International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 10 (2): 77-85, 2015
ISSN 1811-9743 / DOI: 10.3923/ijds.2015.77.85

Effect of *Spirulina platensis* Powder on Whey Fermented with Streptococci

Anandi Kavimandan
Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior, 474005 MP, India

ABSTRACT

The main purpose of this research was to monitor the effect of dry *Spirulina platensis* powder in whey inoculated with pure culture of *Streptococcus lactis* NCIM 2080 and *Streptococcus faecalis* NCIM 2093, respectively during their 21 days storage at 4°C. The whey samples were supplemented with 0.3, 0.5 and 0.8% w/v *S. platensis* powder and without *S. platensis* powder (control). All samples were inoculated with 1% v/v single strain of streptococci. All samples were inoculated with same viable counts. Whey samples were filled in 50 mL tightly capped sterilized falcon tubes. Five hours of incubation were given to all samples each in three replicates at 40°C. The effect was studied on following parameters: pH, titratable acidity and redox potential of whey and viability of streptococci in current sample. These attributes were checked on 0th day (before incubation), 1st, 7th, 14th and 21st day of storage. The most significant results were obtained at 0.8% *S. platensis* supplemented samples. At the day 7 both streptococci gave the highest viable counts throughout the 21 days of storage. The lowest pH and highest titratable acidity was observed in *S. lactis* at 21st day of storage.

Key words: *Spirulina platensis*, whey, *Streptococcus lactis*, *Streptococcus faecalis*, probiotics

INTRODUCTION

The dried biomass of *S. platensis* contains 3-7% moisture, 55-60% protein, 6-8% lipids, 12-20% carbohydrate, 7-10% ash, 8-10% fiber, 1-1.5% chlorophyll a and a wide range of vitamins (Belay, 1997; Cohen, 1997; Vonshak, 1997). *Spirulina* have not cellulose in its cell wall, a trait that makes it a suitable and vital foodstuff for patients who have poor intestinal amalgamation and for old patients (Richmond, 1984).

In India, it is predictable that about 100 million kg of whey is annually derived as a byproduct which may cause substantial loss of about 70,000 t of nutritious whey solids (Parekh, 2006), containing valuable nutrients like lactose, proteins, minerals and vitamins etc., which have essential value as human food. Whey constitutes 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk proteins and 70-90% of milk minerals and most important all the water soluble vitamins are present in milk (Horton, 1995). Considerable work has been done throughout the world to utilize whey for production of Whey Protein Concentrate (WPC), whey powder, lactose, lactic acid, whey paste etc. (Panesar *et al*., 2007).

A probiotic is commonly defined as a live microbial supplement that has an effect on the host by developing its intestinal microbial balance (Fuller, 1989). There are many health benefit for regular intake of viable probiotics which includes lactose tolerance (Kim and Gilliland, 1983), antimicrobial (Yildirim and Johnson, 1998), anticarcinogenic (El-Gawada *et al*., 2004), hypercholesterolemic (Kikuchi-Hayakawa *et al*., 2000; El-Gawada *et al*., 2005) and antimutagenic (Hsieh and Chou, 2006) and various others profitable effects.

Probiotic has a good and healthy source of diet for human from centuries. On the other side, algae are emerging as dietary supplements. So, looking at the nutritional values of whey, *S. platensis* powder and probiotics; it is very useful and cost effective to make a probiotic beverage that has sufficient number of lactic acid bacteria from a waste by product e.g., whey.

Schleifer and Kilpper-Balz (1987) applying classification and serological techniques demonstrated three separate, independent and different genera: *Streptococcus*, *Lactococcus* and *Enterococcus* from old streptococci genus (Schleifer and Kilpper-Balz, 1987) lactococci (formerly group N streptococci) are the major mesophilic microorganisms used for acid production in dairy fermentations. Although five species are recognized, only one, *L. lactis*, is of significance in dairy fermentations (Law and Haandrikman, 1997).

The genus *Enterococcus* includes the Lancefield group D (fecal) streptococci. Now *Streptococcus faecalis* and *S. faecium* are known as *E. faecalis* and *E. faecium* (Stiles and Holzapfel, 1997; Klein *et al*., 1998). Enterococci are used as food safety indicators and have a possible involvement in foodborne illness. Enterococci are also used as starter cultures in some southern European cheeses. In addition, they are commercially available as probiotics for prevention and treatment of intestinal disorders. Among enterococci only *E. faecalis* and *E. faecium* are important as probiotics. They are readily differentiated by fermentation of arabinose and sorbitol and by their growth temperatures (Klein *et al*., 1998).

Enterococci are applied in fermentation process of fermented food production because their contribution to ripening and aroma development, probiotic properties and the production of antimicrobial substances (Giraffa, 2002; Klein, 2003; Moreno *et al*., 2006). Many *E. facalis* and *E. faecium* strains isolated from dairy products were shown to be good producer of acetaldehyde, ethanol, diacetyl and acetone when grown in milk, thus further contributing in the development of aroma and flavour of cheese (Andrighetto *et al*., 2001; Sarantinopoulos *et al*., 2001).

Because of their occurrence in gastrointestinal tract, enterococci are often used as indicator of faecal contamination through food production chain (Giraffa, 2002; Moreno *et al*., 2006). However, enterococci are now also considered as normal parts of the food microflora and not only as indicator for poor hygiene (Klein, 2003). The differentiation of apparently safe and non-safe enterococci strains is not simple, especially because virulence genes can be easily exchanged between strains (Messi *et al*., 2006; McGowan *et al*., 2006).

For applications of enterococci in foods, antibiotic resistance is of special concern because genetic determinants of resistance in these bacteria are generally located in conjugative plasmids or transposons (Hasman *et al*., 2005; Zanella *et al*., 2006). Antibiotic multi-resistance has been more commonly reported for *E. faecalis* due to its notorious ability to acquire and transfer antibiotic resistance genes (Citak *et al*., 2004; McBride *et al*., 2007). So it is a dualistic in nature.

MATERIALS AND METHODS

***Spirulina platensis* biomass:** The culture was obtained from Madhav Institute of Technology and Science and grown in Zarrouk's medium and maintained (Zarrouk, 1966; Vonshak, 2002).

Harvesting of biomass and drying: Twenty three days old *S. platensis* culture was taken and biomass was obtained (Arun *et al*., 2012). The collected pellet then washed with acidified water (pH 4) in order to remove insoluble salts particles. Algal pastes (biomass) were air dried in an oven at 60°C for 6 h for making dried powder (Vonshak, 2002).

Procurement and maintenance of streptococci strains: The *Streptococcus lactis* and *Streptococcus faecalis* strain were procured from National Collection of Industrial Microorganism, Pune; having strain numbers NCIM 2080 and NCIM 2093, respectively. Both cultures were maintained in M17 media incubated at 37-40°C for 48 h (Terzaghi and Sandine, 1975).

Preparation of whey: Double toned milk was warmed to 63°C for 30 min (USPHA., 1995). Acidification of the milk was done by adding citric acid 2% (w/v) solution drop by drop until coagulation occurred. Then it was filtered through a muslin cloth to remove coagulated particles. Whey was collected separately and heated at 60°C for 30 min (Shafiee *et al*., 2010).

Sample preparation: After the cooling of whey to 40°C; sterile falcon tubes were taken and whey was poured in it. Each tube was containing 50 mL of whey sample. Then *S. platensis* powder was added in each tube according to 0.30% w/v, 0.50% w/v and 0.80% w/v and control without *S. platensis*. Each concentration has been taken in three replicates.

Inoculation with streptococci stains: Streptococci culture were taken; having cell count 1.2×10^{8} to 1.8×10^{8} CFU mLG^{1} and all whey samples were inoculated with 1% v/v. Each streptococci strain was taken at a time.

Incubation and storage of samples: After preparing the samples kept it for 5 h incubation at 40°C for fermentation after that kept these at 4°C for 21 days of storage in a refrigerator. All analysis of samples was done at 0th day (before fermentation), 1st, 7th, 14th and 21st day of storage.

pH determination: The pH was determined by combined glass electrode pH meter (Systronic 361) (Shafiee *et al*., 2010).

Electrode potential determination: The Electrode Potential (EP) was determined by combined glass electrode pH meter (Systronic 361) (Shafiee *et al*., 2010).

Titratable acidity measurement: Titratable Acidity (TA) was measured by the method described by Dave and Shah (1997).

Enumeration of total viable count: Streptococci present in samples have been enumerated by pour plate technique (Harrigan, 1998; Varga *et al*., 2002).

Detection of contamination: The chance of any kind of contamination was checked in 1st week of storage. Presence of yeast and mold was check by spread plate technique using YGC agar (Varga *et al*., 2002). Presence of coliforms and *E. coli* were checked by using MacConkey and VRB agar, respectively (APHA., 1992).

Statistical analysis: All data were interpreted in Mean±SE. The significant change in the results was calculated by Student's t test (two tailed test) and two way ANOVA at 5% level of significance.

RESULTS AND DISCUSSION

Effect of *Spirulina platensis* powder on whey inoculated with *Streptococcus lactis* and *Streptococcus faecalis*, respectively: The pure culture of *S. lactis* and *S. faecalis* were inoculated in to whey separately at 8.89 log CFU mLG^{1} in 1% v/v ratio. There was no yeast, mold, *E. coli* and coliform contamination found in both *S. lactis* and *S. faecalis* fermented whey samples.

Effect of *S. platensis* on viable counts of *S. lactis* and *S. faecalis*: When the effect of *S. platensis* powder was studied on viability of *S. lactis* and *S. faecalis* then it was clear that all samples were inoculated with same bacterial counts. On the 0th day viability in all samples were approximately 6.89 log CFU mLG^{1} shown. On the 1st day observation it was clear that the viable counts were increased so rapidly. Control was having least viability and other treatments were increases in viable numbers as the concentration increases. From the Table 1 and 2 the highest viability was observed on 7th day observations then viability start to decreases and after 14th day there was major downfall occurred. The decrement in viability was due to the lack of nutrient in whey samples or may be lack of oxygen in tubes or deposition of toxic waste from bacteria. Low pH may be a cause of less viability because lowest pH was observed on 21st day of storage among all four streptococci inoculated samples (Kavimandan and Sharma, 2015). *Streptococcus thermophilus* was lowest in viability at 7th day observation among all streptococci and highest in viable number at 21st day of storage with equal to *S. faecalis*. Most significant results were obtained in 0.8% w/v enriched samples.

Effect on pH of whey inoculated with *S. lactis* and *S. faecalis*, respectively: As shown in Table 3 and 4 at the time of inoculation (before incubation) *S. platensis* powder supplemented samples were shown high pH value as compare to control. These findings indicate that the

Table 1: Effect of *S. platensis* powder on viable counts of *Streptococcus lactis* in whey

	Viable count of *Streptococcus lactis* (log CFU mLG^{1})				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	6.89±0.04	7.71±0.05	8.75±0.06	8.29±0.04	7.49±0.03
0.3%	6.89±0.03	7.79±0.06	8.83±0.05	8.38±0.05*	7.56±0.09
0.5%	6.89±0.02	7.84±0.05*	8.92±0.05*	8.50±0.06*	7.63±0.07*
0.8%	6.90±0.02	7.90±0.01*	8.98±0.04*	8.61±0.07*	7.74±0.09*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 876.27 and F_2 (between row) = 13.82 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

Table 2: Effect of *Spirulina platensis* powder on viable counts of *Streptococcus faecalis* in whey

	Viable count of *Streptococcus faecalis* (log CFU mLG^{1})				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	6.87±0.03	7.65±0.04	8.78±0.02	8.16±0.06	7.63±0.05
0.3%	6.86±0.04	7.74±0.05	8.85±0.03	8.30±0.04*	7.72±0.05*
0.5%	6.88±0.05	7.80±0.04*	8.92±0.04*	8.42±0.05*	7.79±0.06*
0.8%	6.88±0.07	7.86±0.05*	8.98±0.03*	8.50±0.05*	7.82±0.07*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 860.16 and F_2 (between row) = 13.63 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

Table 3: Effect of *S. platensis* powder on pH of whey inoculated with *S. lactis*

	pH value of whey				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	5.95±0.02	4.81±0.03	4.56±0.04	4.38±0.02	4.17±0.02
0.3%	5.98±0.01*	4.68±0.02*	4.32±0.03*	4.19±0.04*	4.05±0.02*
0.5%	6.01±0.02*	4.52±0.03*	4.17±0.02*	4.08±0.05*	3.92±0.06*
0.8%	6.04±0.04*	4.44±0.01*	4.03±0.02*	3.96±0.05*	3.80±0.04*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 247.88 and F_2 (between row) = 8.96 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

Table 4: Effect of *S. platensis* powder on pH of whey inoculated with *S. faecalis*

	pH value of whey				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	5.94±0.02	4.76±0.01	4.54±0.02	4.32±0.03	4.18±0.01
0.3%	5.99±0.01*	4.57±0.02*	4.39±0.02*	4.11±0.03*	4.02±0.01*
0.5%	6.00±0.02*	4.38±0.03*	4.22±0.01*	4.04±0.01*	3.95±0.03*
0.8%	6.01±0.03*	4.29±0.02*	4.11±0.01*	3.97±0.02*	3.82±0.02*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 272.36 and F_2 (between row) = 8.67 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

S. platensis powder is alkaline in nature. From both the tables it was concluded that as the concentration of *S. platensis* powder increases in the whey; samples shows higher value of pH. After the 5 h of incubation the pH falls down drastically as the observation were taken on 1st day. When the observation were taken on 7th, 14th and 21st day of storage period all results shown that the pH value was stable compared to their previous observations. The falling in the pH was very slow but there was a significant difference between the value of control and other *S. platensis* added samples (at 5% level of significance). From the Table 3 and 4 it was clear as the concentration increased the pH value decreased and control was having highest pH value. Most significant change was occurred at 0.8% w/v enriched whey samples. When the data of pH was compared between *S. lactis* and *S. faecalis* then no major difference were found in them. But *S. lactis* show high acidic nature. When the results were compared with previously published results of study by Kavimandan and Sharma (2015), then no large difference was found in pH among *S. thermophilus*, *S. cremoris*, *S. lactis* and *S. faecalis* inoculated whey samples.

Effect on titratable acidity of whey fermented with *S. lactis* and *S. faecalis*, respectively: From the Table 5 and 6 it was depicted that at 0th day observation the TA of control was having highest percentage showing higher acidity in comparison to other three *S. platensis* treated samples for both *S. lactis* and *S. faecalis* fermented samples, respectively. On the next day (1st day), observations control was having less TA with compare to other three concentration samples in both streptococci inoculated whey. On other days observation e.g., 7th, 14th and 21st day it was clear that control was having least TA as compared to other three treatments. As the concentration increased the TA showed higher percentage and on 21st day observation it showed highest acidity. The most significant value was observed at 0.8% w/v enriched samples. In comparison to both streptococci the *S. lactis* show high acidic nature. When the data compared with the previously

Table 5: Effect of *S. platensis* powder on titratable acidity of whey inoculated with *S. lactis*

	TA of whey (%)				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	0.17±0.01	0.46±0.02	0.58±0.03	0.75±0.02	0.85±0.01
0.3%	0.14±0.02*	0.49±0.01*	0.67±0.01*	0.82±0.04*	0.90±0.03*
0.5%	0.11±0.02	0.52±0.03*	0.71±0.04*	0.87±0.04*	0.96±0.06*
0.8%	0.10±0.03*	0.58±0.01*	0.76±0.04*	0.91±0.02*	1.02±0.02*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 186.40 and F_2 (between row) = 5.23 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

Table 6: Effect of *S. platensis* powder on titratable acidity of whey inoculated with *S. faecalis*

	TA (%) of whey				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	0.18±0.02	0.44±0.01	0.57±0.02	0.77±0.03	0.82±0.01
0.3%	0.16±0.01	0.49±0.02	0.63±0.02*	0.80±0.03	0.86±0.02*
0.5%	0.13±0.02*	0.54±0.03*	0.68±0.01*	0.85±0.01*	0.92±0.03*
0.8%	0.11±0.02*	0.58±0.03*	0.72±0.01*	0.90±0.02*	0.98±0.01*

Values are in Mean±SE where n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 196.46 and F_2 (between row) = 5.37 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

Table 7: Effect of *S. platensis* powder on redox potential of whey inoculated with *S. lactis*

	Redox potential of whey (mV)				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	162.33±0.01	178.69±0.03	182.24±0.04	176.33±0.03	145.23±0.05
0.3%	164.24±0.02*	179.23±0.04*	184.35±0.05*	179.33±0.03*	147.88±0.06*
0.5%	167.24±0.02*	182.34±0.04*	189.23±0.03*	183.22±0.05*	150.52±0.06*
0.8%	171.33±0.04*	186.34±0.02*	192.33±0.03*	186.75±0.05*	156.78±0.07*

Values are in Mean±SE where, n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 1384.27 and F_2 (between row) = 137.73 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

performed research of kavimandan and Sharma similar of results occurred in all studied streptococci (Kavimandan and Sharma, 2015). High acidic value shows there was very less chance of contamination or presence of spoilage microorganism in whey.

Effect on redox potential in *S. lactis* and *S. faecalis* fermented whey samples: As shown in the Table 7 and 8 all samples were showed high RP value on 0th day observation. It was clear that as the concentration increases the sample shown high RP value compared to control. It was happened due to the support of *S. platensis* to oxidation capacity of *S. lactis.* As the viability of *S. lactis* increased the RP value of samples also increases. Higher the concentration of *S. platensis* higher the RP value due to the oxidant present in *S. platensis* powder. It was depicted from tables that the RP value decreases on 14th and 21st day of observations. This happened due to the lack of oxygen in sample containers. The highest RP values were observed on the 7th day observation. The highest viability was also found in 7th day observation and as the viability increases (in increment of concentration) RP value also rose. The RP value started to decreases after

Table 8: Effect of *Spirulina platensis* powder on redox potential of whey *S. faecalis*

	Redox potential of whey (mV)				
Treatments	Day 0	Day 1	Day 7	Day 14	Day 21
Control	148.28±0.02	160.22±0.03	154.22±0.04	138.87±0.05	112.23±0.01
0.3%	152.23±0.03*	163.36±0.01*	156.67±0.05*	141.23±0.03*	115.23±0.05*
0.5%	156.78±0.02*	168.47±0.04*	160.10±0.05*	145.38±0.02*	118.56±0.03*
0.8%	160.89±0.02*	172.21±0.04*	163.36±0.04*	149.67±0.03*	121.21±0.02*

Values are in Mean±SE where n = 3 significant at p#0.05 by two way ANOVA. The calculated F_1 (between column) = 2447.80 and F_2 (between row) = 3.25 (F_{1tab} = 3.25 and F_{2tab} = 3.49). *Significant results at 5% level by student's t test (for $t_{0.05}$ = 2.776 for < = 4)

7th day. *Streptococcus faecalis* was having very low RP compared to *S. lactis* may be because of *S. faecalis* is a facultative anaerobe. It was also concluded that *S. faecalis* was having reducing capacity and *S. platensis* powder support the same due to the presence of some reducing agents in it. When the data was compared from the previously published study by Kavimandan and Sharma (2015) then it was clear that *S. thermophilus* is very oxidative in nature and *S. faecalis* is reducing in nature. At the day of inoculation there was a large difference among the RP of all the streptococci enriched whey samples.

CONCLUSION

This is concluded that *S. platensis* enhances the growth of streptococci species. As the concentration increases; difference between the results of control and concentration increases. There was no large difference between the pH and TA of all species observed after 1st day. The acidity of sample increase slowly. With that effect there were not any spoilage microorganism found. So, the role of *S. platensis* powder as prebiotics was justified. Most significant results were found at 0.8% w/v enriched samples. The study also supports the role of *S. platensis* powder as enhancer of storage life of whey for prolong period (more than seven days). High acidity was one of the factors to deplete the growth of probiotic microorganism in whey after 1st week of storage. The 21st day viability also proved that there was sufficient number of microorganisms present in whey samples which are up to the minimum limit of probiotic at time of consumption. When compared to current study with previously performed study the same results were concluded (Kavimandan and Sharma, 2015). With that present study also shows some more opportunities for beverages and other products made from combination of probiotic microorganism and microalgae.

ACKNOWLEDGMENT

This study was done in completion of degree of M. Tech. by author from Madhav Institute of Technology and Science, Gwalior, India. The author is grateful toward Institute for the fulfillment of facilities provided during research and the author was also pay her gratitude to National collection of Industrial Microorganism (NCIM), Pune for providing Streptococcus cultures.

REFERENCES

APHA., 1992. Standard Method for Examination of Dairy Products. 16th Edn., American Public Health Association, Washington, DC..

Andrighetto, C., E. Knijff, A. Lombardi, S. Torriani and M. Vancanneyt *et al*., 2001. Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. J. Dairy Res., 68: 303-316.

Arun, N., S. Gupta and D.P. Singh, 2012. Antibacterial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi. Int. J. Pharm. Sci. Res., 3: 4866-4875.

Belay, A., 1997. Mass Culture of *Spirulina* Outdoors-the Earthrise Farms Experience. In: *Spirulina platensis* (*Arthrospira*): Physiology, Cell-biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis Ltd., London, pp: 131-158.

Citak, S., N. Yucel and S. Orhan, 2004. Antibiotic resistance and incidence of *Enterococcus* species in Turkish white cheese. Int. J. Dairy Technol., 57: 27-31.

Cohen, Z., 1997. The Chemicals of *Spirulina*. In: *Spirulina Platensis* (*Arthrospira*): Physiology, Cell-biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis Ltd., London, pp: 175-204.

Dave, R.I. and N.P. Shah, 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Int. Dairy J., 7: 31-41.

El-Gawad, A.I.A., E.M. El-Sayed, S.A. Hafez, H.M. El-Zeini and F.A. Saleh, 2004. Inhibitory effect of yoghurt and soya yoghurt containing bifidobacteria on the proliferation of Ehrlich ascites tumour cells *in vitro* and *in vivo* in a mouse tumour model. Br. J. Nutr., 92: 81-86.

El-Gawada, A.I.A., E.M. El-Sayeda, S.A. Hafez, H.M. El-Zeinia and F.A. Saleh, 2005. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int. Dairy J., 15: 37-44.

Fuller, R., 1989. Probiotics in man and animals. J. Applied Microbiol., 66: 365-378.

Giraffa, G., 2002. *Enterococci* from foods. FEMS Microbiol. Rev., 26: 163-171.

Harrigan, W.F., 1998. Laboratory Methods in Food Microbiology. Academic Press, London, ISBN: 13-9780123260437, Pages: 532.

Hasman, H., A.G. Villadsen and F.M. Aarestrup, 2005. Diversity and stability of plasmids from glycopeptide-resistant *Enterococcus faecium* (GRE) isolated from pigs in Denmark. Microb. Drug Resistance, 11: 178-184.

Horton, B.S., 1995. Whey processing and utilization. Bull. IDF., 308: 2-6.

Hsieh, M.L. and C.C. Chou, 2006. Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria. Int. J. Food Microbiol., 111: 43-47.

Kavimandan, A. and S. Sharma, 2015. Role of *Spirulina platensis* as an enhancer of probiotic organisms in whey. Int. J. Dairy Sci. Res., 3: 1-6.

Kikuchi-Hayakawa, H.N., S. Onodera, M. Masuoka, S. Kano, Matsubara, E. Yasuda and F. Ishikawa, 2000. Effect of soymilk and Bifidobacterium-fermented soymilk on plasma and liver lipids in ovariectomozed Syrian hamsters. J. Nutr. Sci. Vitam., 46: 105-108.

Kim, H.S. and S.E. Gilliland, 1983. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. J. Dairy Sci., 66: 959-966.

Klein, G., A. Pack, C. Bonaparte and G. Reuter, 1998. Taxonomy and physiology of probiotic lactic acid bacteria. Int. J. Food Microbial., 41: 103-125.

Klein, G., 2003. Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. Int. J. Food Microbiol., 88: 123-131.

Law, J. and A. Haandrikman, 1997. Proteolytic enzymes of lactic acid bacteria. Int. Dairy J., 7: 1-11.

McBride, S.M., V.A. Fischetti, D.J. LeBlanc, R.C. Moellering Jr. and M.S. Gilmore, 2007. Genetic diversity among *Enterococcus* faecalis. PLoS One, Vol. 2. 10.1371/journal.pone.0000582

McGowan, L.L., C.R. Jackson, J.B. Barrett, L.M. Hiott and P.J. Fedorka-Cray, 2006. Prevalence and antimicrobial resistance of enterococci isolated from retail fruits, vegetables and meats. J. Food Protec., 69: 2976-2982.

Messi, P., E. Guerrieri, S. De Niederhaeusern, C. Sabia and M. Bondi, 2006. Vancomycin-Resistant Enterococci (VRE) in meat and environmental samples. Int. J. Food Microbiol., 107: 218-222.

Moreno, M.R.F., P. Sarantinopoulos, E. Tsakalidou and L. de Vuyst, 2006. The role and application of *Enterococci* in food and health. Int. J. Food Microbiol., 106: 1-24.

Panesar, P.S., J.F. Kennedy, D.N. Gandhi and K. Bunko, 2007. Bio-utilization of whey for lactic acid production. Food Chem., 105: 1-14.

Parekh, J.V., 2006. Emerging new technologies in the dairy industry in India. http://www.fnbnews.com/article/detarchive.asp?articleid=19393§ionid=40

Richmond, A., 1984. Spirulina.. In: Micro-algal biotechnology, Borowitzka, M.A. and L.J. Borowitzka, (Ed.), Cambridge University Press, UK., pp: 85-121.

Sarantinopoulos, P., C. Andrighetto, M.D. Georgalaki, M.C. Rea and A. Lombardi *et al*., 2001. Biochemical properties of enterococci relevant to their technological performance. Int. Dairy J., 11: 621-647.

Schleifer, K.H. and R. Kilpper-Balz, 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: A review. Syst. Applied Microbiol., 10: 1-19.

Shafiee, G., A.M. Mortazavian, M.A. Mohammadifar, M.R. Koushki, A. Mohammadi and R. Mohammadi, 2010. Combined effects of dry matter content, incubation temperature and final pH of fermentation on biochemical and microbiological characteristics of probiotic fermented milk. Afr. J. Microbiol. Res., 4: 1265-1274.

Stiles, M.E. and W.H. Holzapfel, 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol., 36: 1-29.

Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacteriophages. Applied Microbiol., 29: 807-813.

USPHA., 1995. Grade A Pasteurized Milk Ordinance. United States Public Health Service, Washington, DC.

Varga, L., J. Szigeti, R. Kovacs, T. Foldes and S. Buti, 2002. Influence of a *Spirulina platensis* biomass on the microflora of fermented ABT milks during storage (R1). J. Dairy Sci., 85: 1031-1038.

Vonshak, A., 1997. Appendices... In: *Spirulina platensis* (*Arthrospira*) Physiology, Cell-biology and Biotechnology, Vonshak, A. (Ed.). Taylor and Francis Ltd., London, pp: 213-226.

Vonshak, A., 2002. *Spirulina platensis* (*Arthrospira*) Physiology, Cell-Biology and Biotechnology. Taylor and Francis e-Library, London.

Yildirim, Z. and M.G. Johnson, 1998. Characterization and antimier spectrum of *BIfidocin* B, a bacteriocin produced by *Bifidobacterium* bifidum NCFB 1454. J. Food Prot., 61: 47-51.

Zanella, R.C., M.D.J.C. Lima, L.S. Tegani, A. Hitomi, M.C.D.C. Brandileone, I.C.V. Palazzo and A.L.D.C. Darini, 2006. Emergence of vanB phenotype-vanA genotype in vancomycin-resistant enterococci in Brazilian hospital. Brazilian J. Microbiol., 37: 117-118.

Zarrouk, C., 1966. Contribution a letude dune cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de Spirulina maxima. Ph.D. Thesis, Universite de Paris, Paris.