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# EFFECTS OF MILK FERMENTED WITH *Lactobacillus acidophilus* AND *Bifidobacterium* spp., ON LACTIC ACID AND ACETIC ACID CONTENT AND ON *Staphylococcus aureus* AND *Pseudomonas aeruginosa*

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**Abstract:** The aim of the research concerning „Effects of Milk Fermented with *Lactobacillus acidophilus* and *Bifidobacterium* spp., on lactic and acetic acid content, and on *Staphylococcus aureus* and *Pseudomonas aeruginosa*”, were to know the lactic and acetic acid content in this probiotic yoghurt, and also the effect to the pathogenic bacteria, e.g. *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Results indicated that the probiotic yoghurt has lower lactic acid content than the control, but the acetic acid content was higher than the control; and also has ability as bio-preservative against the pathogenic bacteria.

**Key words:** acetic acid, lactic acid, *Bifidobacterium* spp., *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

## Introduction

One of the criteria that the culture used to inoculate a fermentation it must be in a healthy, active state thus minimizing the length of the lag phase in the subsequent fermentation. Another important factor in obtaining an inoculum fulfilling these criteria is the choice of the culture medium (*Stanbury and Whitaker, 1984*).

Lactose or milk sugar is a disaccharide, composed of the monosaccharide dextrose (glucose) and galactose. Lactose in milk may be fermented by micro-organism. Lactic acid bacteria (LAB) convert lactose into lactic acid and a number of by-products until the accumulated acid prevents further reproduction and activity of the bacteria. At this point, 15 to 40% of the lactose has been fermented, depending on the type of bacteria. The acid produced by lactic acid bacteria will prevent the development of certain putrefying bacteria and actually preserves the milk, although it becomes sour. The LAB can be characterized by the way they ferment lactose in milk after this disaccharide has been transformed into two

monosaccharides, viz. glucose and galactose. *Lactobacillus acidophilus* is a slow acid producer in cultures used for the production of acidophilus milk. Acidophilus milk is recommended as a dietary product, because *Lactobacillus acidophilus* functions in the large intestine of man. This product has a plain acid flavor, and may be combined with other bacteria to obtain a more palatable product (van den Berg, 1988). *Lactobacillus acidophilus* grows very slowly in milk and therefore it is important to protect the milk against contamination. In bioghurt, *Lactobacillus bulgaricus* is replaced by *Bifidobacterium* and *Lactobacillus acidophilus*. The reason for using *Bifidobacterium* in place of *Lactobacillus bulgaricus* is that *Bifidobacterium* spp. produces the physiologically active D(+)-lactic acid, whereas *Lactobacillus bulgaricus* produces the physiologically inactive D(-)-lactic acid (Cross and Overby, 1988).

Most bacteria important to the milk processing industry belongs to the Gram-positive cocci e.g. *Staphylococcus* spp. The genus *Staphylococcus* deserves special attention, because it includes many pathogens that may cause infections or produce toxins; *Staphylococcus aureus* is notorious in the dairy industry. The Gram-negative aerobic rods and cocci, are Gram-negative and putrefactive bacteria, such as psychrotropic *Pseudomonas* species, which produces lipases and proteinases. *Pseudomonas* spp. are often rather proteolytic and lipolytic, the milk will easily deteriorate.

Lactic acid bacteria produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic acid fermentation (Oyetayo et al., 2003). Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria (Tagg et al., 1976). The bacteriocins from the Generally Recognized as Safe (GRAS) lactic acid bacteria have arisen a great deal of attention to control pathogens in foods (Schillinger et al., 1996).

## Materials and Methods

**The milk.** Raw milk (cow milk) from the faculty farm animals.

**The bacteria.** The bacteria are pure cultivated *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The identification of the cultures was based on the characteristics of the lactobacilli and streptococci as described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

**Lactic acid and acetic acid content,** was analyzed by HPLC 1050. ODS Hypersil; 250 x 4.6 mm column, UV detector,  $\lambda$  220 nm, eluen methanol + water (1.3% as phosphate) 70 : 30; pH : 2.2 flow 0.5 ml/minute; comp: 120 bar.

**Antibiogram of Lactic acid bacteria on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.** The isolates were inoculated into MRS broth individually and incubated for 24h. About 20ml MRS agar was seeded with the cultures of Lactic acid bacteria isolates, mixed well, poured into sterile Petri plates and stored at 4°C for 1h to solidify the media. OCTA-discs (OXOID) were placed up side down, pressed on the top of the agar plates and kept again at 4°C for 1h. The plates were incubated at 37°C over night. Resistance was defined as the absence of a growth inhibition zone around the discs.

## Results and Discussion

### The effect of the treatment on lactic acid and acetic acid

The lactic acid and acetic acid of yoghurt indicates its real acidity. In Table 1, presents the results of the lactic acid and acetic acid content (%) for each yoghurt (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and *Bifidobacterium* spp. and *Lactobacillus acidophilus*).

**Table 1. The effect of the treatment on lactic acid and acetic acid**

No.	Yoghurt starters	Lactic acid (%)	Acetic acid (%)
1	<i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>	0.4272	0.5873
2	<i>Bifidobacterium</i> spp. and <i>Lactobacillus acidophilus</i>	0.3996	1.6641

*Streptococcus thermophilus* is homofermentative and is especially found in thermophilic starters and cultures, that are particularly suitable for products culture such as yoghurt, and producing diacetyl. *Lactobacillus bulgaricus* which, together with *Streptococcus thermophilus* is known for its capacity to produce the flavour component acetaldehyde in addition to diacetyl. Greater acid production is obtained when the two organisms are growing together than if grown separately. This is due to the proteolytic action of *Lactobacillus bulgaricus*, whereby valin, which stimulates the growth and acid production of the streptococci, is released. *Streptococcus thermophilus*, however, also produces a stimulant for the growth of lactobacilli; this agent has been proved to be formic acid.

*Lactobacillus acidophilus* is a slow acid producer in cultures used for the production of yoghurt that has a plain acid flavor, and may be combined with other bacteria to obtain a more palatable product. *Lactobacillus acidophilus* grows very slowly in milk and therefore it is important to protect the milk against contamination. The milk has no real aromatic flavor and is frequently recognized as too sour, so it is sometimes mixed with other bacteria. The reason for using



*Bifidobacterium* spp. in place of *Lactobacillus bulgaricus* is that *Bifidobacterium* spp. produces the physiologically active D(+)-lactic acid. The lactic acid of the yoghurt using *Bifidobacterium* spp. and *Lactobacillus acidophilus*, are lower than the yoghurt with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starters; because they produced slightly lower of lactic acid and higher acetic acid, especially *Bifidobacterium* spp. that is heterofermentative which producing 40% lactic acid and 60% acetic acid.

### Antibiogram of Lactic acid bacteria on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

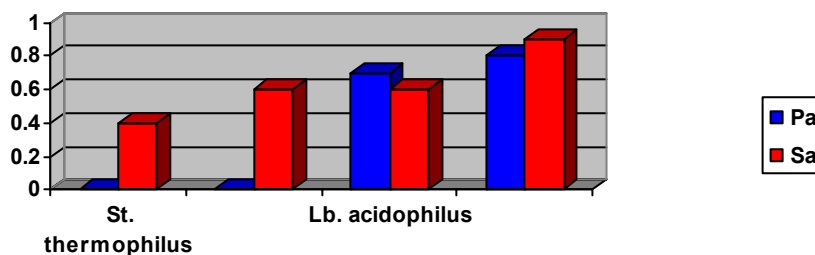
In Table 2, presents the results of the sensitivity of lactic acid bacteria on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Table 2.** The sensitivity of Lactic acid bacteria on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Lactic acid bacteria	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
<i>Streptococcus thermophilus</i>	0 mm	0.4 mm
<i>Lactobacillus bulgaricus</i>	0 mm	0.6 mm
<i>Lactobacillus acidophilus</i>	0.7 mm	0.6 mm
<i>Bifidobacterium</i> spp.	0.8 mm	0.9 mm

From Table 2 *Bifidobacterium* spp. showed high sensitivity reaction on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and *Streptococcus thermophilus* showed the lowest sensitivity reaction on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. According to Mitsuoka (1989) and Fuller (1992), has the ability as bacteriocin.

In the Graphic 1, showed the results about the sensitivity of the lactic acid bacteria on *Pseudomonas aeruginosa* and *Staphylococcus aureus*.



Notes:

Pa = *Pseudomonas aeruginosa*

Sa = *Staphylococcus aureus*

**Graph 1.** Diameter of inhibition zone to the *Pseudomonas aeruginosa* and *Staphylococcus aureus*

From the Graph 1, showed that *Lactobacillus acidophilus* and *Bifidobacterium* spp. has sensitivity on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but *Streptococcus thermophilus* and *Lactobacillus bulgaricus* only on *Staphylococcus aureus*.

## Conclusion

The lactic acid of the yoghurt using *Bifidobacterium* spp. and *Lactobacillus acidophilus*, are lower than the yoghurt with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* starters; because they produced slightly lower of lactic acid and higher acetic acid, especially *Bifidobacterium* spp. that is heterofermentative which producing 40% lactic acid and 60% acetic acid.

*Bifidobacterium* spp. and *Lactobacillus acidophilus*, showed sensitivity reaction on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed sensitivity reaction only to *Staphylococcus aureus*, but no sensitivity to *Pseudomonas aeruginosa*; because *Bifidobacterium* spp. and *Lactobacillus acidophilus* has the ability as bacteriocin.

## Uticaj mleka fermentisanog sa *Lactobacillus acidophilus* i *Bifidobacterium* spp. na sadržaj mlečne i sirćetne kiseline i *Staphylococcus aureus* i *Pseudomonas aeruginosa*

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## Rezime

Cilj istraživanja u ovom radu, «Uticaj mleka fermentisanog sa *Lactobacillus acidophilus* i *Bifidobacterium* spp., na sadržaj mlečne i sirćetne kiseline i na *Staphylococcus aureus* i *Pseudomonas aeruginosa*», je bio određivanje sadržaja mlečne i sirćetne kiseline u probiotskom jogurtu, kao i uticaj na patogene bakterije, *Staphylococcus aureus* i *Pseudomonas aeruginosa*. Rezultati su pokazali da je probiotski jogurt imao niži sadržaj mlečne kiseline u odnosu na kontrolu, ali sadržaj sirćetne kiseline je bio viši nego u kontroli, i poseduje kapacitet bio-prezervacije od patogenih bakterija.

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## References

- CROSS H.R., OVERBY A.J. (1988): Meat Science, Milk Science and Technology. Elsevier Science Publ. Amsterdam. 207, 293.
- HOLT J.G., KRIEG N.R., SNEATH P.H.A., STALEY J.T., WILLIAMS S.T. (1994): Bergey's Manual of Systematic Bacteriology. Ninth ed. Williams and Williams. Baltimore. Pp. 566.
- FULLER R. (1992): History and Development of Probiotics. In Probiotics the Scientific Basis. Edited by Fuller, R. Chapman and Hall.
- MITSUOKA T. (1989): Microbes in the intestinal. Our life long partners. Japan.
- OYETAYO V.O., ADETUYI F.C., AKINYOSOYE F.A. (2003): Safety and Protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent in vivo. Afr. J. Biotech., 2, 448-452.
- SCHILLINGER U., GEISEN R., HOLZAPFEL W.H. (1996): Trends Food Sci. Tech., 7, 158-164.
- STANBURY P.F., WHITAKER A. (1984): Principles of Fermentation Technology. Pergamon Press. Oxford. pp. 108.
- TAGG J.R., DAJANI A.S., WANNAMAKER L.W. (1976): Bacteriocins of Gram-positive bacteria. Bacteriol. Rev., 40, 722-756.
- VAN DER BERG J.C.T. (1988): Dairy Technology in the tropics and subtropics. Pudoc, Wageningen. The Netherlands. pp. 146.

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