGTTCCT AAAAGGNNNNCCCNACCGACTTTGGGTGTTACAAACT CTCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGA ACGTATTCACCGCGGCATGCTGATCCGCGATTACTAG CGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAA TCCGAACTGAGAATGGCTTTAAGAGATTAGCTTACTC TCGCGAGTTCGCAACTCGTTGTACCATCCATTGTAGC ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTT GACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCA GTCTCACCAGAGTGCCCAACTTAATGCTGGCAACTGA TAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC ATCTCACGACACGAGCTGACGACAACCATGCACCACC TGTATCCATGTCCCCGAAGGGAACGTCTAATCTCTTA GATTTGCATAGTATGTCAAGACCTGGTAAGGTTCTTC GCGTAGCTTCGAATTAAACCACATGCTCCACCGCTTG TGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTG CGGCCGTACTCCCCAGGCGGAATGCTTAATGCGTTAG CTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAG CATTCATCGTTTACGGTATGGACTACCAGGGTATCTA ATCCTGTTTGCTACCCATACTTTCNAGCCTCAGCGTC AGTTACAGACCAGACAGCCGCCTTCGCCACTGGTGTT CTTCCATATATCTACGCATTTCACCGCTACACATGNN NTTCCACTGTCCTCTTCTGCACTCNAGTTTCCCAGTT TCCGATGCACTTCNTCGGTTGAGCCGAANGNTTTCAC ATCNNANTTAAAAAANCGCCTGCGCTCGCTTTACNCC CAATAA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 47. Genotypic identification of TH48 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH48 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCT TGCATCATGATTNNNATNNGAGTGAGTGGCGAACT GGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGG GGGATAACACCTGGAAACAGATGCTAATACCGCAT AACAACTTGGACCGCATGGTCCGAGCTTGAAAGAT GGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCG TATTAGCTAGATGGTGGGGTAACGGCTCACCATGG CAATGATACGTAGCCGACCTGAGAGGGTAATCGGC CACATTGGGACTGAGACACGGCCCAAACTCCTACG GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGA AAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAG GGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGA ACATATCTGAGAGTAACTGTTCAGGTATTGACGGT ATTTAACCAGAAAGCCACGGCTAACTACGTGCCAG CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC GGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTT TTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGA AGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAG AAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAA TGCGTAGATATATGGAAGAACACCAGTGGCGAANG CGGCTGTCTGGTCTGTAACTGACGCTGANGCTCGA AAGTATGGGTAGCAAACAGGATTAGATACCCTGGT AGTCCATACCGTAAACGATGAATGCTAAGTGTTGG AGGGTTTCCGCCCTTCAGTGCTGCAGCTNACGCAT TAAGCATTCNNCCTGGGGAGTANNGCCGCAAGGCT GAAACTCAAAGGAANTGANGGGGGNCCGCA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 99 % 98 % 93 %
TH48 Reverse	GACTTCNCCCTAATCNTCTGTCCCACCTTAGGCGG CTGGTTCCTAAAAGGTNNCCCNACCGACTTTGGGT GTTACAAACTCTCATGGTGTGACGGGCGGTGTGTA CAAGGCCCGGGAACGTATTCACCGCGGCATGCTGA TCCGCGATTACTAGCGATTCCGACTTCATGTAGGC GAGTTGCAGCCTACAATCCGAACTGAGAATGGCTT TAAGAGATTAGCTTACTCTCGCGAGTTCGCAACTC GTTGTACCATCCATTGTAGCACGTGTGTAGCCCAG GTCATAAGGGGCATGATGATTTGACGTCATCCCCA CCTTCCTCCGGTTTGTCACCGGCAGTCTCACCAGA GTGCCCAACTTAATGCTGGCAACTGATA ATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC ATCTCACGACACGAGCTGACGACAACCATGCACCA CCTGTATCCATGTCCCCGAAGGGAACGTCTAATCT CTTAGATTTGCATAGTATGTCAAGACCTGGTAAGG TTCTTCGCGTAGCTTCGAATTAAACCACATGCTCC ACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGT TTCAGCCTTGCGGCCGTACTCCCCAGGCGGAATGC TTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAAC CCTCCAACACTTAGCATTCATCGTTTACGGTATGG ACTACCANGGTATCTAATCCTGTTTGCTACCCATA CTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAG CCGCCTTCGCCACTGGTGTTCTTCCATATATCTAC GCATTTCACCGCTACACATGGNNTTCCACTGTCCT CTTCTGCACTCAAGTTTCCCAGTTTCCNATGCACT TCTTCGGTNGAGCCGANNNTTTCACATCNNANTNN AAAACCGCCTGNNCTCGCTTTACNCCCANNAAATC CNNANAANGCTNGNCANCTNCGTATTACC	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	100 % 100 % 100 %

Table 48. Genotypic identification of TH49 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH49 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCT TGCATCATGATTNNNNATTTNAGTGAGTGGCGAAC TGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCG GGGGATAACACCTGGAAACAGATGCTAATACCGCA TAACAACTTGGACCGCATGGTCCGAGCTTGAAAGA TGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGC GTATTAGCTAGATGGTGGGGTAACGGCTCACCATG GCAATGATACGTAGCCGACCTGAGAGGGTAATCGG CCACATTGGGACTGAGACACGGCCCAAACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG AAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAA GGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG AACATATCTGAGAGTAACTGTTCAGGTATTGACGG TATTTAACCAGAAAGCCACGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTT TTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCG AAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAA ATGCGTAGATATATGGAAGAACACCAGTGGCGAAN GCGGCTGTCTGGTCTGTAACTGACGCTGANGCTCG AAAGTATGGGTAGCAAACAGGATTAGATACCCTGG TAGTCCATACCGTAAACGATGAATGCTAAGTGTTG NAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCA TTAAGCATTCCNCCTGGGGAGTACGGNCGCANGGN TGAAACTCAAAGGAATTGANNGGGGCCCGCACAAG CGNNGNANCATGNGGTTTAATTCGAA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 99 % 98 % 98 %
TH49 Reverse	ACTTNNCCCTAATCNTCTGTCCCACCTTAGGCGGC TGGTTCCTAAAAGGTTACCCCACCGACTTTGGGTG TTACAAACTCTCATGGTGTGACGGGCGGTGTGTAC AAGGCCCGGGAACGTATTCACCGCGGCATGCTGAT CCGCGATTACTAGCGATTCCGACTTCATGTAGGCG AGTTGCAGCCTACAATCCGAACTGAGAATGGCTTT AAGAGATTAGCTTACTCTCGCGAGTTCGCAACTCG TTGTACCATCCATTGTAGCACGTGTGTAGCCCAGG TCATAAGGGGCATGATGATTTGACGTCATCCCCAC CTTCCTCCGGTTTGTCACCGGCAGTCTCACCAGAG TGCCCAACTTAATGCTGGCAACTGATAATAAGGGT TGCGCTCGTTGCGGGACTTAACCCAACATCTCACG ACACGAGCTGACGACAACCATGCACCACCTGTATC CATGTCCCCGAAGGGAACGTCTAATCTCTTAGATT TGCATAGTATGTCAAGACCTGGTAAGGTTCTTCGC GTAGCTTCGAATTAAACCACATGCTCCACCGCTTG TGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCT TGCGGCCGTACTCCCCAGGCGGAATGCTTAATGCG TTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAAC ACTTAGCATTCATCGTTTACGGTATGGACTACCAG GGTATCTAATCCTGTTTGCTACCCATACTTTCGAG CCTCAGCGTCAGTTACAGACCAGACAGCCGCCTTC GCCACTGGTGTTCTTCCATATATCTACGCATTTCA CCGCTACACATGGAGTTCCACTGTCCTCTTCTGCA CTCNAGTTTCCCAGTTTCCNATGCACTTCTTCGNT NGAGCCNAAGGNTTTNNCATCANACTTAAAAACCG CCTGNGCTCGCTTTACGCNANNAAATCCNGA	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum*	99 % 99 % 99 %

Table 49. Genotypic identification of TH58 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH58 Forward	TGCAAGTCGAGCGCATCGGCCCAACTGATTGAAGATGCTT GCATCCNNNTGANNNTGGTTTACCGATGAGCGGCGGACGG GTGAGTAACACGTAGGTAACCTGCCCAGAAGCGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAGGTCATTTGA CCGCATGGTCAAATGATTAAAGATGGCTCTGCTATCACTT CTGGATGGACCTGCGGCGTATTAGCTAGTTGGTAAGGTAA CGGCTTACCAAGGCAATGATACGTAGCCGAGTTGAGAGAC TGATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAA GTCTGATGGAGCAACGCCGCGTGAGCGAAGAAGGTCTTCG GATCGTAAAACTCTGTTGTTAGAGAAGAACACGGGTGAGA GTAACTGTTCACCTGTTGACGGTATCTAACCAGCAAGTCA CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTANGTG GCAAGCGTTATCCGGATTTATTGGGCGTAAAGGGAACGCA GGCGGTTCTTTAANTCTGATGTGAAAGCCTTCGGCTTAAC CGAAGATGTGCATTGGAAACTGGGGAACTTGANTGCAGAA NANGAGAGTGGAACTCCNTGTGTAGCGGTGAAATGCGTAN ATATATGG	*Lactobacillus saerimneri*	99 %
TH58 Reverse	CCCCAATCATCTGTCCCACCTTAGACGGCTGGCTCCAAAA GGTTACCCCACCGGCTTTGGGTGTTACAAACTCTCATGGT GTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC GCGACATGCTGATTCGCGATTACTAGCGATTCCGACTTCG TGCAGGCGAGTTGCAGCCTGCAGTCCGAACTGAGAGCAGC TTTAAGAGATTTGCTAAACCTCGCGGTTTCGCGACTCGTT GTACTGCCCATTGTANCACGTGTGTAGCCCAGGTCATAAG GGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTT TGTCACCGGCAGTCTCGCCNNAGTGCCCAACTGAATGCTG GCAACTGACAACAAGGGTTGCGCTCGTTGCGGGACTTAAC CCAACATCTCACGACACGAGCTGACGACAACCATGCACCA CCTGTCATTTTGTCCCCGAAGGGAAAACCTAATCTCTTAG GTGGTCAAAAGATGTCAAGACCTGGTAAGGTTCTTCGCGT AGCTTCNAATTA	*Lactobacillus saerimneri*	97 %

Table 50. Genotypic identification of TH61 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH61 Forward	TGCNAGTCGAACGAACTCTGGTATTGATTGGTGCT TGCATCATGATTNNNCATNNNAGTGAGTGGCGAAC TGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCG GGGGATAACACCTGGAAACAGATGCTAATACCGCA TAACAACTTGGACCGCATGGTCCGAGTTTGAAAGA TGGCTTCGGCTATCACTTNTGGATGGTCCCGCGGC GTATTAGCTAGATGGTGAGGTAACGGCTCACCATG GCAATGATACGTAGCCGACCTGAGAGGGTAATCGG CCACATTGGGACTGAGACACGGCCCAAACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG AAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAA GGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG AACATATCTGAGAGTAACTGTTCAGGTATTGACGG TATTTAACCAGAAAGCCACGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTT TTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCG AAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCA GAAGAGGACAGTGGAACTCCNTGTGTAGCGGTGAA ATGCGTAGATATATGGAAGAACACCAGTGGCGAAN GNGGCTGTCTGGTCTGTAACTGACGCTGANGCTCG AAAGTATGGGTAGCAAACANGATTAGATACCCTGG TAGTCCATACCGTAAACGANGAATGCTAAGTGTTG GAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCA TTAANCATTCCGCCTGGGGAGTANNNCNNCANGGN TGAAACTCAAAGGAATTGANNGGGNCCNCNCAAGC GGTGGANCATGNNNNTTAATTCGAA	*Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacilllus plantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	99 % 99 % 98 % 93 %
TH61 Reverse	ACTTCNCCCTAATCATCTGTCCCACCTTAGGCGGC TGGTTCCTAAAAGGNNNNNNNNNNNNGACTTTGGGT GTTACAAACTCTCATGGTGTGACGGGCGGTGTGTA CAAGGCCCGGGAACGTATTCACCGCGGCATGCTGA TCCGCGATTACTAGCGATTCCGACTTCATGTAGGC GAGTTGCAGCCTACAATCCGAACTGAGAATGGCTT TAAGAGATTAGCTTACTCTCGCGAGTTCGCAACTC GTTGTACCATCCATTGTAGCACGTGTGTAGCCCAG GTCATAAGGGGCATGATGATTTGACGTCATCCCCA CCTTCCTCCGGTTTGTCACCGGCAGTCTCACCAGA GTGCCCAACTTAATGCTGGCAACTGATAATAAGGG TTGCGCTCGTTGCGGGACTTAACCCAACATCTCAC GACACGAGCTGACGACAACCATGCACCACCTGTAT CCATGTCCCCGAAGGGAACGTCTAATCTCTTAGAT TTGCATAGTATGTCAAGACCTGGTAAGGTTCTTCG CGTAGCTTCGAATTAAACCACATGCTCCACCGCTT GTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCC TTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGC GTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAA CACTTANCATTCATCGTTTACGGTATGGACTACCA GGGTATCTAATCCTGNTTGCTACCCATACTTTCGA GCCTCAGCGTCAGTTACAGACCANACAGCCGCCNT CGCCACTGGTGTTCTTCCNTATATCTACGCANTTC ACCGCTACACATGNAGTTCCACTGTCCTCTTCNGC ACTCAAGTTTCCCAGTTTCCGANGCACTTNNTCNG TTGAGCCGAANGNTTTNNCNTCANANTTAAAAAAC CGCCTGNNNTCGCTTTACGCCCANTAAATNCGGAN ANGCTNGNCACCTACGTATTACC	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 100 % 100 % 99 %

Table 51. Genotypic identification of TH62 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH62 Forward	TGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTG CATCATGATTNNNCATTNNAGTGAGTGGCGAACTGGT GAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGAT AACACCTGGAAACAGATGCTAATACCGCATAACAACT TGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGC TATCACTTCTGGATGGTCCCGCGGCGTATTAGCTAGA TGGTGAGGTAACGGCTCACCATGGCAATGATACGTAG CCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAG ACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGA ATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCT GTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG CGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGG CGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAA CCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGC AGAAGAGGACAGTGGAACTCCNTGTGTAGCGGTGAAA TGCGTAGATATATGGAAGAACACCAGTGGCGAANGNG GCTGTCTGGTCTGTAACTGACGCTGANGCTCGAAAGT ATGGGTAGCAAACAGGANTAGATACCCTGGTAGTCCA TACCGTNNNNATGAATGCTAAGTGNTGGAGGGTTTCC GCCCTTCANTGCTGCAGCTAACGCATTAAGCATTCCG CCTGGGGNGTANGGCCGC	*Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum*	98 % 98% 98 %
TH62 Reverse	ACTTNNCCCTAATCATCTGTCCCACCTTAGGCGGCTG GTTCCTAAAAGGNNNNCCNACCGACTTTGGGTGTTAC AAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCC CGGGAACGTATTCACCGCGGCATGCTGATCCGCGATT ACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCC TACAATCCGAACTGAGAATGGCTTTAAGAGATTAGCT TACTCTCGCGAGTTCGCAACTCGTTGTACCATCCATT GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGAT GATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCAC CGGCAGTCTCACCAGAGTGCCCAACTTAATGCTGGCA ACTGATAATAAGGGTTGCGCTCGTTGCGGGACTTAAC CCAACATCTCACGACACGAGCTGACGACAACCATGCA CCACCTGTATCCATGTCCCCGAAGGGAACGTCTAATC TCTTAGATTTGCATAGTATGTCAAGACCTGGTAAGGT TCTTCGCGTAGCTTCGAATTAAACCACATGCTCCACC GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAG CCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGC GTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACA CTTAGCATTCATCGTTTACGGTATGGACTACCANGGT ATCTAATCCTGTTTGCTACCCATACTTTCGAGCCTCA GCGTCNGTTACAGACCAGACAGCCGCCTTCGCCACTG GTGTTCTTCCATATATCTACGCATTTCACCGCTACNC ATGNNNTTCCACTGTCCTCTTCTGNACTCAAGTTTCC CAGTTTNCGATGCACTTCNTNNNTTGAGCCNANNNNT TCACATNNNANTTAAAAAACCGCCTGCGCTCGCTTTA NGCCCAATAAATCCGGANAANGCTTGCCACCNACGTA NTACCG	*Lactobacillus plantarum* *Lactobacillus pentosus* *Lactobacillus paraplantarum* *Lactobacillus plantarum* subsp. *argentoratensis*	100 % 100 % 100 % 98 %

Table 52. Genotypic identification of TH64 strain based on 16S rRNA gene dideoxy sequencing; organisms matched of 90-100 % identities were displayed

Strain	Nucleotide sequences of 16S rRNA gene	Match organism	Identity
TH64 Forward	TCGAACGCTTTGTGGTTCAACTGATTTGAAGAGCTTGCTCANNA TATGACGATGGACATTGCAAAGAGTGGCGAACGGGTGAGTAACA CGTGGGAAACCTACCTCTTAGCAGGGGATAACATTTGGAAACAG ATGCTAATACCGTATAACAATGACAACCGCATGGTTGTTATTTA AAAGATGGTTCTGCTATCACTAAGAGATGGTCCCGCGGTGCATT AGCTAGTTGGTAAGGTAATGGCTTACCAAGGCGATGATGCATAG CCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACGGC CCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGG GCGAAAGCCTGATGGAGCAACGCCGCGTGTGTGATGAAGGGTTT CGGCTCGTAAAACACTGTTGTAAGAGAAGAATGACATTGAGAGT AACTGTTCAATGTGTGACGGTATCTTACCANAAAGGAACGGCTA AATACGTGCCANCANCCGCGGTAATACGTATGTTCCAAGCGTTA TCCGGATTTATTGGGCGTAAAGCGAGCGCAGACGGTTAT	*Weissella confusa*	94 %
TH64 Reverse	CTGTCCCACCTTANACGGCTGGCTCCCGAAGGNNACCCCACCGG CTTTGGGTGTTACAAACTCTCATGGTGTGACGGGCGGTGTGTAC AAGACCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTA CTANCGATTCCGANTTCATGTAGGCGAGTTGCANCCTACAATCC GAACTGAGACGTACTTTANNANATTAGCTCACCCTCNCGGGTTG GCNNNNCGTTGTATACGCCATTGTANCACGTGTGTANCCCANGT CATAANGGGCATGNTGA	*Weissella cibaria* *Weissella confusa*	100 % 98 %

Table 53. Genotypic identification of SB42-6, BJ48-5, RT49-5 and RT49-7 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
SB42/6 V1	CACTCAAATGTAAATCATGATGCAAGCACC	*Lactobacillus pentosus* *Lactobacillus plantarum*	100 % 100 %
SB42/6 V3	Less than 15 good quality bases	-	-
BJ48/5 V1	CACTCAAATGTAAATCATGATGCAAGCACC	*Lactobacillus pentosus* *Lactobacillus plantarum*	100 % 100 %
BJ48/5 V3	Less than 15 good quality bases	-	-
RT49/5 V1	CACTCAAATGTAAATCATGATGCAAGCACC	*Lactobacillus pentosus* *Lactobacillus plantarum*	100 % 100 %
RT49/5 V3	Less than 15 good quality bases	-	-
RT49/7 V1	CACTCAAATGTAAATCATGATGCAAGCACC	*Lactobacillus pentosus* *Lactobacillus plantarum*	100 % 100 %
RT49/7 V3	Less than 15 good quality bases	-	-

Table 54. Genotypic identification of TH14, TH24 and TH27 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
TH14 V1	AAGCTTCTTTCGGTGAATGCAAGCATTCGGT	*Lactobacillus ruminis*	100 %
TH14 V3	AGGTCTTGACATCTTCTGACAATTCCAGAGA	*Lactobacillus ruminis*	100 %
TH24 V1	CACTCAAATGTAAATCATGATGCAAAGCCA ACCCC	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH24 V3	AGGTCTTGACATACTATGCA	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %
TH27 V1	CACTCAAAATGTAAATCATGATGCAAAG	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH27 V3	AGGTCTTGACATACTATGCA	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %

Table 55. Genotypic identification of TH33, TH39, TH43 and TH45 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
TH33 V1	CAACTTCTTACGGTGAATGCAAGC	*Lactobacillus salivarius*	100 %
TH33 V3	AGGTCTTGACATCCTTTGAC CACCTAAGAGATTAGGCTTT TCCCCTTT	*Lactobacillus salivarius*	81 %
TH39 V1	CACTCAAATGTAAATCATGA TGCAAGAACCC	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH39 V3	Less than 15 good quality bases		
TH43 V1	CACTCAAATGTAAAATCATGA	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH43 V3	AGGTCTTGACATACTAT	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %
TH45 V1	CACTCAAATGTAAATCATGA TGCAAAGCAACCC	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH45 V3	AGGTCTTGAC	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %

Table 56. Genotypic identification of TH47 and TH48 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
TH47 V1	CACTCAAATGTAAATCATGTGC	*Lactobacillus plantarum* *Lactobacillus pentosus*	81 % 81%
TH47 V3	AGGTCTTGACATACTATGCA AATCTAAGAGATTAG	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100% 100 %
TH48 V1	CACTCAAATGTAAATCATGA	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH48 V3	AGGTCTTGACATACTATGCA	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %

Table 57. Genotypic identification of TH49, TH58 and TH61 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
TH49 V1	CACTCAAATGTAAATCATGA (TAGCAAGACACC)	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH49 V3	AGGTCTTGACATACTAT	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %
TH58 V1	CATCGGTAAACCATCGTCAATCG GATGCAA GCAT	*Lactobacillus saerimneri*	100 %
TH58 V3	AGGTCTTGACATCTTTTGACCAC CTAAGAGA	*Lactobacillus saerimneri* *Lactobacillus aviarius*	100 % 100 %
TH61 V1	CACTCAAAATGTAAATCATG ATGCAAAGGCCAACCCC	*Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 %
TH61 V3	AGGTCTTGACATACTAT	*Lactobacillus paraplantarum* *Lactobacillus plantarum* *Lactobacillus pentosus*	100 % 100 % 100 %

Table 58. Genotypic identification of TH62 and TH64 strains based on pyrosequencing of 16S rRNA gene V1 and V3 variable regions. Organisms matched of 80-100 % identities were displayed.

Strain	Nucleotide sequences	Match organism	Identity
TH62 V1	CACTCAAATG TAAATCATGA	*Lactobacillus plantarum*	100 %
		Lactobacillus pentosus	100 %
TH62 V3	AGGTCTTGAC ATACTATGCA	*Lactobacillus paraplantarum*	100 %
		Lactobacillus plantarum	100 %
		Lactobacillus pentosus	100 %
TH64 V1	CTTTGCAATGTCCATCGTCA TATCTGAGC	*Weissella cibaria*	100 %
		Weissella confusa	100%
		Weissella viridescens	100 %
TH64 V3	AGGTCTTGACATCCCTTG	*Anaerofustis stercorihominis*	100 %
		Anoxybacillus contaminans	100 %
		Anoxybacillus voinovskiensis	100%
		Facklamia sourekii	100 %
		Vagococcus salmoninarum	100 %
		Weissella thailandensis	100 %
		Weissella confusa	100 %
		Weissella hellenica	100 %
		Weissella cibaria	100 %

Table 59. Identification of anti-pathogenic *Lactobacilllus* strains by API, 16 S rRNA gene sequencing and pyrosequencing

***Lactobacillus* strain**	**Identification**		
	API	**16S rRNA gene dideoxy DNA sequencing**	**DNA Pyrosequencing**
SB42-6	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. paraplantarum* 100% *L. pentosus* 100% *L. plantarum* 99%	*L. pentosus* 100% *L. plantarum* 100%
BJ48-5	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. plantarum* 100% *L. pentosus* 99% *L. paraplantarum* 97%	*L. pentosus* 100% *L. plantarum* 100%
RT49-5	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. plantarum* 100% *L. pentosus* 99% *L. paraplantarum* 98%	*L. pentosus* 100% *L. plantarum* 100%
RT49-7	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. plantarum* 100% *L. pentosus* 99% *L. paraplantarum* 98%	*L. pentosus* 100% *L. plantarum* 100%

Table 60. Identification of anti-pathogenic *Lactobacilllus* strains by API, 16 S rRNA gene sequencing and pyrosequencing.

Strains	Identification		
	API 50 CHL	**16S rRNA gene dideoxy DNA sequencing**	**DNA Pyrosequencing**
TH14	*Leuconostoc lactis* 98%, *L. acidophilus* 1%	*L. ruminis* 98%	*L. ruminis* 100%
TH24	*L. plantarum* 99% *L. pentosus* 1%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH27	*L. plantarum* 53% *L. pentosus* 47%	*L. plantarum* 99%, *L. pentosus* 100%, *L. paraplantarum* 99%	*L. plantarum* 100% *L. pentosus* 100%
TH33	*L. salivarius* 99.9%	*L. salivarius* 99%	*L. salivarius* 100%
TH39	*L. plantarum* 53% *L. pentosus* 47%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH43	*L. paracasei ssp paracasei* 61% *L. plantarum* 37%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH45	*L. plantarum* 99% *L. pentosus* 0.4%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH47	*L. plantarum* 92% *L. pentosus* 8%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH48	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100% *L. pentosus* 100%
TH49	*L. plantarum* 99.9% *L. pentosus* 0.1%	*L. plantarum* 100%, *L. pentosus* 99%, *L. paraplantarum* 98%	*L. plantarum* 100% *L. pentosus* 100%
TH58	*Pediococcus damnosus*22% *L. acidophilus* 21% *Weissella viridescens* 18% *L. delbrueckii* ssp *lactis* 13% *L. delbrueckii* spp *delbrueckii* 13%	*L. saerimneri* 99%	*L. saerimneri* 100%
TH61	*L. plantarum* 91% *L. brevis 8%* *L. pentosus* 0.4%	*L. plantarum* 100%, *L. pentosus* 100%, *L. paraplantarum* 100%	*L. plantarum* 100%, *L. pentosus* 100%,
TH62	*L. brevis 90%*, *L. pentosus* 3%	*L. plantarum* 98%, *L. pentosus* 100%, *L. paraplantarum* 98%	*L. plantarum* 100%, *L. pentosus* 100%
TH64	*L. brevis* 62 %, *Pediococcus pentosacceus* 17% *L. lactis* ssp *lactis* 15%, *Weissella confusa* 5%	*Weissella cibaria* 100% *Weissella confusa* 98%	*Weissella cibaria* 100% *Weissella confusa* 100%

9.2 Genotyping of selected *Lactobacillus* strains based on 16S rRNA gene sequencing and rep-PCR genotyping

Twelve anti-inflammatory strains including TH24, TH27, TH33, TH39, TH43, TH45, TH47, TH48, TH49, TH58, TH61 and TH62 were chosen to determine phylogenetic relationships based on 16S rRNA gene sequencing compared to TH14, immunostimulatory strain and TH64, non-anti-inflammatory and non-immunostimulatory strain. Phylogenetic analysis was performed by using MEGA 4.0 software package [161]. Multiple sequence alignment of nucleotide sequences were using Clustral W program [162] and phylogenetic tree was constructed with the neighbour-joining method with 1,000-replicates bootstrap analysis. Phylogenetic tree as showed in Figure 23 indicated genetic distances of these strains. Isolates divided into 3 distinct clusters including one cluster of *L. plantarum* with closely related species, one cluster of *L. salivarius* and *L. ruminis*, and one cluster of *L. saerimneri*. *W. cibaria*, member of lactic acid bacteria was as outlier species.

Strains described above and anti-pathogenic strains were selected to perform rep-PCR genotyping. Dendrogram of genomic fingerprinting was generated by DiversiLab software. In Figure 24 demonstrated genomic fingerprinting analyses of anti-pathogenic strains, SB42-6, BJ48-5, RT49-5, RT49-7 and *L. reuteri* strains. *L. plantarum*, BJ48-5, RT49-5, RT49-7 strains, were displayed 100% similarity within these strains but *L. plantarum*, SB42-6 strain, was showed 90% similarity to those 3 strains. All of the *L. plantarum* species were different from *L. reuteri* strains which showed approximately 55% similarity.

In Figure 25, the immunomodulatory strains also displayed genomic fingerprinting analyses compared with *L. reutri* strains, MM41-A, TNF-α inhibitory strain and SD2112, non-TNF-α inhibitory strain. Anti-inflammatory strains of *L. plantarum* group could be divided into 3 clusters including a cluster of TH43, TH45, TH61, TH62 which displayed 97% similarity. Cluster of TH39 which was closely related to TH47 and displayed 90% similarity. Cluster of TH24, TH48 and TH49 were closely related together and displayed 94-97% similarity. TH58, the most potent TNF-α inhibitory strain showed similarity with a cluster of TH39 and TH47 with 78% similarity. TH58 showed 70% and 60% similarity to SD2112, MM4-1A, (TNF-α inhibitory strain) and TH14 (*L. ruminis*; non-TNF-α inhibitory strain), respectively.

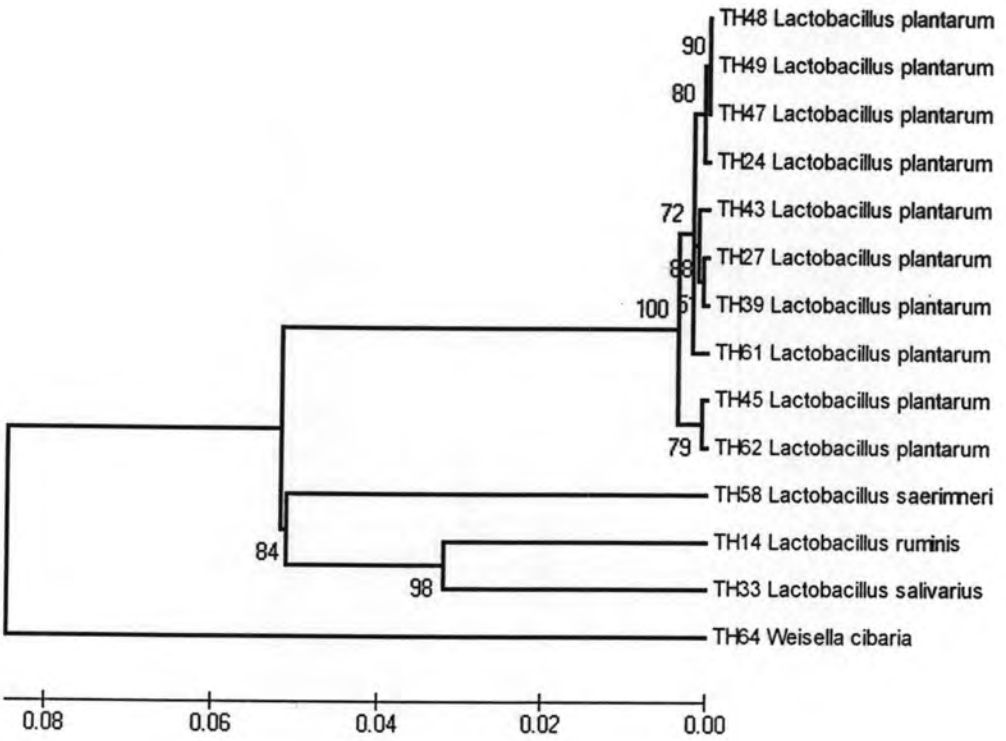


Figure 23. Phylogenetic relationships based on the 16S rRNA gene sequences between anti-inflammatory strain of TH24, TH27, TH33, TH39, TH43, TH45, TH47, TH48, TH49, TH58, TH61 and TH62; TH14, immunostimulatory strain; TH64, non-anti-inflammatory and non-immunostimulatory strain. The tree was generated by using neighbour-joining method for 1,000 bootstrapping iteration. The number indicated bootstrap value. The scale bar represents nucleotide substitution

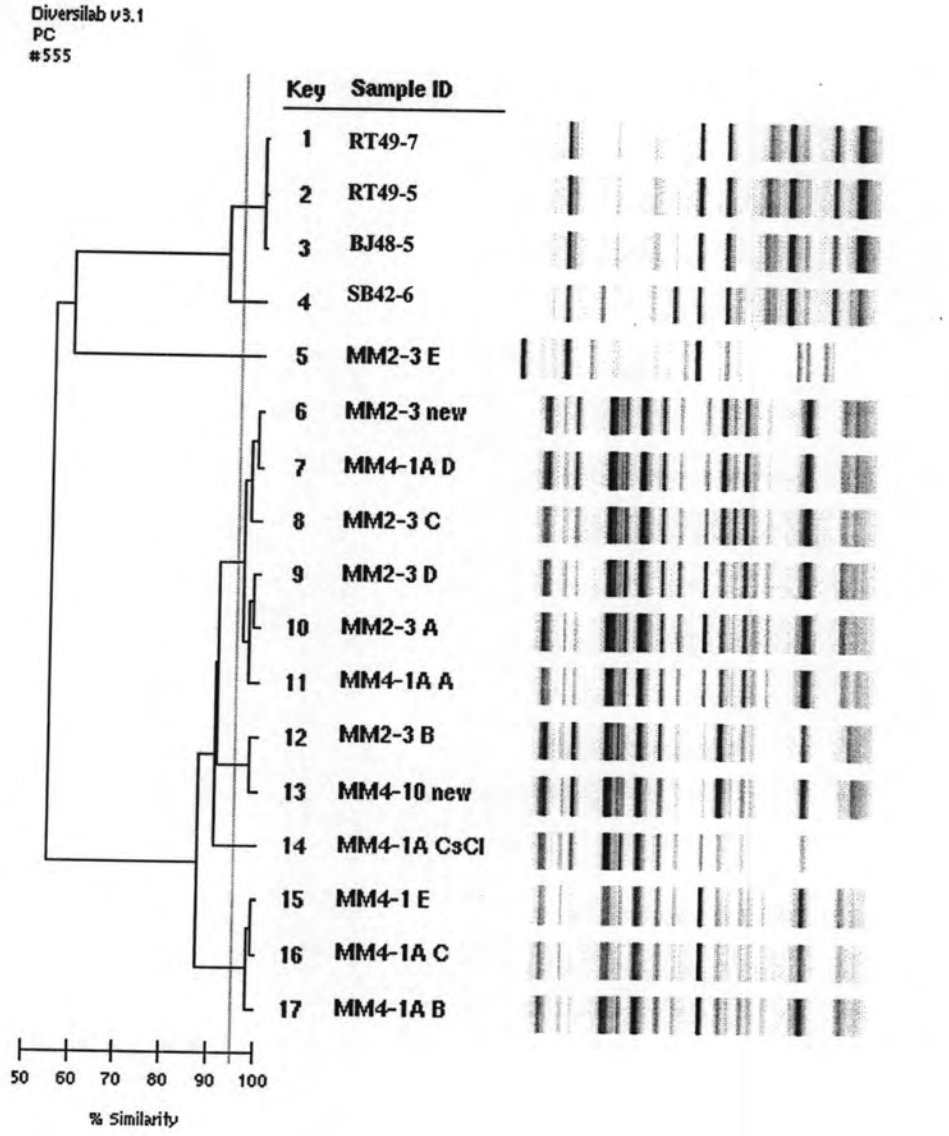


Figure 24. The rep-PCR genomic fingerprinting analyses of SB42-6, BJ48-5, RT49-5, RT49-7 anti-pathogenic strains (*L. plantarum)* and *L. reuteri* strains (key: 5-17).

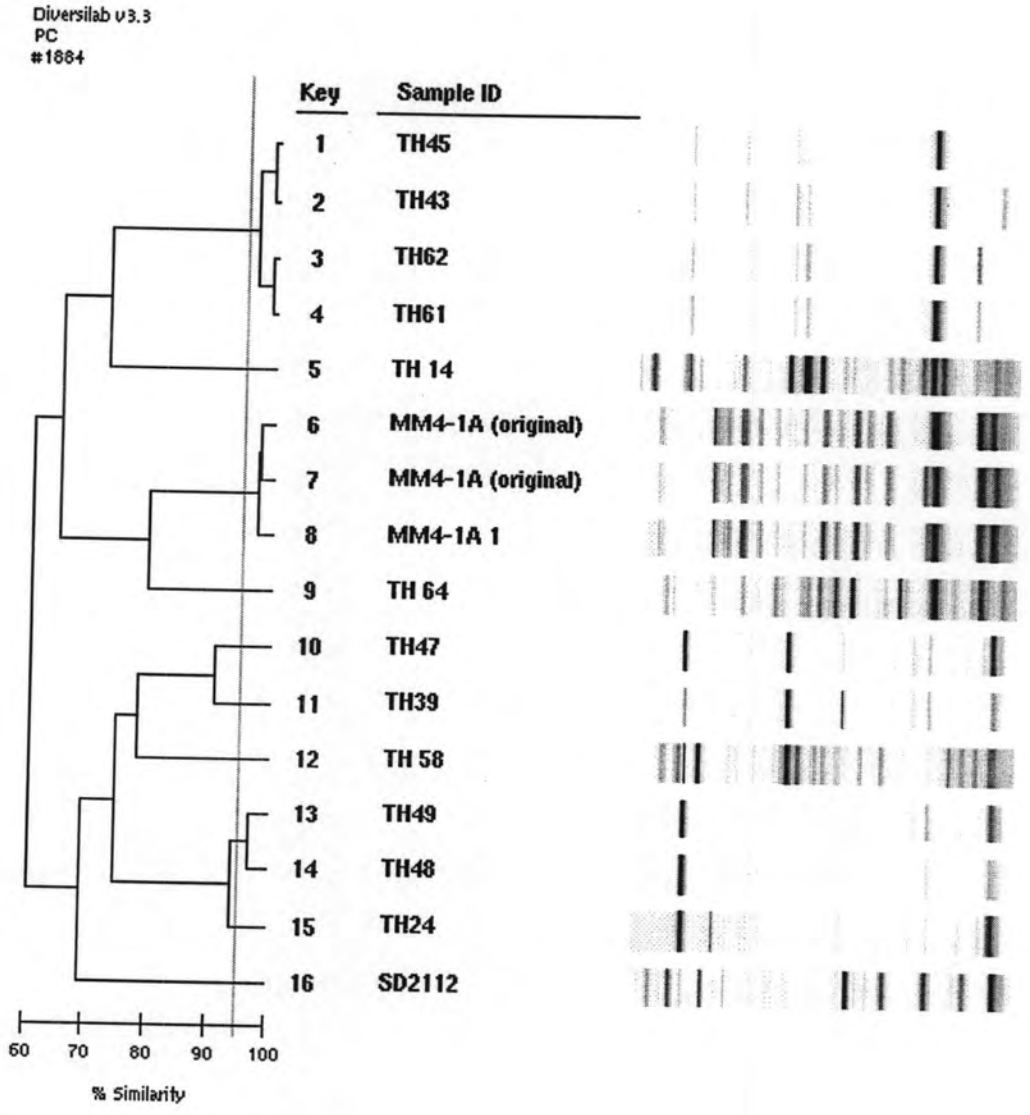


Figure 25. The rep-PCR genomic fingerprinting analyses of immunomodulatory strains (key: 1-5, 9-15), the TNF-α inhibitory strain (*L. reuteri* MM4-1A) and non-TNF- α inhibitory strain (*L. reuteri* SD2112)