2002 MAFMA Final Report

Project Title	**The Use of Probiotic Bacteria to Improve the Nutritional Value and Quality of Swiss Cheese**
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Academic Institution	**Iowa State University**
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Objective Accomplishments

Four different probiotic bacteria, *Bifidobacteria breve, B. longum, B. infantis,* and *Pediococcus acidilactici* (obtained from Institut Rosell Inc.) were screened using a cheese slurry model system to evaluate the viability of the bacteria in conditions characteristic of Swiss cheese. The model systems were incubated for 10 days at 37°C under anaerobic conditions. Microbiological assays have been developed to differentiate the probiotic bacteria from the lactic acid bacteria of the Swiss cheese. Total bacteria in the cheese slurries were enumerated by plating on MRS agar. Probiotic bacteria were plated on MRS agar plates with added streptomyocin. Volatile flavor compounds were isolated using solid-phase microextraction techniques, with gas chromatographic analysis. Free amino acids were also determined.

Table 1 compares the growth of the probiotic and lactic acid bacteria in the cheese slurry model systems. Of the probiotic bacteria, *B. breve* and *B. longum* demonstrated the highest growth in the model systems. The presence of the probiotic bacteria contributed to a slight decrease in the counts of the background starter cultures in the Swiss cheese, however, the counts for the cheese slurries with *B. breve* and *B. longum* were most similar to that of the control cheese slurry.

Table 1. Growth[a] of probiotic and lactic acid bacteria in cheese slurry model systems

	Probiotic Bacteria			Lactic Acid Bacteria		
Bacteria	0 Day	7 Days	10 Days	0 Day	7 Days	10 Days
Control				8.83	10.13	10.46
B. breve	8.45	9.53	10.22	9.49	9.82	10.98
B. infantis	9.11	9.19	9.35	9.53	9.52	9.99
B. longum	8.76	9.49	9.84	9.22	9.86	10.29
P. acidilactis	8.84	8.79	9.64	9.96	9.95	9.83

[a] $\log_{10}$ CFU/ml

Volatile flavor compounds identified in the Swiss cheese slurry included ketones, alcohols, aldehdyes, esters, fatty acids, and sulfur compounds, which are characteristic of Swiss cheese. In general contents of the volatile flavor compounds increased with ripening time. Table 2 indicates that there was a significant difference seen in bacterial treatments ($P<0.05$) for several of the key volatile flavor compounds. Butyl propionate, dimethyl disulfide, dimethyl trisulfide and 2-heptanone were among common compounds present in Swiss cheese.

Table 2. Effect of probiotic bacteria on the content of volatile flavor compounds in Swiss cheese curd slurrries[a]

	Bacterial Treatment				
	Control	*B. breve*	*B. infantis*	*B. longum*	*P. acidilactici*
Dimethyl disulfide	116.8a	117.3a	279.7c	214.7b	233.8bc
Dimethyl trisulfide	80.3a	192.1b	234.4c	262.2c	150.6b
4-Methyl-2-pentanol	19.7	9.9	11.2	16.5	14.4
2-Hexanone	465.6a	927.5c	978.0c	920.1c	660.8b
2-Heptanone	148.3b	97.8a	148.5b	95.0a	151.0b
1-Butanol	28.7	15.8	11.0	12.0	13.2
1-Pentanol	5.0	6.6	11.2	8.2	1.3
1-Octanol	658.0a	803.6a	738.5a	851.8aa	1406.7b
2-Methyl-1-butanol	33.2	52.2	82.1	74.2	61.9
Butyl propionate	9.8	9.1	13.7	6.0	11.3
Acetic acid	122.2a	356.0b	240.8ab	319.8b	175.0a
Propionic acid	5544.7a	4854.1a	4686.8a	5342.2a	9540.1b
2-Methylbutanoic acid	649.0a	1438.8b	1117.7b	1188.0b	1413.8b
Heptanoic acid	63.2a	87.0a	115.9ab	115.5ab	163.3b
Octanoic acid	56.3b	120.1c	98.3bc	73.4b	23.2a

[a]Content of volatile flavor compounds reported as GC peak area. Means are duplicate analyses of 3 replications, with data for ripening time pooled. Means followed by different letters are significantly different ($P<0.05$).

Ripening time was shown to have a significant effect on the content of a majority of the free amino acids. The three Swiss cheese curd slurries containing bifidobacteria showed an increase in free amino acid content during the 10-day ripening period in comparison to *Pediococcus acidilactici* and the control sample. However, only the contents of leucine, thiaproline, and serine were significantly increased through the addition of the bifidobacteria. The increase in free amino acid content of bifidobacteria spp. indicates an increase in proteolytic acitivity in those cheese slurries. Methionine, proline, lysine, and cysteine contribute to the formation of key volatile flavor compounds in Swiss cheese.

The use of the Swiss cheese curd slurry model systems allowed us to evaluate the effects of the different probiotic strains on the microbial viability and flavor characteristics. From these studies, it was determined that the incorporation of the bifidobacteria do maintain their viability and do not adversely affect the flavor characteristics of the Swiss cheeses. The cheese slurries with the additional bifidobacteria did result in higher contents of free amino acids and volatile flavor compounds, indicating that these bacteria may contribute to accelerating the ripening process.

Throughout this project, we have had several meetings with Swiss Valley Farms to discuss concerns and issues related to the incorporation of bifidobacteria into Swiss cheese, using their current process. Additional experiments were designed to evaluate the heat and pH stability of the probiotic bacteria. Since the Swiss cheese process involves heating the curds at 49°C (120°F), the viability of the probiotic bacteria held at 49°C for 3 hours was determined (Figure 1). In the cheese slurry model systems, the pH ranged from 5.9 to 6.6 during the 10-day incubation period. During processing of Swiss cheese, the pH drops to 5.1. Therefore, to simulate the decrease in pH during the processing of the Swiss cheese, a model system was designed to decrease the pH of the cheese slurry from 6.3 to 5.1 over a 21-hour incubation period (Figure 2). Both the heat and pH stability experiments demonstrated that the probiotic bacteria would remain their viability over temperature and pH conditions typical of Swiss cheese processing.

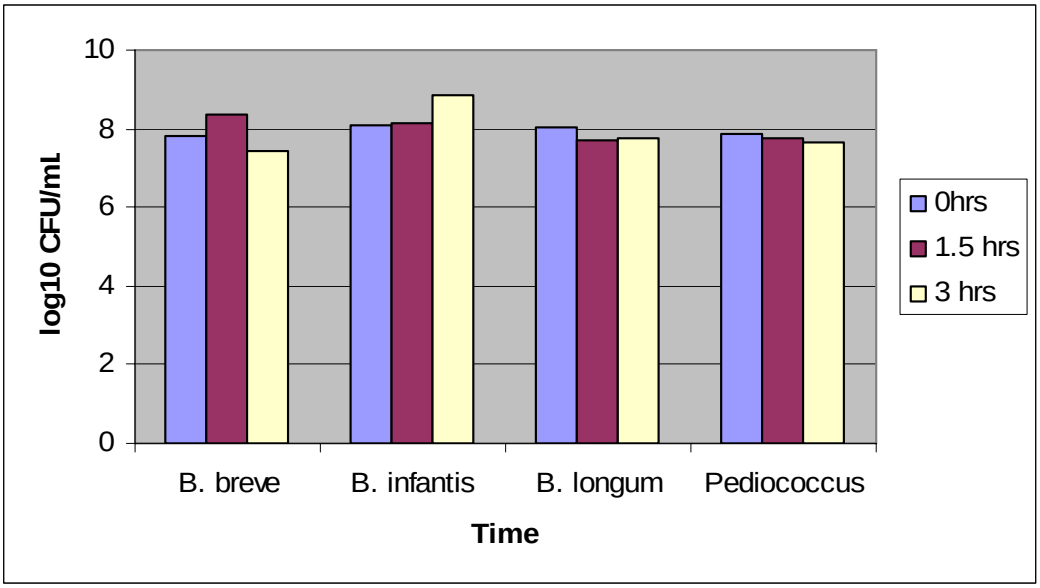

Figure 1. Temperature stability of probiotic bacteria in cheese slurry model systems at 49°C for 3 hours.

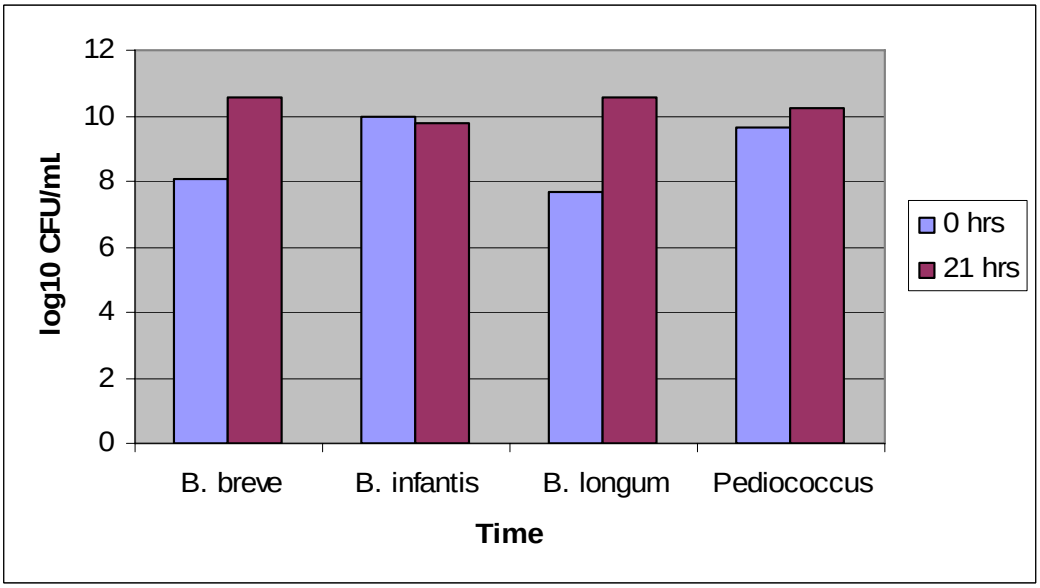

Figure 2. pH Stability of probiotic bacteria in cheese slurry model systems. pH at 0 hrs was 6.3, pH at 21 hrs was 5.1

A production run was conducted at Swiss Valley Farms processing facility in Luana, IA during summer 2004. Due to the lack of Kosher certification for the bifidobacteria strains from Institut Roselle, *Bifidobacterium longum* was obtained from Chr. Hansen and used in this processing run. This product did have Kosher certification as required by the Swiss Valley Farms processing facility. Swiss cheese processed with the added B. longum had the same processing and quality characteristics as the control cheese (without added Bifidobacteria).

Unexpected findings, if any

We had unexpected delays in adapting the results of the model system to the production run at the Luana plant. When we initially obtained the Bifidobacteria strains from Institut Roselle, the company confirmed that the cultures were Kosher, as required to maintain the Kosher status of the Swiss Valley plant. Therefore, an extensive amount of time was spent to identify Bifidobacteria sources that met the Kosher requirement. Eventually, a Bifidobacteria strain (*B. longum*; BB-46) was identified from Chr. Hansen and used in a plant production run.

4. Practical impacts of research efforts.

a. Short Term Impacts

Further research on the incorporation of probiotic bacteria into dairy products was obtained by the principal investigators to study the improved nutritional benefits of milk products produced through the addition of probiotic bacteria. The project was funded by a USDA Special Grant through the Center for Designing Foods to Improve Nutrition (Iowa State University).

b. Long Term Impacts

The funded research demonstrated the technological feasibility of incorporating bifidobacteria into Swiss cheese as a means of improving the nutritional value of dairy products. The economic feasibility of the incorporation of the bifidobacteria is currently under study by Swiss Valley Farms.