Classification of Swiss cheese starter and adjunct cultures using Fourier transform infrared microspectroscopy

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ABSTRACT

The acceptability of Swiss cheese largely depends on the flavor profile, and strain variations of cheese cultures will affect the final quality. Conventional biochemical methods to identify the cultures at the strain level are time-consuming and expensive, and require skilled labor. Our objective was to develop rapid classification methods of starter cultures at the strain level by using a combination of hydrophobic grid membrane filters and Fourier transform infrared (FTIR) spectroscopy. Forty-four pulsed-field gel electrophoresis-verified strains of starter and nonstarter cultures including *Streptococcus thermophilus*, *Propionibacterium freudenreichii*, and *Lactobacillus* spp. were analyzed. The strains were grown on their respective agar media, transferred to broth media, and incubated. Then, cultures were centrifuged and the pellets were resuspended in saline solution (10 μ L). Aliquots (2 μ L) of the suspended bacterial solution were placed onto a grid of the hydrophobic grid membrane filters, having 6 grids per each strain analyzed. The dried filters were read by FTIR microspectroscopy fitted with an attenuated total reflectance probe. Collected spectra were statistically analyzed by a soft independent modeling of class analogy (SIMCA) for pattern recognition. Classification models were developed for *Streptococcus thermophilus*, *Propionibacterium freudenreichii*, and *Lactobacillus* spp. strains. The models showed major discrimination in the spectral region from 1,200 to 900 cm^{-1} associated with signals from phosphate-containing compounds and various polysaccharides in the cell wall. The developed method allowed for rapid classification of several Swiss cheese starter and nonstarter cultures at the strain level. This information provides a detailed overview of microbiological status, which would enable corrective measures to be taken early in the cheese making process, limiting production of inferior quality cheese and minimizing defects. This method could

be an effective tool to identify and monitor activity of cheese and other dairy starter cultures.

Key words: Fourier transform infrared spectroscopy, Swiss cheese, microbiological identification, starter and nonstarter culture

INTRODUCTION

Swiss cheese is a hard cheese prepared from pasteurized milk and has holes, or eyes, that develop throughout the cheese due to microbiological activity (USDA, 2001). Although the Swiss cheese-making process differs among various countries, the basic steps involved in Swiss cheese making are milk coagulation, removal of whey, acid production, salt addition, and ripening. Initial ripening is carried out in warm room for the eye formation to take place by the action of propionibacteria, and the cheese is moved to cold room to finish the ripening process (Beresford et al., 2001). The quality of Swiss cheese essentially depends on fermentation by lactic acid and propionic acid bacteria, and proteolysis (Steffen et al., 1987). Thermophilic lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus helveticus*, and propionic acid bacteria, mostly *Propionibacterium freudenreichii* ssp. *shermanii*, are common starter cultures in Swiss cheese making (Ji et al., 2004).

In addition to the starter cultures, Swiss cheese microflora also comprises nonstarter lactic acid bacteria (Fox et al., 2000) that usually consist of facultatively heterofermentative lactobacilli such as *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, or *Lactobacillus rhamnosus* (Weinrichter et al., 2004). Although nonstarter lactic acid bacteria are generally indigenous to the raw milk or cheese-manufacturing facility, intentional addition of selected strains as adjunct cultures has been implemented because of their ability to accelerate proteolysis for cheese flavor development (Kieronczyk et al., 2003). It has been reported that facultatively heterofermentative lactobacilli adjunct starters may influence the characteristics of Swiss cheeses with respect to various properties (Weinrichter et al., 2004) and that cheese manufactured with adjunct lactobacilli showed better flavor intensity than cheeses made

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from pasteurized or microfiltered milk, suggesting that indigenous nonstarter lactic acid bacteria play a role in the development of cheese flavor (Lynch et al., 1997).

Overall, the complex microflora has a major effect on cheese ripening and hence the characteristic features of Swiss cheese. Lortal and Chapot-Chartier (2005) reported that lysis of LAB is an essential parameter to control and accelerate cheese ripening. However, knowledge about genetic similarities and differences are needed for rational culture selection because genetic variations are correlated with cheese making and cheese quality parameters (Jenkins et al., 2002). Selection of suitable strains would enable the cheese maker to control or modify flavor development (Beresford et al., 2001). Evaluating the presence, numbers, and types of starter bacteria is of utmost importance for the dairy industry because it allows for identification of the bacterial flora of starter cultures, detection of the total numbers in dairy samples, and detection of particular strains or biotypes present in dairy products.

Conventional methods used to characterize the cheese starter cultures are summarized in many publications (Holzapfel et al., 2001; Tannock, 2001; Jenkins et al., 2002). These methods include SDS-PAGE of whole-cell proteins, DNA-DNA hybridization, and PCR. Strain-level differentiation typically uses PCR-based methods or methods based on restriction enzyme analysis (Kahala et al., 2008). Several studies report that pulsed-field gel electrophoresis (PFGE) is a highly reproducible method for characterization of starter cultures at the strain level (Tanskanen et al., 1990; Tilsala-Timisjarvi and Alatossava, 1997; Mannu and Paba, 2002; Lick, 2003; Ward et al., 2004). However, these biochemical tests and molecular typing techniques are expensive and time-consuming, and require extensive skill, especially for the identification of bacteria at the subspecies or strain level (Oust et al., 2004; Dziuba et al., 2007).

Fourier transform infrared (FTIR) spectroscopy is an attractive technology for the rapid, inexpensive, sensitive, and high-throughput analysis of microorganisms. The FTIR method allows for the chemically based discrimination of intact microbial cells and produces complex biochemical fingerprints that are distinct and reproducible for different bacteria. Bacteria have shown highly specific mid-infrared spectral patterns that may be unique for individual strains (Helm et al., 1991; Naumann et al., 1991). Fourier transform infrared spectroscopy combined with chemometrics has been used for the accurate classification of microorganisms (Wenning et al., 2002; Mossoba et al., 2003; Ngo-Thi et al., 2003). Moreover, FTIR microspectroscopy combined with hydrophobic grid membrane filters (HGMF) was successfully used for rapid characterization of *Salmonella* serovars (Männig et al., 2008). Hydrophobic grid

membrane filters have a special printed grid pattern that separates bacterial colonies from one another, thus preventing lateral growth, spreading, and confluence.

The objective of this study was to classify Swiss cheese starter and adjunct cultures at the strain level using a combination of HGMF, FTIR microspectroscopy, and multivariate analysis.

MATERIALS AND METHODS

Bacterial Cultures

Thirteen strains of *Streptococcus thermophilus*, 21 strains of *Lactobacillus* spp., and 10 strains of *Propionibacterium freudenreichii* were used for this study (Table 1). Detailed information of the bacterial cultures can be

Table 1. List of Swiss cheese starter and nonstarter culture strains

Strain
<i>Streptococcus thermophilus</i> S363
<i>Strep. thermophilus</i> S804
<i>Strep. thermophilus</i> A054
<i>Strep. thermophilus</i> S341
<i>Strep. thermophilus</i> S392
<i>Strep. thermophilus</i> 884
<i>Strep. thermophilus</i> S869
<i>Strep. thermophilus</i> S838
<i>Strep. thermophilus</i> S847
<i>Strep. thermophilus</i> S728
<i>Strep. thermophilus</i> S765
<i>Strep. thermophilus</i> S731
<i>Strep. thermophilus</i> S794
<i>Lactobacillus casei</i> A2
<i>L. casei</i> A26
<i>L. casei</i> A3
<i>L. casei</i> A34
<i>Lactobacillus fermentum</i> B4
<i>L. fermentum</i> B15
<i>L. casei</i> B21
<i>Lactobacillus delbrueckii</i> C2
<i>Lactobacillus rhamnosus</i> D56
<i>L. delbrueckii</i> E3
<i>L. delbrueckii</i> F1
<i>L. delbrueckii</i> F44
<i>L. casei</i> G2
<i>L. rhamnosus</i> H1
<i>L. rhamnosus</i> H2
<i>Lactobacillus helveticus</i> S1
<i>L. helveticus</i> S2
<i>L. helveticus</i> S9
<i>L. helveticus</i> S14
<i>L. helveticus</i> S15
<i>L. helveticus</i> S5
<i>Propionibacterium freudenreichii</i> P891
<i>P. freudenreichii</i> P812
<i>P. freudenreichii</i> P728
<i>P. freudenreichii</i> P843
<i>P. freudenreichii</i> P835
<i>P. freudenreichii</i> P859
<i>P. freudenreichii</i> P318
<i>P. freudenreichii</i> P745M1
<i>P. freudenreichii</i> P745M2
<i>P. freudenreichii</i> ATCC 6207

found in Tables 2, 3, and 4. The cultures were provided by the J. T. Parker Chair Swiss cheese culture collection. The cultures and company names were coded for confidentiality. The letter in the code refers to the strain genus (L = *Lactobacillus*, P = *Propionibacterium*, and S = *Streptococcus*), the first number of each code indicates the culture manufacturer, and the last 2 numbers are the strain designation. Cultures containing multiple strains were designated with an “M” following the numerical code. All the culture strains were previously identified by PFGE (Jenkins et al., 2002). *Streptococcus thermophilus* strains were grown in M17 agar containing 0.5% lactose and 0.15% lithium chloride for 2 d at 42°C. Colonies were transferred to M17 broth containing 0.5% lactose and 0.15% lithium chloride and incubated at 42°C for 24 h. Strains of *Lactobacillus* spp. were grown on Rogosa SL agar for 2 d at 37°C in an anaerobic chamber (Forma Scientific Inc., Marietta, OH) purged with a 5% carbon dioxide, 10% hydrogen, 85% nitrogen gas mixture. Colonies were transferred to lactobacilli de Man, Rogosa, and Sharpe broth and incubated for 24 h at 37°C in an anaerobic chamber. *Propionibacterium freudenreichii* strains were grown anaerobically in sodium lactate agar for 5 to 7 d at 30°C. Colonies were transferred to sodium lactate broth and incubated anaerobically for 2 d at 30°C. Bacterial cultures were analyzed on 4 separate days.

Sample Preparation

Individual bacterial cultures grown in broth medium (1 mL) were centrifuged (5,934 × g; 4°C; 5 min) and washed with 0.85% saline solution; the resulting bacterial pellets were resuspended in 10 µL of saline solution. An aliquot (2 µL) of the suspension was applied onto 1 grid of HGMF (0.45-µm porous, Neogen Corp., Lansing, MI) placed on an Iso-Grid filtration unit (Iso-Grid, Neogen Corp.). Six grids were used for each bacterial strain and the HGMF containing the cultures

were dried in a vacuum desiccator for 5 min before analysis.

FTIR Microspectroscopy

The HGMF containing bacterial cultures were analyzed using an FTIR microscope (UMA 600 series infrared microscope interfaced with a FTS Excalibur 3100GX FTIR spectrometer; Varian, Walnut Creek, CA) equipped with a mercury cadmium telluride detector in attenuated total reflectance mode. The microscope was equipped with a motorized x-y stage, 4× and 16× objectives, and a slide-on attenuated total reflection germanium (Ge) objective (600 UMA, Varian, Palo Alto, CA). The instrument had a Permaglow (ceramic; Varian Inc., Palo Alto, CA) mid-infrared source and an extended-range potassium bromide beam splitter. The FTIR spectra were collected using Varian Resolutions Pro (v4.05, Varian Inc., Palo Alto, CA) in the mid-infrared region (4,000 to 700 cm⁻¹) at 8 cm⁻¹ resolution. The spectrum of the Ge surface alone was measured as a background reference before sample analysis. To enhance the signal-to-noise ratio, the sample and background single beam Fourier-processed spectra consisted of 128 scans that were co-added and averaged. The absorbance spectra were determined by the ratio of the sample’s single beam spectra to background. Six spectra per individual cheese culture strain were collected. The reproducibility of cultures grown on 3 to 5 different days was also examined, resulting in 18 to 30 spectra per strain to construct the training model.

Multivariate Analysis

Multivariate analysis of the collected spectral data was performed using the Pirouette software (Infometrix Inc., Bothel, WA). The FTIR spectra were mean-centered, transformed to their second derivative

Table 2. Interclass distances between 13 *Streptococcus thermophilus* strains

Strain	S363	S804	A054	S341	S393	S884	S869	S838	S847	S728	S765	S731	S794
S363	0.0												
S804	6.2	0.0											
A054	3.9	5.9	0.0										
S341	9.5	5.0	7.5	0.0									
S393	7.3	7.1	4.4	6.2	0.0								
S884	10.5	5.6	10.4	7.2	10.4	0.0							
S869	7.5	6.7	7.3	9.2	7.7	9.1	0.0						
S838	3.0	5.1	3.4	7.7	6.0	9.0	7.3	0.0					
S847	3.1	5.3	3.5	8.2	6.0	9.8	6.4	1.4	0.0				
S728	6.1	6.6	3.3	7.4	5.1	11.0	9.3	5.2	5.7	0.0			
S765	2.3	7.2	3.6	10.8	7.6	12.1	8.0	3.5	3.5	5.4	0.0		
S731	2.4	6.5	3.5	9.7	6.8	10.3	6.3	3.6	3.2	5.4	1.4	0.0	
S794	4.4	6.0	3.2	8.9	6.8	10.3	6.5	4.6	4.5	6.7	5.2	4.0	0.0

Table 3. Interclass distances between 21 *Lactobacillus* spp. strains¹

Strain	A2	A26	A3	A34	B4	B15	B21	C2	D56	E3	F1	F44	G2	H1	H2	S1	S2	S9	S14	S15	S5
A2	0.0																				
A26	4.4	0.0																			
A3	6.3	1.2	0.0																		
A34	0.7	4.1	5.9	0.0																	
B4	4.8	3.3	4.2	4.5	0.0																
B15	13.7	9.9	13.4	12.6	8.4	0.0															
B21	2.8	3.4	5.1	2.4	3.5	13.4	0.0														
C2	6.2	4.9	6.4	5.7	4.9	12.1	5.2	0.0													
D56	5.7	1.8	2.6	5.2	4.2	13.7	4.5	6.3	0.0												
E3	11.2	8.3	10.1	10.5	6.8	8.7	10.7	10.1	11.0	0.0											
F1	8.8	5.9	7.5	8.2	5.3	10.4	8.0	7.1	8.7	4.3	0.0										
F44	6.5	5.1	6.9	6.1	5.8	13.6	6.1	4.8	6.3	10.9	8.1	0.0									
G2	4.9	3.2	4.9	4.4	4.8	14.9	3.6	5.9	4.3	11.6	8.9	5.6	0.0								
H1	7.2	3.6	5.0	6.8	6.0	15.2	7.3	8.4	4.9	12.8	10.5	8.4	7.7	0.0							
H2	3.3	4.3	6.6	3.0	4.4	14.4	3.2	6.1	4.6	11.7	9.7	6.4	4.8	7.7	0.0						
S1	6.7	6.7	8.8	6.3	5.1	12.0	6.6	4.9	7.8	10.8	9.1	7.4	8.0	10.3	6.4	0.0					
S2	7.3	5.3	6.9	6.9	4.9	11.0	6.5	4.7	7.0	7.8	5.2	5.1	6.8	9.0	7.6	6.0	0.0				
S9	6.5	5.1	6.4	6.0	5.6	13.2	5.8	2.8	6.6	11.0	7.5	3.3	5.6	8.5	6.7	6.4	4.9	0.0			
S14	6.3	4.4	5.6	5.9	4.1	12.0	5.3	3.8	5.1	9.8	6.9	4.9	5.8	7.5	6.3	5.6	4.7	3.3	0.0		
S15	5.0	4.5	5.8	4.6	4.8	11.7	4.2	2.8	5.4	9.5	6.8	2.4	4.2	7.5	4.8	5.4	4.6	1.9	3.7	0.0	
S5	5.3	4.1	5.2	4.9	3.9	9.9	4.1	2.1	5.4	8.1	5.5	3.3	4.6	6.9	5.2	4.9	3.2	1.8	2.7	1.7	0.0

¹Strains A2, A26, A3, A34, B21, and G2 = *Lactobacillus casei*; strains B4 and B15 = *Lactobacillus fermentum*; strains C2, E3, F1, and F44 = *Lactobacillus delbrueckii*; strains D56, H1, and H2 = *Lactobacillus rhamnosus*; strains S1, S9, S14, S15, and S5 = *Lactobacillus helveticus*.

using a 5-point Savitzky-Golay polynomial filter, and normalized before analysis. Soft independent modeling of class analogy (**SIMCA**) was used to build classification models for the cheese cultures. The SIMCA analysis procedure consists of assigning a separate class for each sample and creating a principal component analysis model to explain the majority of variation in the data set and grouping samples based on similarities (De Maesschalck et al., 1999). The first 3 principal components are used to project the classes; however, more principal components may be used for the actual differentiation. Inter-class distances were calculated using between-class residuals, and variable importance was determined by comparing the average residual variance of each class to all classes and the residual variance of all classes to themselves. Variable importance, also known as discriminating power, was used to define

the variables that have a predominant effect on sample classification (Dunn and Wold, 1995).

RESULTS AND DISCUSSION

Swiss cheese cultures were isolated using HGMP, and infrared microspectroscopy was used for direct fingerprinting of starter and nonstarter (adventitious bacterial flora) cultures involved in cheese production. The application of HGMP allowed for isolation of the bacterial culture in a hydrophobic square, limiting horizontal spread and overlap of microcolonies. Representative raw spectrums of *Strep. thermophilus*, *L. delbrueckii*, and *P. freudenreichii* are shown in Figure 1. Transformation of raw bacterial spectra to its second derivative using Savitzky-Golay second derivative algorithm (5-point gap size) allowed for reduced variability between rep-

Table 4. Interclass distances between 10 *Propionibacterium freudenreichii* strains

Strain	P764	P745M2	P745M1	P318	P859	P835	P843	P873	P728	P812	P891
P891	0.0										
P745M2	12.0	0.0									
P745M1	11.4	4.5	0.0								
P318	6.3	12.8	12.1	0.0							
P859	12.5	6.6	7.9	12.8	0.0						
P835	9.6	11.2	10.9	6.2	11.3	0.0					
P843	27.1	34.0	37.6	27.6	38.5	16.1	0.0				
P873	5.5	10.1	10.2	8.8	10.5	9.3	21.4	0.0			
P728	7.2	9.2	9.9	9.1	9.5	10.1	25.7	3.9	0.0		
P812	4.4	7.7	6.5	6.4	6.7	9.3	17.3	4.2	4.2	0.0	
P891	23.8	30.3	30.7	23.4	32.7	15.3	3.5	19.3	23.0	13.3	0.0

Figure 1. Typical Fourier transform infrared raw spectra of *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, and *Propionibacterium freudenreichii* bacteria.

fications by removing baseline variation and resolving overlapping peaks (Kansiz et al., 2001).

Multivariate analysis of spectra resulted in classification models for differentiating between strains of *Strep. thermophilus*, *Lactobacillus* spp., and *P. freudenreichii*, the main starter and adjunct cultures used in making Swiss cheese (Ji et al., 2004). This protocol allowed for the collection of reproducible infrared absorption spectra directly from biomass of individual colonies isolated with HGMP, thus overcoming the labor-intensive and time-consuming biochemical and phenotypic characterization of bacteria of traditional methods for identification of cheese microorganisms such as PFGE and DNA fingerprinting. Species differentiation based on infrared spectroscopic data has been shown to be consistent with the analysis results obtained by the 16S RNA sequencing, regarded as the gold standard (Kirschner et al., 2001).

The SIMCA analysis of FTIR spectral data from 13 *Strep. thermophilus* cultures showed good clustering at the strain level (Figure 2A) and good separation between classes, evidencing the potential of infrared spectroscopy and multivariate analysis to detect subtle differences for intraspecific differentiation of strains. The SIMCA model (Figure 2A) showed close grouping between the strains S363, A054, S838, S847, S765, S731, and S794, suggesting high similarities between these 7 strains that was confirmed by interclass distance (ICD) values (Table 2) close to 3; generally, ICD >3 are indicative of good discrimination (Kyalheim and Karstang, 1992). All other strains showed ICD values >3. Cluster analysis based on PFGE for *Strep. thermophilus* cultures used for Swiss cheese manufac-

ture has shown 30 to 40% similarity among cultures (O'Sullivan and Fitzgerald, 1998; Jenkins et al., 2002). The FTIR approach permitted collection of complex biochemical fingerprint-like spectra derived from the total cell composition (Becker et al., 2006), allowing for rapid and reliable typing of *Strep. thermophilus* starter cultures.

Multivariate analysis of *Lactobacillus* spp. spectra generated a model that classified the strains according to starters and nonstarters. The model (Figure 3) showed clear separation between *L. helveticus* starter cultures from the remaining nonstarter strains. However, the nonstarter *L. delbrueckii* strains formed a separate cluster distinct from both the starters and no-starters. The ICD values between the 3 clusters are shown in Table 5. We observed that *L. delbrueckii* clustered closer to the *L. helveticus* starter cultures (ICD 5.1) than to the other nonstarter cultures (ICD 6.7). *Lactobacillus delbrueckii* strains are used as starter cultures along with *L. helveticus* in the manufacture of Swiss-type cheeses (Khalid and Marth, 1990). Marked differences were observed among the *L. delbrueckii* strains: the strains were separated in 2 groups with significant interclass separation between strains C2 and F44 and strains E3 and F1 (ICD 15.4), indicating phenotypic differences between these strains. Strains of *L. delbrueckii* C2 and F44 showed greater relatedness to the starters than the remaining 2 strains (E3 and F1). Thus, SIMCA analysis provided a model that distinguished the *L. delbrueckii* strains according to their closeness to the starters (Figure 3).

The SIMCA analysis of the spectral data from *Lactobacillus* strains also permitted development of a

Figure 2. Soft independent modeling of class analogy plot for (A) *Streptococcus thermophilus* (13) strains, (B) *Lactobacillus* spp. (21) strains, and (C) *Propionibacterium freudenreichii* (10) strains. Color version available in the online PDF.

model that classified all strains into individual clusters (Figure 2B). The clustering pattern showed similarities to the SIMCA model that classified the strains according to starters and nonstarters. The 6 starter *L. helveticus* strains and *L. delbrueckii* C2 and F44 strains formed close clusters (8, 12, and 16–21 in Figure 2B). *Lactobacillus delbrueckii* E3 and F1 strains clustered together (10 and 11 in Figure 2B). These data were also supported by the ICD values (Table 5) provided by multivariate analysis. Interclass difference values <3 were observed in closely related lactobacilli strains. *Lactobacillus* strains are more closely related to each other (55–100% similarity) than other cheese starter cultures, indicating less heterogeneity within species by PFGE (Jenkins et al., 2002).

The SIMCA analysis of the spectral data from *P. freudenreichii* strains generated a model that classified

them into 10 classes (Figure 2C). The model showed good class separation and tight clustering among the 10 strains with between-class distances ranging from 4.2 to 38.5. The ICD values of this model are indicated in Table 4. Clustering between *P. freudenreichii* ssp. *shermanii* strains was similar to that reported by Jenkins et al. (2002) using PFGE. Strains clustered into 3 groups that included P859, P745M1, and P745M2 (group 1), P843 and P891 (group 2), and P764, P812, P873, and P728 (group 3), suggesting similar biochemical characteristics among strains within each group. Jenkins et al. (2002) reported that *P. freudenreichii* strains showed >30.5% similarity by PFGE.

The pattern recognition model showed good discrimination in the infrared region from 1,300 to 800 cm^{-1} , and the wavenumbers having the greatest influence on the classification model can be identified in the dis-

Table 5. Interclass distances between *Lactobacillus* starters and nonstarter cultures

Class ¹	1	2	3
1	0.0		
2	5.1	0.0	
3	6.7	5.9	0.0

¹Class 1 = nonstarter lactic acid bacteria; class 2 = starter lactic acid bacteria; class 3 = *Lactobacillus delbrueckii*.

criminating power plot (Figure 4A). The major bands responsible for the classification models were associated to C-O, C-C stretching, and C-O-H and C-O-O deformation of the cell components involving cell wall polysaccharide components (Udelhoven et al., 2000; Sahu et al., 2006). Classification of 13 *Strep. thermophilus* strains showed major discriminating bands at 1,146, 1,065, 1,045, and 991 cm^{-1} (Figure 4A). Signals at 1,065 and 991 cm^{-1} are characteristic of carbohydrates, with the former band characterized on a biosurfactant produced by *Strep. thermophilus* strains (Rodrigues et al., 2006; McCann et al., 2007). The classification of *Lactobacillus* spp. strains (Figure 4B) showed a major band at 1,150 cm^{-1} that can be associated with signals from carbohydrate C-O stretching vibrations of cell wall carbohydrate components (Nichols et al., 1985; Nau-

mann et al., 1995, 1996). Work done by Luginbuhl et al. (2006) reported that the infrared region from 1,300 to 900 cm^{-1} was the most effective in classifying, at the species level, 102 strains of the *Lactobacillus acidophilus* group, including *L. acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus crispatus*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *L. helveticus*, and *Lactobacillus johnsonii*. It was reported by Amiel et al. (2001) that the region 1,200 to 900 cm^{-1} , containing signals C-O-C and C-O-P stretching vibration from polysaccharides, were selective for classification of dairy *Lactobacillus* spp. at the species and strain levels. Figure 4C shows the discriminating power plot indicating the important discrimination bands for the classification model of *P. freudenreichii* strains. The infrared region from 900 to 1,300 cm^{-1} resulted in a major discrimination band at 1,010 cm^{-1} associated with the vibrational mode (ν) of the C=O bond ($\nu_{\text{C-OH}}$) of alcohol (C6-O6H; Haxaire et al., 2003). Alcohols display intense $\nu_{\text{C-O}}$ bands in the 1,000–1,120 cm^{-1} region (Haxaire et al., 2003). This research is the first attempt to classify *Propionibacterium* cultures using FTIR spectroscopy. Overall, the SIMCA discrimination power showed that classification of Swiss cheese cultures was attributed to vibrations due to differences in the carbohydrate backbone related to bacterial cell wall phenotype. Strong complex absorp-

Figure 3. Soft independent modeling of class analogy plot for *Lactobacillus* starter and nonstarter cultures.

Figure 4. Discriminating power plot for the classification of (A) *Streptococcus thermophilus* strains, (B) *Lactobacillus* spp. strains, and (C) *Propionibacterium freudenreichii* strains. Infrared wavenumbers that were different between classes and the relative extent of the difference are shown.

tion in the 1,100 to 1,000 cm^{-1} region is ascribed to vibrations of ring structures, and bands between 1,200 and 1,100 cm^{-1} are assigned to $\nu\text{C-C}$ and $\nu\text{C-O}$ weakly coupled to $\delta\text{C-O-H}$ modes, including the $\nu\text{C-O-C}$ of the glycosidic linkage ($\sim 1,124 \text{ cm}^{-1}$).

CONCLUSIONS

Application of FTIR spectroscopy to classify microorganisms had been successfully implemented by many researchers. This research was aimed at building classification models for several starter and nonstarter cultures used in making Swiss cheese. A rapid and simple method was developed to classify more than 40 Swiss cheese cultures at the strain level using a combination of HGMF and FTIR microspectroscopy. The developed classification model also indicated biochemical similarities between the strains by forming close clusters. The models showed major discrimination in the spectral region from 1,100 to 900 cm^{-1} associated with stretching vibration signals from polysaccharides in the cell wall. Implementing this technique would enable the Swiss cheese manufacturer to select cheese cultures at the strain level. This information will enable the cheese maker to have a detailed overview of the microbiological status of the starter and nonstarter cultures, which in turn would enable corrective measures to be taken early in the process, limiting production of inferior quality cheese and minimizing defects. Furthermore, this technique could be extended to classify other cheese and dairy cultures.

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