Acta Veterinaria (Beograd), Vol. 51. No. 1, 53-66, 2001.

UDK 619:637.146

CHARACTERISTICS OF NATURAL ISOLATES OF LACTIC ACID BACTERIA SELECTED FOR CONSTRUCTION OF STARTER CULTURES FOR SEMI-HARD CHEESE OF THE TRAPIST TYPE

VUKAŠINOVIĆ MAJA, FIRA Đ. and TOPISIROVIĆ LJ.

Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, P.O. BOX 446, 11001 Belgrade, Yugoslavia, LAB6@eunet.yu

(Received 16. December 2000)

Starter cultures (S12, S19 and S21) for semi-hard cheese of the Trapist type were constructed by using natural isolates of lactic acid bacteria (LAB) that were mostly isolated from home-made cheese. These starter cultures caused rapid acidification under technological conditions of cheese manufacture and greatly contributed to the ripening of the products. Incubation of these starter cultures under a temperature gradient showed that the treatment stimulated the curd cooking phase. The reduced cell viability was probably due to heat stress during the heat treatment of curd during the temperature gradient. All semi-hard cheeses produced using these starters had good flavor and a few small eyes.

Key words: Lactic acid bacteria, semi-hard cheese, starter culture, Trapist

INTRODUCTION

Lactic acid-fermented foods (including cheeses) have proved their wholesome value for hundreds of years and are accepted without restriction by the consumer. The use of starter cultures to initiate or "start" the cheese manufacturing process was practised long before anything was known about the role of the bacteria they contained. The starters used until the end of the 19th century were either naturally soured milk or whey from a previous well produced batch of cheese. This type of starter is still used today in countries with a long tradition of cheese making in small production units (Italy, France and Switzerland). Such a method of production is not reliable and the quality of the end product can vary tremendously (Teuber, 1987).

In the modern manufacture of cheese and fermented milks, the trend is towards larger production units and a controlled manufacturing process. Over the past 15-20 years, starter cultures have evolved from a heterogeneous mix of undefined strains into the small number of highly characterized, uniform strains that support todays intensive cheese-making practices.The successful production of cheese and other fermented dairy products relies completely on choosing the right organisms for the specific type of required fermentation (Tamime, 1981).

The formulation of particular starter cultures for different types of cheeses is based on the physiological and technological characteristics of the lactic acid bacteria (LAB). The LAB used in starter cultures possess numerous metabolic properties (acidification rate, proteolytic activity, diacetyl formation, production of exopolysaccharide, synthesis of bacteriocin, resistance to bacteriophages) which are strain dependent. All of these metabolic activities contribute to the flavor, texture and frequently, the nutritional attributes of the products (Gilliland, 1985).

Today, it is possible to construct improved strains using recombinant DNA technology, because several of the desired properties are plasmid-encoded, which facilitates genetic manipulation. This approach allows for the development of a new generation of starter cultures with precise and easily controlled product formation. However, genetically modified microorganisms are still far away from broad application. Most consumers would still choose conventional food (food without genetically modified microorganisms), because genetically modified microorganisms will be consumed in the live state and this information can have considerable influence on public acceptance. Taking this situation into consideration and knowing that there is a pool of naturally available strains of LAB, there is still no need for wide use of genetically modified strains (Bueckenhuskes, 1993). Instead, elucidation of the properties of natural isolates of LAB is opening a new possibilities in the construction of desirable starter cultures.

In this paper, we describe the characterization of natural isolates of LAB, and their use for construction of new starter cultures for manufacturing the Trapist type semi-hard cheese. Starter cultures S12, S19 and S21 were prepared by combination of natural isolates of LAB and their quality was tested in laboratory and industrial conditions.

MATERIALS AND METHODS

Bacterial strains and media

The list of bacterial strains selected for this study, including their origin, is given in Table 1. Natural isolates of lactic acid bacteria (LAB), designated as BGTM1, BGTM4, BGJAV11, were collected from different home made hard cheeses. It is worth mentioning here that all LAB were isolated from traditionally home made cheeses that were manufactured without addition of any starter culture. In addition, LAB were isolated from home made butter (BGMM) and the human intestinal tract (BGRA43). Analysis of the bacterial species in BGMM revealed three lactococcal species (Table1). Standard microbiological procedures were used for the isolation, detection and selection of LAB. Isolates were characterized by Gram staining, catalase test and ability to grow at 15°C, 30°C and 45°C. In addition, the carbohydrate fermentation pattern was determined by API 50 CH (API System S., Montelieu, Vercieu, France). The isolates were identified from the characteristics of LAB presented in Bergeys Manual of Systematic Bacteriology (Kandler and Weiss, 1986, Mundt, 1986a). All *Lactobacillus* strains were grown in MRS broth (Difco, Detroit, Mich.) (DeMan *et al.*, 1960). *Lactococcus lactis* strains were grown in M17 medium (Terzaghi and Sandine, 1975) supplemented with glucose (0.5% w/v) (GM17 broth) (Merck, GmbH, Darmstadt, Germany). The cultures were maintained by routine subculturing in reconstituted skim milk containing 10% solids (w/v) (RSM) and

stored at 4^{0}C between subculturing. Agar plates were prepared by adding agar (1,5% w/v) (Difco) to each broth when used as a solid medium.

Table 1: List of bacterial strains used in the study.

Strain	Properties	Source
L. casei subsp. *rhamnosus* BGTM1	Natural isolate	Home made hard cheese
L. helveticus BGTM4	Natural isolate	Home made hard cheese
L lactis subsp. *lactis* BGJAV11	Natural isolate	Home made hard cheese
butter milk starter cultures BGMM *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* biovar.diacetylactis *L. lactis* subsp. *cremoris*	Natural starter	Home made butter
L. acidophilus BGRA43	Natural isolate	Human intestinal tract

Assay of proteolytic activity

Proteolytic activity of BGTM1, BGTM4, BGJAV11,BGRA43, BGMM and three constructed starter cultures were assayed as described previously (Kojic *et al.*, 1991, Kojic *et al.*, 1995). For enzymatic assays, the isolates were grown on milk-citrate agar (MCA) plates containing 4.4% RSM, 0.8% Na-citrate, 0.1% yeast extract, 0.5% glucose and 1.5% agar (w/v) for 48h at 30 ^{0}C and 37^{0}C for induction of proteinase production. Collected cells (5 mg) were resuspended in 100 mM Na-phosphate buffer (pH 7,2) to an approximate density of 10^{10} cells per ml. The cell suspension was mixed with substrate dissolved in the same buffer in a 1:1 volume ratio. The resulting mixtures were incubated at 30^{0}C and 37^{0}C for various time intervals (3 and 8 h).Total casein (12 mg ml^{-1}) (Sigma Chemie GmbH, Deisenhofen, Germany) was employed as the substrate. After incubation, samples were taken, centrifuged, and degradation products were analyzed by SDS-PAGE by loading 12% (w/v) acrylamide gel with prepared samples. Gels were run on vertical slab electrophoresis cells (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, MD, USA) for 7h at 20mA constant current and stained with Coomassie brilliant blue G250 (SERVA, Heidelberg, Germany).

Plasmid isolation

The plasmid content of isolates was examined by separation of plasmids from the strains and running them on agarose gel (1%) as described by Anderson and McKay (1983). A logarithmic culture was prepared in 4 ml of MRS or GM17 broth. Cells were pelleted by centifugation (1 min at 12000g), resuspended in 1.5 ml TEN buffer (50mM Tris-HCl, 10mM EDTA, 50 mM NaCl, pH 8) and pelleted again by centrifugation. Cells were resuspended in 100 µl PP buffer (0.5 M sucrose, 40 mM NH4-acetate, 1 mM Mg-acetate, pH 8) to which 4 mg ml^{-1} of lysozyme was added. This suspension was incubated for 15 min at 37^{0}C. After

incubation 200l of 1% SDS in TE-1 buffer (100 mM Tris-HCl, 10 mM EDTA, pH 12) was added and the tubes were gently inverted. Glacial acetic acid (100 çl) was added and the contents mixed gently by inversion followed by addition of 120 µl NaCl (5M). The resultant obtained mixture was again gently inverted twice and centrifuged (15 min at 12000g). The clear supernatant fluid (400 µl) was transferred into a new tube (taking care to avoid disturbing the pelleted material) to which 2 volumes of cold 96% EtOH was added. After immediate centrifugation (10 min at 12000g), the resulting pellet was washed twice in 1 ml 70% EtOH, dried and resuspended in 20 µl H_2O. Electrophoresis was performed on 1% (w/v) gels. TBE (89 mM Tris, 89 mM boric acid, 25mM EDTA-Na_2, pH 8.5) was used as the running buffer. Alternatively, plasmids were isolated following the previously described procedure for the isolation of large plasmids (O' Sullivan and Klaenhammer, 1993).

Starter cultures S12, S19 and S21

Starter cultures S12, S19 and S21 were grown in 300 ml of RSM with a 3% inoculation rate. The composition of the starters was: S12- BGRA43: BGTM4: BGMM (2:1:1), S19- BGMM: BGTM1 (1:3), S21- BGMM: BGTM1: BGJAV11 (2:1:1). Starter cultures S19 and S21 were mesophilic with an optimal growth temperature of 30^{0}C, while starter culture S12 was thermophilic with optimal growth at 37^{0}C. The semi-hard Trapist types cheeses produced from these starter cultures (S12, S19 and S21) were manufactured according to standard procedures for this type of cheese in the dairy factory in 1000 L vats. Pasteurised cows milk adjusted to 2,8% fat and at 30^{0}C was inoculated with 1% starter which was stirred in for 5 min. At this stage, coagulant (1,5%), i.e. Fromase 150TL, $CaCl_2$ (0,02%) and cheese colour (1%) were added and stirred for 5 min. The milk was allowed to coagulate for 30 min. The coagulum was cut to the size of pea grains, heated to 38^{0}C and stirred for 50 min. The cheeses, weighing 2.6 to 2.8 kg were pressed for 15h and salted by immersion in 20% brine solution at 12^{0}C for 2 days. Immediately after salting, the cheeses were covered with a protective layer of polyvinyl acetate and ripened in rooms at 80% humidity at 912^{0}C for 60 days.

Acidification activity of starter cultures for semi-hard cheese at constant temperature and under a temperature gradient

The acidification activities and growth of starter cultures at constant temperature and under technological conditions was investigated as follows. The freshly curdled culture was inoculated (3%, vol/vol) in 400 ml of sterile skim milk and incubated at a constant temperature (30^{0}C for mesophilic starter cultures S19 and S21 and 37^{0}C for thermophilic starter culture S12) and under a temperature gradient. After inoculation, the temperatures of 30°C and 37°C were held for 40 min and then increased slowly over 40 min to 37°C (starters S19 and S21) and 45°C (starter S12) (1°C for 4 min) "cooking phase". The higher temperature was held for 120 min and then the cultures were gradually cooled to 30°C and 37°C, respectively, during 24 h. The sourness (°SH) was continuously recorded in all starter cultures for 24h. During the tests samples of starter cultures were taken for determination of the colony forming units per ml (CFU ml^{-1}), at times identical to those used for measurement of sourness (°SH) Each sample, collected at different incubation times, was plated on MRS agar (Difco) (Neviani *et al*, 1995).

RESULTS

Strain characterization

The natural isolates BGTM1, BGTM4, BGJAV11, BGMM and BGRA43 were Gram-positive and catalase-negative. BGTM1, BGTM4 and BGRA43 were rod-shaped bacteria while BGJAV11 contained cocci. Microbiological analysis confirmed that BGMM consisted of three lactococcal species, *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* biovar. diacetylactis and *L. lactis* subsp. *cremoris*. BGJAV11 and BGMM grew at 15^{0}C, but did not grow at 45^{0}C, 4% or 6.5% NaCl nor at pH 9.2 or pH 9.6. BGTM4 and BGRA43 did not grow at 15^{0}C, but grew at 45^{0}C in MRS broth. All isolates grew very fast in RSM. The isolate BGTM1 grew well at both temperatures (15^{0}C and 45^{0}C). Isolates BGTM4 and BGRA43 did not grow in 4% and 6.5% (w/v) NaCl, while BGTM1 was able to grow, i.e. was resistant to NaCl (4%, w/v). None of the isolates produced gas from glucose. The isolate BGJAV11 produced bacteriocin that inhibited growth of closely related lactococci. When isolates BGTM1, BGTM4, BGRA43 and BGMM were grown in sterile RSM, the viscosity of the curd increased considerably. Taking into consideration these characteristics and carbohydrate fermentation patterns the isolates were characterised as: *L. casei* susbp. *rhamnosus* BGTM1, *L. helveticus* BGTM4, *L. acidophilus* BGRA43, *L lactis* susbp. *lactis* BGJAV11.

Plasmid profile

Analysis of plasmid content of isolates BGTM1, BGTM4, BGJAV11 and BGRA43 regardless of the procedure used for plasmid isolation, revealed that only strains BGJAV11 and BGRA43 contained plasmid. The plasmid in strain BGRA43 appeared to be very stable and it seems that it did not harbour gene(s) encoding proteinase production, since proteinase non-producing derivatives of BGRA43 contained the same plasmid profile (Banina *et al.*, 1998). Analysis of the plasmid content of BGTM1 and BGTM4 showed that these natural isolates appeared to be plasmid free (data not shown).

Proteolytic activity of selected strains and starter cultures S12,S19 and S21

To test the ability of selected strains (BGTM1. BGTM4, BGJAV11, BGMM and BGRA43) and constructed starter cultures S12, S19 and S21 to hydrolyse total casein, cells of these strains were incubated in the presence of total casein for 3 h and 8 h at 30^{0}C and 37^{0}C (Fig. 1). The results showed that the isolate *Lactobacillus acidophilus* BGRA43 had the highest proteolytic activity. Complete hydrolysis of total casein was tested after 3 h of incubation at 37^{0}C (Fig 1, line 9). It has been shown previously that strain BGRA43 efficiently hydrolyzed all three major casein fractions (α1-casein, β-casein and κ-casein) (Banina *et al*, 1998). On the other hand, there was no visible degradation of total casein by isolates BGTM1, BGTM4, BGJAV11 and BGMM after 3h of incubation. After 8h of incubation strains BGTM1, BGTM4, BGAV11 and BGMM showed lower degradation of total casein than that with BGRA43. Strain BGTM4 hydrolyzed only beta-casein. This could be explained either by lower activity of BGTM1, BGJAV11 and BGMM proteinases or smaller quantities of enzymes synthesized by these isolates (Kojic *et al.*, 1995).

Thermophilic starter culture S12 completely hydrolysed total casein after incubation for 3 h at 37^{0}C (Fig 1, line 13). In contrast, mesophilic starter cultures S19 and S21 expressed very low, if any, proteolytic activity towards total casein within 3 h at 37^{0}C (Fig 1, line 15 and 17 respectively).

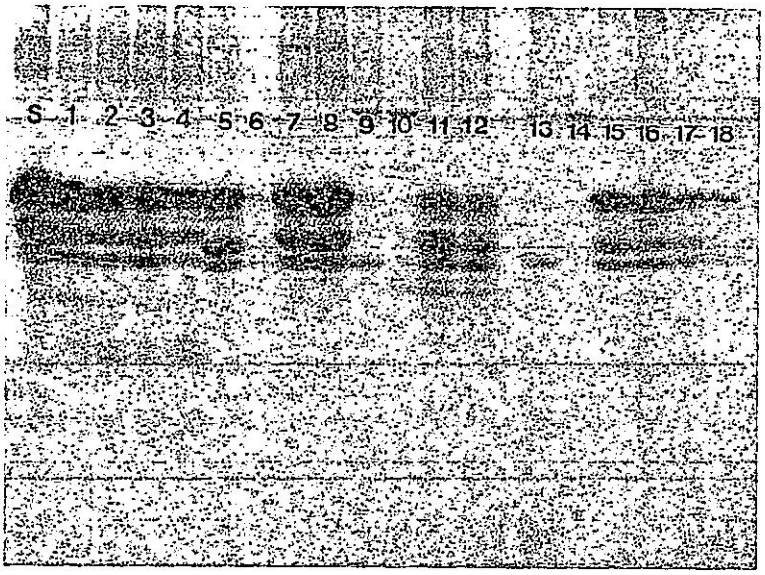

Figure 1. Proteolytic activity towards total casein of the selected LAB and starter cultures S12, S19 and S21
Lane S: starting substrate -total casein. Lanes 1 and 2: butter milk starter culture BGMM after 3 h and 8h of incubation, at 30°C respectively,. Lanes 3 and 4: butter milk starter culture BGMM after 3 h and 8h of incubation, at 37°C respectively,. Lanes 5 and 6: BGTM1 incubation at 30°C for 3 h and 8h respectively, Lanes 7 and 8: BGJAV11 after 3 h and 8h of incubation respectively, at 30°C. Lanes 9 and 10: BGRA43 after 3 h and 8h of incubation respectively, at 37°C. Lanes 11 and 12 BGTM4 after 3 h and 8h of incubation respectively, at 37°C. Lanes 13 and 14: starter culture S12 after 3 h and 8h of incubation respectively, at 37°C. Lanes 15 and 16: starter culture S19 after 3 h and 8h of incubation respectively, at 30°C. Lanes 17 and 18 : starter culture S21 after 3 h and 8h of incubation respectively, at 30°C.

Acidification activity of starter cultures S12, S19 and S21 at constant temperature and under temperature gradient

The acidification activity of starter cultures at a constant temperature was evaluated to obtain a reference level to compare with the test carried out simultaneously under a temperature gradient. Generally, all starter cultures incubated at constant temperature (S19 and S21 at 30°C, and S12 at 37°C), showed good acidification activity, which, however, varied among the starter cultures used (Table 2).

During the first 2h of incubation under the temperature gradient all starter cultures exhibited negligible acidification. Starter culture S12 showed almost overlapping acidification profiles between 2 h and 24 h of incubation under the two different temperature regimes applied (Fig 2c). In contrast, starter cultures

Table 2: Activity of starter culture at constant temperature.

Starter culture	Coagulation time (h)	After coagulation		Synthesis of diacetyl
		pH	sourness (°SH)	
S12	4	4.63	23,0	+
S19	8	4.75	25.0	+
S21	8	4.83	26.0	+

The starter cultures S19 and S21 were incubated at 30°C whereas starter culture S12 was incubated at 37°C

S19 and S21 showed faster acidification rates by more than 2 h under the temperature gradient in comparason to the process at constant temperature (Table 2 and 3, Fig 2 a, b).

Table 3: Activity of starter culture under the temperature gradient regime

Starter culture	Coagulation time (h)	After coagulation		Synthesis of diacetyl
		pH	sourness (°SH)	
S12	4	4.44	30.0	+
S19	6	4.64	31.0	+
S21	6	4.71	33.0	+

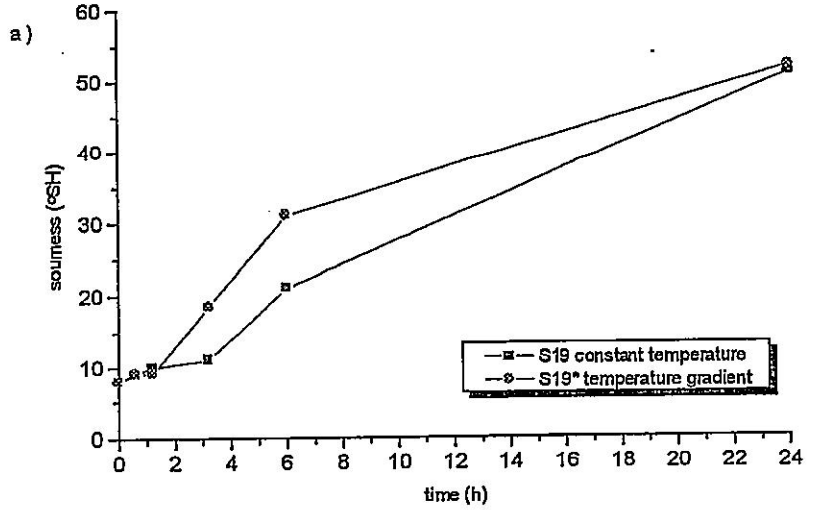

Figure 2a. The acid production (°SH) of starter culture S19 during growth in milk at a constant temperature and under a temperature gradient.

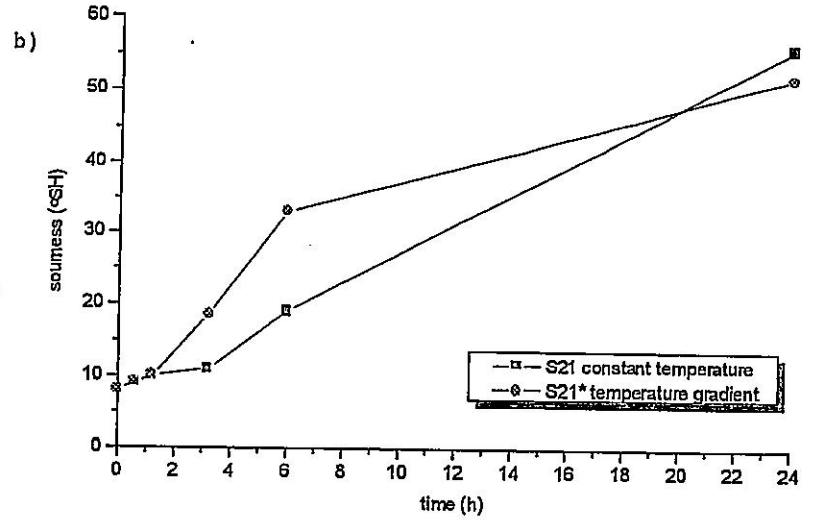

Figure 2b. The acid production (^{0}SH) of starter culture S21 during growth in milk at a constant temperature and under temperature gradient.

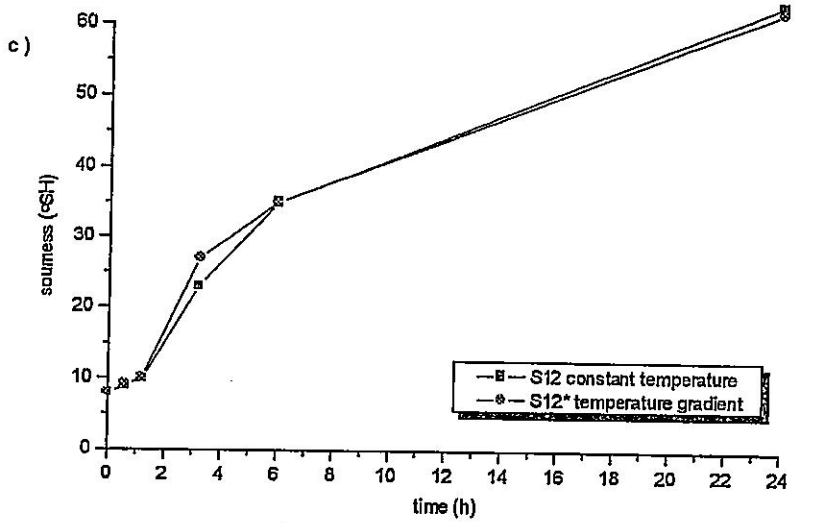

Figure 2c. The acid production (^{0}SH) of starter culture S12 during growth in milk at a constant temperature and under a temperature gradient.

Regardless of the temperature regime of incubation most acidifying starter cultures S12 was best fitted for the conditions and had a higher growth rate (Fig 3c). as shown by increases in colony forming units per ml (CFU ml^{-1}) after 24h of incubation. The delayed and reduced (CFU ml^{-1}) of starter cultures S19 and S21 were probably due to heat stress during the temperature gradient that resulted a slower rate of cellular growth (Fig 3 a,b) (Neviani *et al.*, 1995). All starter cultures had very high viscosity curd and produced diacetyl.

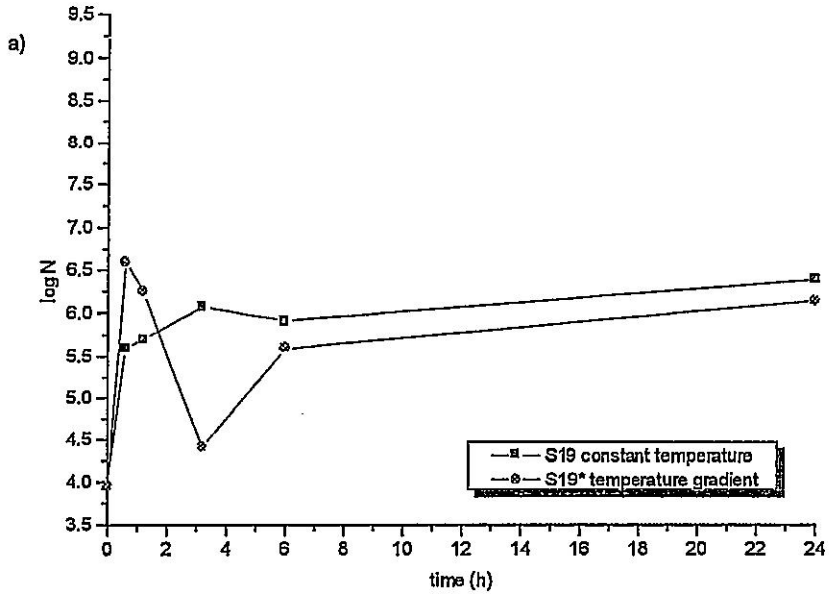

Figure 3a. The growth of starter culture S19 in milk at constant temperature and under a temperature gradient.

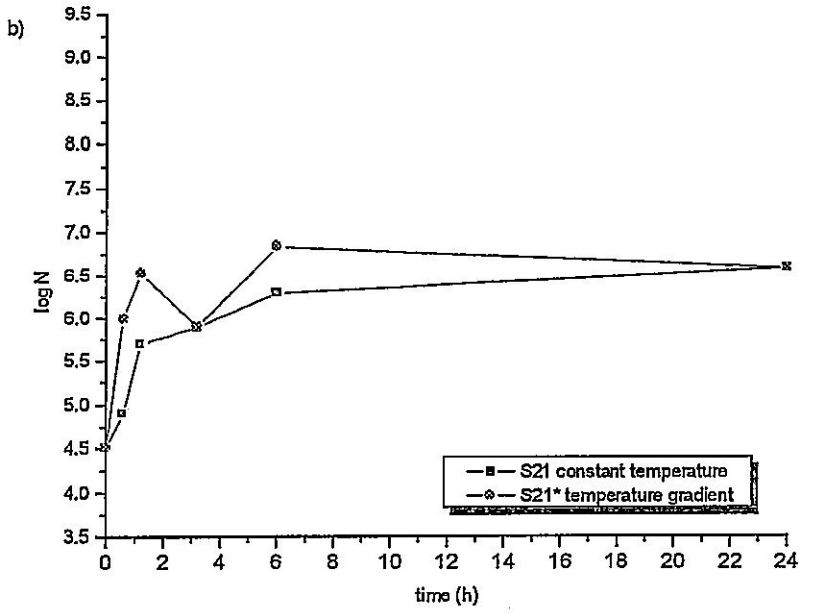

Figure 3b. The growth of starter culture S21 in milk at the constant temperature and under a temperature gradient.

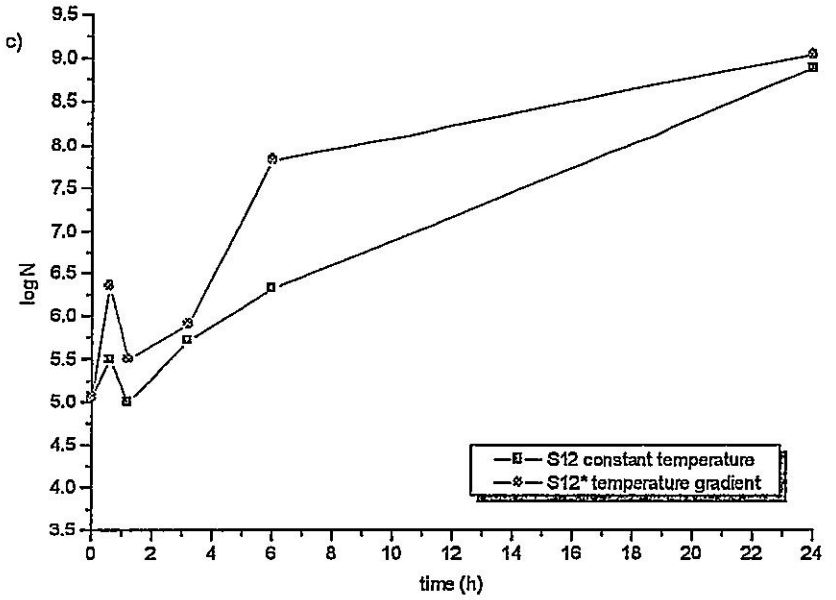

Figure 3c. The growth of starter culture S12 in milk at the constant temperature and under a temperature gradient.

DISCUSSION

Food fermentations, which were traditionally carried out by spontaneous growth and metabolic activity of microorganisms in special environments such as milk, are today carefully controlled microbial processes for which selected cultures have been developed. Knowledge about lactic acid bacteria growth in milk, their influence on milk acidification and subsequent curd acidification are essential for the successful outcome of cheese making. Recognition of the main bacterial species involved in starter cultures is the first attempt to control acid and flavor development in cheese (Stadhouders, 1986).

The group of semi-hard cheeses contains a number of varieties (Edam, Gouda, Lancashire), which are quite different, and demonstrate the diverse role of LAB in cheesemaking, ripening and flavor development (Dilaman, 1974). The lactic acid starter cultures used for production Gouda and Edam type cheese, primarily consist of mesophilic strains of *Lactococcus lactis* subsp. *cremoris* and *Lactoccus lactis* subsp. *lactis*. Our starter cultures for semi-hard cheese of the Trapist type consisted of mesophilic LAB (*Lactococcus lactis* subsp. *cremoris* and *Lactoccus lactis* subsp. *lactis*), and thermophilic LAB (*Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Lactobacillus casei* susbp. *rhamnosus*).

From an industrial point of view, the investigated starter cultures for Trapist type semi-hard cheese (S12, S19 and S21) grew very fast in milk and had proper acid formation (Table 2 and 3). They are therefore suitable for making semi-hard cheeses since they produce enough lactic acid and make an adequatly acid gel. Activity of the starter has a great effect on the moisture content of cheese which is most important for manufacturing semi-hard cheese. A fast starter decreases

the moisture content, which is also desirable for inhibition of undesired bacteria during production of semi-hard cheese (Gilliland, 1985b, Radke-Mitchell and Sandine, 1985).

Starter cultures can also be classified according to their ability to adapt to cheese-making conditions. Some starter cultures that showed good acidification activity at their optimal temperature were not able to decrease pH under the technological conditions of cheese manufacture. Incubation conditions simulating the temperature gradient within the first 24h of cheese making are very different from the optimal growing conditions of LAB. Incubation under a temperature gradient showed that the treatment simulating the curd cooking phase supported the acidification process for almost all constructed starters (S12, S19 and S21) even if this treatment resulted in decreased cell viability, probably because of thermal stress (Neviani *et al.*, 1995). It was observed that coagulation of milk with many starter cultures occurred sooner at temperatures much higher than those temperatures at which the greatest amount of cell growth occurred. This indicated that the optimum temperature for growth did not coincide with the temperature at which the rate of acid production was greatest (Radke-Mitchell and Sandine, 1985). From comparison of the acidification curves that were obtained under the temperature gradient and those that were obtained at constant temperature, we concluded that starter culture S12 was not influenced by modified incubation conditions.

The conditions applied in our experimental procedure were close to those during the initial phase of cheese making. Nevertheless, the actual conditions of the technological process cannot be easily simulated. In practice, the milk used does not present the chemical and technological characteristics of the curd. Also, parameters for the temperature gradient are a mean of those occurring in freshly made cheese, where the temperature varies considerably between the core and the surface of the cheese, especially as a result of its size (Neviani *et al.*, 1995).

Compatibility between strains was another characteristic that needed to be checked to prevent inhibition of one strain by another (Gilliland, 1985). Although the isolate *Lactococcus lactis* susbp. *lactis* (BGJAV11) produced bacteriocin which inhibited the growth of closely related lactococci, this bacteriocin did not show an effect on the growth of *Lactobacillus casei* susbp. *rhamnosus* (BGTM1) and butter milk starter culture BGMM in starter culture S21.

All starter cultures had high viscosity that appeared to be due to casein particle formation rather than exopolysaccharide production. High viscosity of starter cultures plays a key roles in the rheological behavior and texture of semi-hard cheese (Ramaswamy and Basak, 1991). Texture is an important component of flavor. It changes the mouthful of the product and encourages flavor volatiles to remain in the mouth for a long period (Marshall, 1987). All semi-hard cheeses produced by using starter cultures S12, S19 and S21 had good flavor and a few small eyes produced by the butter milk starter culture BGMM. It is well know, that butter milk starter cultures consist of *Lactococcus lactis* susbp. *lactis* biovar. diacetylactis or *Leuconostoc cremoris*, which can utilize citrat present in milk and produce the flavor compound diacetyl (an important aroma compound) and CO_2. Diacetyl gives a specific "nut like" or butter flavour and contributes to the freshness of these cheeses. The formation of eyes is due to CO_2 production by the *Lactococcus lactis* susbp. *lactis* biovar. diacetylactis or *Leuconostoc cremoris* (Tamime, 1981).

The most important biochemical process for flavour and texture formation in hard type and semi-hard-type cheeses is proteolysis. Products of proteolysis, i.e. peptides and amino acids, together with other non volatile and volatile compounds make the greatest contribution to cheese flavour (Marshall, 1987). Many investigators have agreed that the limited proteolytic activity of some starter cultures may be the reason for the frequently occurring fault in cheese known as bitterness. Bitterness is a problem in many cheese varieties, especially those made with mesophilic cultures, and has been associated with production of bitter peptides, which contain predominantly hydrophobic amino acid residues, by rennet and starter bacteria. Apparently all starter strains are potentially capable of causing bitterness but this does not occur if the rate of multiplication during manufacture is controlled (e.g. by higher cooking temperatures) such that relatively low cell numbers are found in the curd (Kunji, *et. al.*, 1996, Pritchard and Coolbear, 1993). In the case of semi-hard cheese produced with starter cultures S12, S19 and S21 flavor defect e.g bitterness may be avoided, and provide accelerated ripening of cheeses with associated savings in storage costs.

In summary, data from the present study suggest that starter cultures S12, S19 and S21 could be used for preparation semi-hard cheeses of the Trapist type. A major technological development in recent years has been the provision of cultures in a diversity of forms (Gilliland, 1985a, Hunger and Peitersen, 1993). The next step in our research is to determine storage conditions required to achieve a higher number of cells that retain activity over extended periods.

REFERENCES

1. *Anderson DG, Mc Kay LL,* 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci, *Appl Envir Microbiol.* 46, 549-52
2. *Banina A, Vukasinovic M, Brankovic S, Fira D, Kojic M, Topisirovic L,* 1998. Characterization of natural isolate *Lactobacillus acidophilus* BGRA43 useful for acidophilus milk production, *J Appl Microbiol,* 84, 593-599
3. *Buckenhuskes JH,* 1993. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities, *FEMS*,12, 253-272
4. *DeMan JC, Rogosa M, Sharpe ME,* 1960. A medium for the cultivation of lactobacilli. *J Appl Bacteriol,* 23, 130-35.
5. *Dilaman Ch Z,* 1974. Role of bacterial starters in the manufacture of different types of hard cheese, *Milchwissenschaft* , 29, 337-40.
6. *El Soda AM,* 1993. The role of lactic acid bacteria in accelerated cheese ripening, *FEMS Microbiol Rev*, 12, 239-52
7. *Gilliland ES,* 1985a. Concentrated starter cultures, In: Bacterial Starter Cultures for Food, Ed. Stanley E. Gilliland, CRC Press, Inc/ Florida, 146-55.
8. *Gilliland ES,* 1985b. Role of starter culture bacteria in food preservation, In: Bacterial Starter Cultures for Food, Ed. Stanley E. Gilliland, CRC Press, Inc/ Florida, 176-84.
9. *Gilliland ES,* 1985c. The Lactobacilli: Milk products, In: Bacterial starter cultures for food, Ed. Stanley E. Gilliland, CRC Press, Inc/ Florida, 42-52.
10. *Gilliland ES,* 1990. Health and nutritional benefits from lactic acid bacteria, *FEMS*, 87, 175-188.
11. *Bergey's Manual of Systematic Bacteriology, Genus Lactobacillus.* 1986. Vol 2 (PHA Sneath, NS Mair, ME Sharpe, JG Holt, eds) The Williams and Wilkins Company, Baltimore, MD, USA)
12. *Hunger W, Peitersen N,* 1993. New technical aspects of the preparation of starter cultures, *Bulletin of the IDF* ,277
13. *Kojic M, Fira D, Banina A, Topisirovic L,* 1991. Characterization of the cell wall-bound proteinase of *Lactobacillus casei* HN14, *Appl Envir Microbiol,* 57, 1753-7
14. *Kojic M, Fira D, Bojovic B, Banina A, Topisirovic L,* 1995. Comparative study on cell envelope-associated proteinases in natural isolates of mesophilic lactobacilli, *J Appl Bacteriol,* 79, 61-68

15. *Kunji SRE, Mierau I, Hagting A, Poolman B, Konings NW,* 1996. The proteolytic systems of lactic acid bacteria, *Antonie van Leewenhoek,* 70, 91-125
16. *Marshall MV,*1987. Lactic acid bacteria: Starters for flavour, *FEMS*, 46, 327-336
17. *Mundt JO,* 1986. Lactic acid streptococci. In: Bergey's Manual of Systematic Bacteriology, Vol 2 (PHA Sneath, NS Mair, ME Sharpe, JG Holt, eds) The Williams and Wilkins Company, Baltimore, MD, USA
18. Nevia*ni E, Divizia R, Abbiati E,* 1995. Acidification activity of thermophillic Lactobacilli under the temperature gradient of Grana cheese making, *J Dairy Sci,* 78, 1248-52
19. *OSullivan DJ, Klaenhammer TR,* 1993. Rapid mini-prep isolation of high-quality plasmid DNK from *Lactococcus* and *Lactobacillus* sp., *Appl Envir Microbiol,* 59, 2730-33
20. *Pritchard GGG, Coolbear T,* 1993. The physiology and biochemistry of the proteolytic system in lactic acid bacteria, *FEMS Microbiol Rev,* 12, 179-207
21. *Radke-Mitchell CL, Sandine EW,* 1985. Influences of temperature on associative growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, *J Dairy Sci*, 69, 2558-68
22. *Ramaswamy HS, Basak S,* 1991. Rheology of stirred yogurts, *J Text Stud*, 22, 231-35
23. *Stadhouders J,* 1986. Control of cheese starter activity, *Neth Milk Dairy J*, 40, 155-73
24. *Tamime YA,* 1981. Microbiology of Starter Cultures, Dairy Microbiology, volume 2, The Microbiology of Milk products, Ed. Robinson K.R., Applied Science Publishers/ London and New York, 113-56.
25. *Terzaghi BE, Sandine WE,* 1975. Improved medium for lactic streptococci and their bacteriophages. *Appl Microbiol,* 29, 807-13.
26. *Teuber M,* 1987. Starter cultures: Fundamental aspects, Milk - The vital force, 75-81

KARAKTERISTIKA PRIRODNIH IZOLATA BAKTERIJA MLEČNE KISELINE NAMENJENIH ZA FORMIRANJE STARTER KULTURA ZA PROIZVODNJU POLUTVRDOG SIRA TIPA TRAPIST

VUKAŠINOVIĆ MAJA, FIRA Đ. I TOPISIROVIĆ LJ.

SADRŽAJ

Starter kulture S12, S19 i S21 formirane su od bakterija mlečne kiseline izolovanih iz tradicionalno proizvedenih fermentisanih mlečnih proizvoda, za čiju proizvodnju nisu korićene komercijalne starter kulture. Na osnovu dobijenih rezultata može se zaključiti da starter kulture S12, S19 i S21 pokazuju dobru adaptaciju na uslove koji vladaju u toku prvih 24 časa proizvodnje sira tipa Trapista. To se posebno odnosi na mezofilne starter kulture S19 i S21, koje pri uslovima variranja temperature skraćuju vreme zgruavanja za dva sata, i pored toga to ukupan broj bakterija u toku dogrevanja opada usled preživljenog termičkog stresa. Poznavanje ponaanja starter kultura u početnoj fazi tehnološkog procesa značjno je za njihovu selekciju pri proizvodnji polutvrdog sira tipa Trapist.