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PRESERVING DOMIATI CHEESE USING METABOLITES OF *PROPIONIBACTERIUM THOENII* P-127

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In many developed and developing countries, sedulous trails are running for achieving high quality dairy products without using chemical preservatives. Microbial metabolites, such as that produced by *Propionibacteria* are coming in need to achieve such an aim. Therefore, utilization of permeate supplemented with different nutrients to improve production of metabolites under controlled conditions in a fermentor was studied. Metabolites of *Propionibacterium thoenii* P-127 proved to have an antimicrobial activity against the tested microorganisms.

Permeate supplemented with casein hydrolysate supported maximum production of antimicrobial substances by *P. thoenii*. Antifungal activity of lyophilized metabolites stored at 25°C showed a drop of activity (approximately 25%) compared to metabolites stored at 4°C. The addition of lyophilized *P. thoenii* P-127 metabolites by 1.5% to Domiati cheese milk obviously prolonged the shelf-life of soft cheese.

The investigators proposed the name "Propiogard" for the product, to differentiate it from commercial name "Microgard".

INTRODUCTION

Dairy propionibacteria are commercially important in the production of "eye" and typical flavours in Swiss type cheeses. They also produce organic acids leading to an effective "natural" antifungal ingredient that could be used in dairy industry [Mantere-Alhonen, 1995].

Whey and permeate by-products of cheese manufacture, create a worldwide problem of waste disposal of considerable proportions. Permeate usually contains 45 g/L of lactose. Lactose in permeate can be fermented by various microorganisms to organic acids, alcohol or biogas [Schuppert *et al.*, 1992; Sharaf *et al.*, 1999]. Propionibacteria can use both lactose from milk and the lactic acid produced by lactic acid starter cultures as substrates, but usually prefer lactic acid [Glatz, 1992]. Antimicrobial food additive can be obtained from milk cultures of *Propionibacterium thoenii*. The resulting growth liquid is then added to food or feed to inhibit yeast, mould, spoilage and pathogenic bacteria [Ayres *et al.*, 1993]. To accelerate metabolites production from permeate prior conversion of lactose into lactic acid, the use of mixed inocula of propionibacteria and lactic acid bacteria is beneficial [Begin *et al.*, 1992]. Also, the presence of commercial casein hydrolysate in media has a positive effect on growth or metabolite production of propionibacteria [Marcoux *et al.*, 1992]. To facilitate storage and shipment, the resulting growth liquid may be dried to form powder or maybe be frozen before use as an antimicrobial food additive [Ayres *et al.*, 1993]. Powdered or liquid natural metabolites of propionibacteria can be incorporated into various foods including some dairy products. They may be added at various concentrations between 0.01 and 10% of total weight of food where their function is to inhibit growth of yeast, mould or certain bacteria.

Therefore, the goals of this study were to detect antimicrobial activity of metabolites produced by *P. thoenii*, to seek the best conditions to improve metabolites production during fermentation of permeate supplemented with different nutrients and to evaluate the efficiency of the *P. thoenii* metabolites to extend the shelf-life of Domiati cheese as a natural preservative.

MATERIAL AND METHODS

Source of cultures. *Streptococcus thermophilus* and *Lactobacillus casei* were obtained from Chr. Hansen's Lab., Denmark. A strain of *Propionibacterium thoenii* P-127 was provided by the Department of Food Technology, Propionibacteria Culture Collection, Iowa State University. Indicator strains used for the assay were: *Escherichia coli*, *Pseudomonas fluorescens*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger*, and *Saccharomyces cerevisiae*. These were from our stock collection.

Fermentation media. The following media were tested: (a) the standard laboratory medium, sodium lactate broth (NLB) was prepared as described by Hsieh *et al.* [1996]; (b) fresh permeate was obtained from Domity Company (6 October City).

The permeate was divided into three equal portions and supplemented with 0.5% of each yeast extract, casein hydrolysate and soya protein to obtain the culture medium. All three media were sterilized at 110°C for 10 min.

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Fermentations. Fermentations were performed in a Bioflo 3000 fermentor (5 L working volume, New Brunswick Scientific Co. Inc., Edison, N.J. USA). When NLB was used as fermentation medium, fermentation was started with a 1% (vol/vol) inoculum of *P. thoenii* P-127 culture. While in the case of using permeate medium as a substrate, the fermentation was started with a 1% (vol/vol) inoculum of *Str. thermophilus* culture and *P. thoenii* P-127. Incubation was carried out for 3 days at a temperature of 32°C. During fermentation pH was controlled at 7.0±0.1. Agitation was at 150 rpm without aeration [Paik & Glatz, 1997]. The resulting culture was then pasteurized.

Antimicrobial activity. Antimicrobial activity was assayed against the tested microorganisms using agar well diffusion assay as described by Lyon and Glatz [1993]. All assays were performed in duplicate, and the results presented are the means of duplicate trials.

Freeze drying of metabolites. A sterile reconstituted non-fat dried milk (7%) was added to the neutralized heated metabolites of *P. thoenii* P-127 at 71°C for 10 min [Effat *et al.*, 2001]. Thereafter, it was lyophilized by freeze drying technique using an LY5 FM-RB freeze dryer (Snijders Scientific). Lyophilized metabolites were examined for the inhibitory activity against *S. cerevisiae* and *A. niger* periodically (monthly) during storage for 6 months at 25 and 4°C.

Evaluation of utilization of *P. thoenii* metabolites as dairy products preservative. Domiati cheese was manufactured according to Fahmi and Sharara [1950] from a heated mixture of cow-buffalo milk (1:1) (72°C/15 sec). Cheese milk was immediately cooled to 37°C and divided into 2 equal portions. The first portion was treated as a control. The metabolites produced from *P. thoenii*, as described previously, were added to the second portion to achieve the concentration of 1.5% (w/v) of cheese milk. Then 1% starter (*Str. thermophilus* and *L. casei*) was added and followed by rennet. Salt was added at a level of 3% of milk. The resultant cheeses were distributed into sterile plastic screw-cap containers and pickled in their own whey. Then, the plastic containers were tightly capped and stored at 7°C for 4 months. Trials were carried out in triplicate.

Cheese analysis. Cheese samples were taken when fresh and monthly during the storage period.

Microbiological analysis. (a) Yeast and mould count was determined according to Standard Methods for Examination of Dairy Products [APHA, 1994]; (b) lactic acid bacteria were enumerated according to Elliker *et al.* [1956].

Organoleptic score. Domiati cheese products were judged by 10 panelists of the experienced staff members of the Food Technology and Dairy Science Dep., National Research Centre and the Faculty of Agric., Ain Shams Univ. as described by Ammar [1999] with scores for flavour (40 points), body and texture (50 points), and appearance (10 points).

RESULTS AND DISCUSSION

Antimicrobial activity of propionibacteria

Table 1 shows that metabolites of *P. thoenii* P-127 proved to have an antimicrobial activity against the indicator strains tested. This effect was more obvious against the Gram-negative strains and yeast and mould. Also, *L. monocytogenes*, the Gram-positive strain was sensitive to this antimicrobial substance. On the other hand, *B. cereus* and *Staph. aureus* were poorly inhibited by *P. thoenii* P-127 metabolites.

Generally, our results confirm the results obtained by Lyon and Glatz [1993] and El-Kholy [2000] who recorded that metabolites produced by *P. thoenii* P-127 were active against some Gram-positive organisms, Gram-negative organisms and some yeasts and moulds. Moreover, the foregoing results are in accordance with other reports which stated that fermentation by propionibacteria is important as a mould and yeast inhibitor [Perlman, 1978; El-Kholy, 2000].

TABLE 1. Antimicrobial spectrum of propionibacteria metabolites.

Selected indicators	Inhibition by metabolites of *P. thoenii* P-127
E. coli	++
Pseudomonas fluorescens	++
Listeria monocytogenes	++
B. cereus	+
Staph. aureus	+
A. niger	++
S. cerevisiae	++

+ weak inhibition; ++ strong inhibition

Modification of growth medium to improve antimicrobial production

Results on the effect of supplementation of different nutrients in permeate are shown in Table 2. They indicate that permeate supplemented with casein hydrolysate allowed maximum production of antimicrobial substances followed by supplementation with yeast extract. These findings are in accordance with those of Marcoux *et al.* [1992] and Cecchi *et al.* [1998]. Also, Piveteau *et al.* [1995] found that *Str. thermophilus* stimulated the growth of all propionibacteria strains. On the other side, the application of soy protein for permeate supplementation resulted in slight inhibition of growth of the indicator organisms tested.

TABLE 2. Antimicrobial spectrum of metabolites from *P. thoenii* P-127 grown in permeate supplemented with different nutritional factors.

Selected indicators	Inhibition by metabolites of *P. thoenii* grown in		
	Permeate + 0.5% yeast extract	Permeate + 0.5% casein hydrolysate	Permeate + 0.5% soya protein
E. coli	++	++	+
Pseudomonas fluorescens	+	++	+
Listeria monocytogenes	+	+	+
B. cereus	+	+	+
Staph. aureus	+	+	+
A. niger	+	++	+
S. cerevisiae	+	++	+

+ weak inhibition; ++ strong inhibition

Effect of storage periods and temperatures on antimicrobial activity of *P. thoenii* metabolites

The antimicrobial activity of lyophilized metabolites against *S. cerevisiae* and *A. niger* during storage at 25 and 4°C is shown in Figures 1 a, b. The highest antimicrobial activity was always obtained in lyophilized metabolites stored at 4°C, also no change in the activity was observed during storage period. The antimicrobial activity of metabolites kept at 25°C decreased by approximately 25% compared to their activity when stored at 4°C already at the second month. Thus, storage at different temperatures resulted in significant differences in antimicrobial activity.

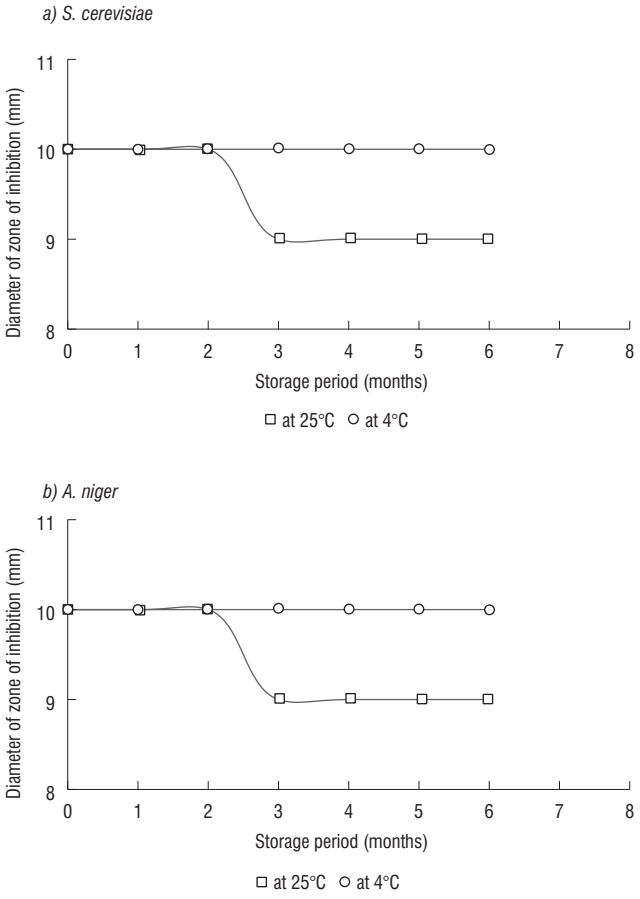

FIGURE 1. Effect of storage period and temperature on antimicrobial activity of lyophilized *P. thoenii* metabolites.

Effect of *P. thoenii* metabolites on quality of Domiati cheese
Microbiological properties

The effect of *P. thoenii* metabolites on microbiological properties of Domiati cheese is shown in Figure 2. Data presented therein clearly showed that no yeasts and moulds could be detected in all fresh and stored cheese in a refrigerator containing *P. thoenii* metabolites, but were detected in control cheese during the storage period. A similar trend was observed by Salih *et al.* [1990]; El-Shafei *et al.* [1995] and Effat *et al.* [2001]. In addition, lactic acid bacteria (LAB) in both control and treated cheese followed the same trend, as increased throughout storage period to reach the maximum counts after 1 month, thereafter counts decreased throughout the rest of storage. It could be noticed that cheese made with the addition of *P. thoenii* metabolites possessed higher counts, especially, lactic acid

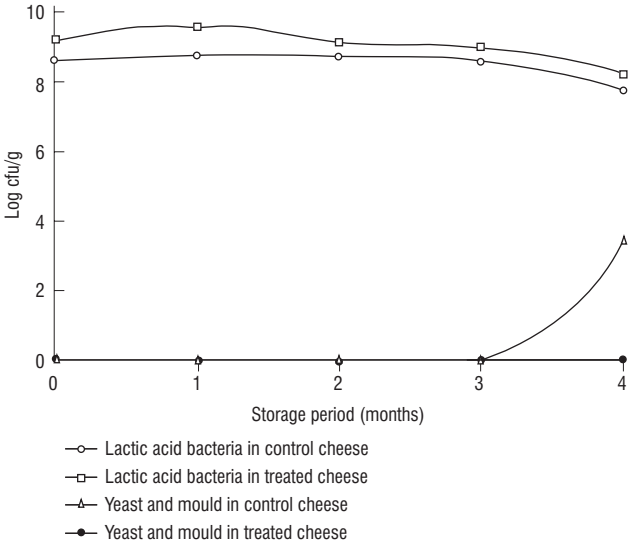

FIGURE 2. Changes in microbial contents in Domiati cheese made with adding *P. thoenii* metabolites.

bacteria counts during storage period comparing with control cheese. These results are in accordance with those of Effat *et al.* [1993] and El-Kholy [2000] who reported that *P. thoenii* metabolites exerted high stimulatory effect on lactic starters.

Organoleptic properties

The cheeses were evaluated for taste, flavour, consistency and appearance. Data pertaining to the overall evaluation and preference of cheeses during storage at 7°C are depicted in Figure 3. As shown, the presence of *P. thoenii* metabolites, raised the score points of the resulting Domiati cheese. The addition of 1.5% of freeze dried *P. thoenii* metabolites to the milk had achieved the highest score for the resulting cheeses. In general, results proved that the addition of *P. thoenii* metabolites enhanced the resulting Domiati cheese quality. This could be attributed to the fact that propionibacteria produce vitamins and some growth factors which stimulate the starter cultures [Glatz, 1992]. These results are in agreement with those of El-Kholy [2000] and El-Sayed *et al.* [1994] who reported that sensory evaluation of Ras cheese slurry was higher when propionibacteria cell free filtrate was added.

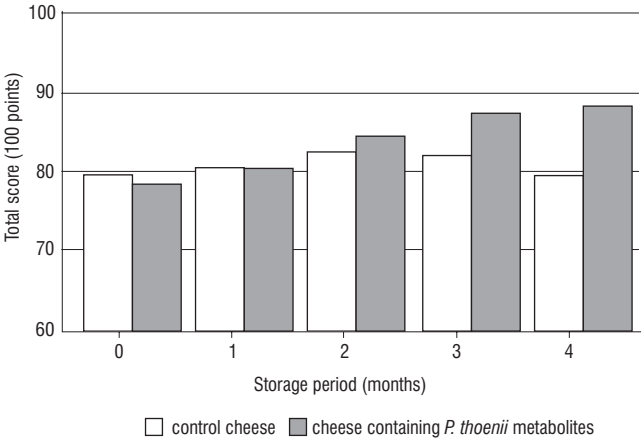

FIGURE 3. Organoleptic properties of Domiati cheese containing *P. thoenii* metabolites.

CONCLUSIONS

In conclusion, results indicate that *P. thoenii* metabolites show antimicrobial effect against spoilage, pathogenic bacteria, yeast and mould in both synthetic media and Domiati cheese. The application of such metabolites could improve the shelf-life, quality and safety of soft cheese products, replacing or reducing the increasing use of chemical additives.

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