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Studying the Effects of Heat and Cold Shock on Cell wall Microstructure and Survival of Some LAB in Milk

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Abstract: In this study we investigated the effect of heat shock (42-45°C for 5, 10, 15, 20, 25, 30 minutes) cold shock (4, 0, -5, -10 and -20°C for 2h) on cell wall Microstructure of LAB. Cell wall Changes and micro-damage behavior of LAB under the impact of heat and cold shock was studied by optical microscope and Transmission Electron Microscope (TEM). Treatment of LAB with heat and cold shock had significant effects on permeability properties of LAB just in long heat exposures and very cold temperatures. The survival of LAB was just affected by the heat shock.

Key words: Micro structural properties . lab . heat shock . cold shock . survival

INTRODUCTION

Probiotic bacteria are defined as living micro-orginsms, which upon ingestion in certain numbers; exert health benefits beyond in herent basic nutrition. Probiotic's ability to grow well in the product and also their survial in the final product is of great importance to show their health benefits. Increasing the entymatic activity of probiotics without any negative effect on their survival is noteworthy [1]. The secretive enzymes of probiotic bacteria improve their survival [2]. Every kind of shock can influence the functionality of the cell wall of bacteria and improve or damage their normal physiological and vital activities [3, 4]. Cold shock changes the lipid phase in the membrane and develops hydrophobic holes in it. The result is the enhanced permeability of cell membrane and release of internal compounds [5, 6].

Freezing shocks in temperatures below -20°C in comparison to-10°C have less impact on cell wall of the bacteria [7]. The reduction of bacterial population is very fast in temperatures near the freezing points especially -2°C [8].

Heat shock also causes holes in the membrane and inactivates the sensitive enzymes and ribosoms, the final result is the reduction of biological activities of the bacteria or their death.

The main purpose of this study was to evaluate the potential of ultrasound cold, freezing and heat shock on cell wall of the bacteria and increasing the cell wall permeability of some probiotic bacteria by TEM and optical microscope. The release of internal enzymes and other internal compounds is very important for different aspects of dairy processing technology as reducing the yoghurt coagulation time.

MATERIAL AND METHODS

Milk with 2/5% fat and 10/5% dry weight was supplied from local market. Pure lyophlised cultures of Lactobacillus acidophilus (strain, LAI), Lactobacillus casei (strains AB) Lactococcus lactis.spp cremoris and lactococus lactis.spp lactis were supplied from local industries.

Packages of bacteria were prepared according to the company's instructions and were added (5%, v/w) to the milk aseptically, then the milk was distributed into tubes with screwing caps to perform the shocks. Cold and freezing shock for 2 hours in 4/0, -5, -10 and -20°C. And Heat shock for 5, 10, 15, 20, 25 and 30 minutes in 42-45°C were performed on the samples. Then samples were diluted serially and plated on MRS agar. Then the samples were prepared to observe under the optical and TEM Microscope [9-11].

RESULTS AND DISCUSSION

Freezing and cold shock: Figure 1-7 show the effect of cold and freezing shock on probiotic bacteria.

According to the results obtained by optical microscope, there was a population balance both in control and treated samples. As discussed before, by decreasing the temperatures, the streptobacillus or filaments are formed. Micro cracks, micro voids and raptures (less than -20°C) are also observed.

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Fig. 1: Microscopic picture of probiotic bacteria before freezing shock

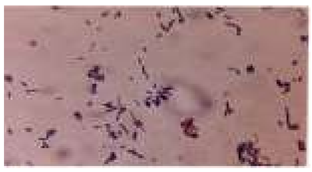

Fig. 2: Microscopic picture of probiotic bacteria after freezing shock (4°C)

Fig. 3: Microscopic picture of probiotic bacteria after freezing shock (0°C)

Fig. 4: Microscopic picture of probiotic bacteria after freezing shock (-5°C)

Fig. 5: Microscopic picture of probiotic bacteria after freezing shock (-10°C)

Heat shock: Figure 8-12 show the effect of heat shock on the probiotic bacteria. Studies showed that, increasing the exposure time, does not change the population to special group of bacteria, other characteristics of bacteria as the shape of colony, length and diameter of cell, was unchanged after heat treatment [10, 11].

Fig. 6: Microscopic picture of probiotic bacteria after freezing shock (-20°C)

Fig. 7: Microscopic picture of probiotic bacteria (cell wall damages are not obvious)

Fig. 8: Microscopic picture of probiotic bacteria before heat shock.The number of Lactococcus and lactobacillus are the same and all of bacteria are safe

Fig. 9: Microscopic picture of probiotic bacteria after 5 minutes exposure to heat shock

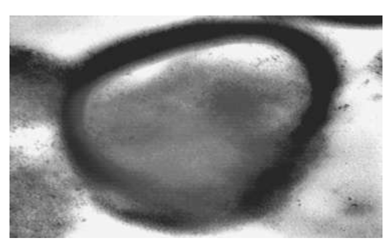

Fig. 10: Microscopic picture of probiotic bacteria before heat shock

Table 1: Changes of pH, acidity and total count of bacteria after the exposure to heat shock

	0 min	5 min	10 min	15 min	20 min	25 min	30 min
pH	6.40	6.06	6.033	6.00	6.00	6.00	6.00
Acidity	0.20	0.30	0.280	0.27	0.28	0.28	0.27
Total count	5.98 CFU	5.80 CFU	5.730	5.55	5.32	5.23	5.22
Dry matter	12.27	12.23	12.230	12.00	12.07	12.00	12.07

Table 2: Changes of pH, acidity and total count of bacteria after the exposure to cold shock

	Control	4°C	0°C	-5°C	-10°C	-20°C
pH	6.40	6.16	6.16	6.13	6.03	6.06
Acidity	0.20	0.20	0.21	0.28	0.28	0.29
Total count	5.98	5.95	5.89	4.96	4.94	4.94
Dry matter	12.27	12.03	12.03	12.00	12.03	12.03

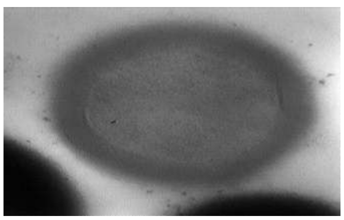

Fig. 11: Microscopic picture of probiotic bacteria after 10 minutes exposure to heat

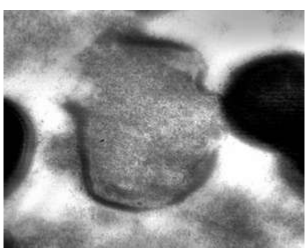

Fig. 12: Microscopic picture of probiotic bacteria more than 10 minutes exposure times to heat shock.

Short exposures just reduced the cell wall diameter, but longer exposures caused micro-cracks and micro-voids in the cell wall. Table 1 and 2 show the changes of pH, acidity and total count of bacteria after the exposure to the heat and cold shock [11].

CONCLUSIONS

- Cell wall permeability, volume and size of the cells are affected by heat and cold shock. different ways (Fig. 10 and 12).
- Surival of probiotic strains decreased as the exposure time increased.Some physiological functions of bacteria depend on their cell wall structures and every kind of damages could affect it.Further studies proved this (data not shown).

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