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Research Note

Incidences of Problematic Organisms on Petrifilm Aerobic Count Plates Used To Enumerate Selected Meat and Dairy Products

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ABSTRACT

Petrifilm aerobic count plates are similar to or better than conventional pour plates. Petrifilm has its problems, however; some microorganisms can liquefy the Petrifilm gel and others do not produce the necessary color change with the indicator dye used. Petrifilm aerobic count plates were compared with the pour plates for determining the incidence and identification of problematic organisms in 329 meat and dairy products. Petrifilm plates produced higher mean counts with better repeatability than did pour plates. There was also close correlation between methods with coefficients of 0.97 to 1.0. *Bacillus subtilis*, *Bacillus licheniformis*, and a group D *Streptococcus* liquefied Petrifilm gels in 17.4% of the samples tested: dairy products accounted for 16.0%, and meats accounted for the remaining 1.4%. Liquefaction hindered enumeration in 3.2% of the Petrifilm plates used. *Streptococcus viridans* was not detectable on Petrifilm plates after the recommend incubation period, and this organism occurred in 0.3% of the Petrifilm plates used. These results indicate that Petrifilm plates would be unsuitable for samples with large numbers of these organisms. Knowledge of the contaminating flora may be an asset when utilizing Petrifilm aerobic count plates for the enumeration of microbes in food.

Petrifilm products are preprepared culture media that require only sample preparation. They have the advantages of easy use, reduced laboratory space requirements, and overall cheaper costs. Petrifilm aerobic count plates (3M Microbiology Products, St. Paul, Minn.) contain standard culture medium components, the cold water–soluble gelling agent, guar gum coated onto a film base, an overlay film coated with gelling agent, and the 2,3,5-triphenyltetrazolium chloride (TTC) indicator. The TTC indicator is contained in a slow-release system that eliminates toxicity of the dye to microorganisms. The reduction of the TTC indicator dye by the growing organism produces a red color (formazan), which facilitates colony enumeration. All red dots regardless of size or intensity are counted as colonies.

In previous studies, Petrifilm aerobic count plates were similar and in some cases better than conventional pour plates for the enumeration of total aerobes in food products *(2, 3, 6, 7, 12, 16, 18, 21, 23, 24, 30).* Petrifilm has been approved for use by a number of international standard organizations *(27).* Petrifilm aerobic count plates do have some problems; the *Petrifilm Aerobic Plate Count Product Manual* states that some organisms can liquefy the gel, allowing colonies of these organisms to spread out and obscure the presence of colonies of other organisms. Blackburn et al. *(2)* reported this problem but made no mention of the causative organisms and the mechanism of gel liquefaction.

The American Public Health Association (APHA) reported that not all bacteria form red colonies when exposed to TTC *(25).* Small white or pale-colored colonies that do not produce the necessary color change with the TTC dye would be extremely difficult to detect against the opaque background of Petrifilm aerobic count plates.

The microbial flora associated with food varies among different industrial practices and geographically. Therefore, prevalent strains of microorganisms isolated in different countries or regions may differ. The suitability of Petrifilm aerobic count plates for testing locally produced foods and identifying problematic organisms was the major focus of this study.

MATERIALS AND METHODS

Foods. A total of 329 samples were tested: 196 samples of dairy products and 133 samples of meat products. The dairy product samples included 55 of powdered skim and whole milk, 71 of pasteurized liquid milk, and 70 of ice cream. The meat product samples were fresh or frozen and included 44 of poultry, 45 of fish, and 44 of beef. All products were produced by local manufacturers and were collected from several retail and factory outlets over a 1-year period. Equal distribution of all brand types was ensured, and samples had different production dates and/or production codes. All samples collected were tested within 24 h after collection. Chilled samples were stored at approximately 4°C, and frozen samples were stored at approximately −24°C. Powdered

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TABLE 1. *Morphological, chemical, and biochemical tests used to identify microorganisms that liquefied or caused problematic reactions on Petrifilm aerobic count plates*

Test
Aerobic and anaerobic growth
Carbohydrate fermentation
Casein hydrolysis
Catalase production
Cell morphology
Citrate utilization
Coagulase production
Colony morphology
Gelatin hydrolysis
Gram strain
Growth in NaCl
Growth temperature range
Indole production test
Lipid hydrolysis
Methyl red Voges-Proskauer
Motility
Oxidase production
pH range for growth
Phenylalanine deamination
Spore production
Starch hydrolysis
Urease production

milk samples were stored at room temperature. Chilled and frozen samples were stored on ice during transport from retail outlets to the testing laboratory.

Sample preparation. All samples were prepared in accordance with the methods described by the APHA *(20, 29).* Before dilution, liquid and powdered samples were shaken to increase homogeneity. Frozen meat samples were thawed at approximately 4°C for no longer than 18 h before analysis. Fresh and thawed meat samples were cut into small pieces and then homogenized using a stomacher. Ice cream was used in the frozen state by removing a portion with a spatula and adding it to diluent, where it was allowed to melt and was then homogenized by shaking. Initial dilutions (1:10) were prepared with 25 g or 25 ml for dairy products and 50 g for meat samples in appropriate volumes of phosphate dilution water and 0.1% peptone water, respectively.

Petrifilm method. Duplicate Petrifilm aerobic count plates were prepared as instructed by the manufacturer. Plates were incubated at 32 ± 1°C for 48 ± 3 h for dairy products and 35 ± 1°C for 48 ± 3 h for meat samples according to APHA standards *(20, 29).* After incubation, all red dots on Petrifilm plates regardless of size or intensity were counted as colonies.

Standard plate count. Duplicate plates were prepared using plate count agar (Difco, Becton Dickinson, Sparks, Md.) for meats and milk agar (Difco, Becton Dickinson) for dairy products in accordance with procedures described by APHA *(20, 29).* The indicator TTC indicator was added to aid in colony identification. Plates were incubated as described in the previous section. After incubation, all colonies were counted regardless of size and color.

Identification of microorganisms. Organisms that liquefied the Petrifilm gel and other problematic organisms were identified with traditional tests using morphological characteristics along with chemical and biochemical tests (Table 1). Organisms that could not be identified in the testing laboratory were sent to the University of the West Indies Hospital Microbiology Laboratory for identification using chemical, biochemical, and serological methods.

Data analysis. Pour plate counts in the range of 25 to 250 CFU per plate was used in the comparisons. Colony counts were calculated by taking an average of duplicate plates of the same dilution and multiplying by the reciprocal of the dilution factor. The result was then rounded to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10^x, where x is the appropriate power of 10. Colony counts were converted to log counts per gram or milliliter to more closely match the underlying assumption of a normal distribution and homogeneous variance. Student's paired t test was used to compare the mean log differences to determine any significant differences ($P < 0.05$) in the recovery of organisms between methods. Standard regression techniques were used to calculate correlation coefficients and the significance of the correlation ($P < 0.05$). Repeatability variance, repeatability standard deviation, and the coefficient of variation were calculated from the differences between duplicate readings. The coefficient of variation was calculated by dividing

TABLE 2. *Summary of statistical analysis for all products tested*

Product	Method[a]	Mean count[b]	Coefficient of variation (%)	t test ($P < 0.05$)	Correlation ($P < 0.001$)
Beef	SPC	4.31	1.5	0.77	0.99
	PAC	4.32	1.0		
Fish	SPC	4.13	1.7	0	0.99
	PAC	4.48	1.4		
Ice cream	SPC	4.14	1.6	0.03	0.99
	PAC	4.15	1.6		
Pasteurized liquid milk	SPC	3.32	3.2	0.19	1.0
	PAC	3.35	0		
Poultry	SPC	3.34	4.8	0	0.97
	PAC	3.71	1.2		
Powdered milk	SPC	5.37	1.7	0.04	0.99
	PAC	5.39	1.0		

[a] SPC, standard plate count; PAC, Petrifilm aerobic plate count.
[b] Log CFU per gram or per milliliter.

TABLE 3. *Incidence of liquefaction on Petrifilm aerobic count plates*[a]

Product	SL	LL	TL	No liquefaction	Total no. of plates	% liquefaction in total plates
Beef	0	3	0	135	138	0.4
Fish	0	0	0	142	142	0
Ice cream	27	1	15	100	143	5.6
Pasteurized liquid milk	2	0	0	76	78	0.3
Poultry	0	6	2	145	153	1.0
Powdered milk	70	1	7	41	119	10.1
Total[b]	99 (12.8)	11 (1.4)	24 (3.2)	639 (82.7)	773 (100)	17.4

[a] SL, small liquefaction; LL, localized liquefaction; TL, total liquefaction.
[b] Numbers in parentheses are the percentage of each liquefaction group in total plates.

the repeatability standard deviation by the mean count and was expressed as a percentage. Estimated counts were not used for the regression and repeatability analysis and the *t* tests.

RESULTS AND DISCUSSION

A total of 773 Petrifilm plates had corresponding pour plate counts within the range of 25 to 250 CFU per plate. Petrifilm gave significantly higher mean counts for all samples except those of beef and pasteurized liquid milk. Overall, Petrifilm had better repeatability, with correlation coefficients between 0.97 and 1.0 (Table 2). These results are consistent with those of previous studies of dairy and meat samples where Petrifilm was better or comparable to standard methods *(2, 4, 5, 16, 18).*

Liquefaction of Petrifilm plates occurred with 17.4% of the samples tested: 16.0% were dairy products and 1.4% were meats. Three categories of liquefaction occurred on these plates. Small liquefaction was defined as plates containing one or more colonies with small zones of liquefaction that did not affect the accuracy of enumerations (Table 3 and Fig. 1). In plates with localized liquefaction, liquefaction zones rendered accurate enumerations impossible, allowing only estimated counts. This type of liquefaction normally affected only a portion of the plate, so estimated counts could be made from the unaffected areas (Table 3 and Fig. 2). Total liquefaction, when liquefaction rendered enumeration impossible, occurred in 3.2% of all Petrifilm plates (Table 3). Approximately 3.3% of the corresponding agar plates were affected by either mold or spreaders.

Changes in gelling properties have major implications for the use of Petrifilm, because Petrifilm cannot be used for samples with large numbers of organisms that cause liquefaction. Three organisms were implicated in liquefaction: *Bacillus subtilis, Bacillus licheniformis,* and a group D *Streptococcus*; *B. licheniformis* was the most abundant, and group D *Streptococcus* was the least abundant (Table 4). The gelling agent on Petrifilm is the cold water–soluble guar gum, a galactomannan formed by a backbone of D-mannose with a D-galactose side chain attached to every other D-mannose residue: mannose (1-4) plus galactose (1-6). Removal of the galactose side chains results in an increase in the gelling properties of guar gum; however, disruption of the β 1-4 linkages of the mannose subunits results in a loss of gelling properties. Hydrolysis of the subunits can be affected by β-mannanase and multicomponent hemicellulase with β-mannanase activity *(10, 19, 26),* both of which are produced by *B. subtilis* and *B.*

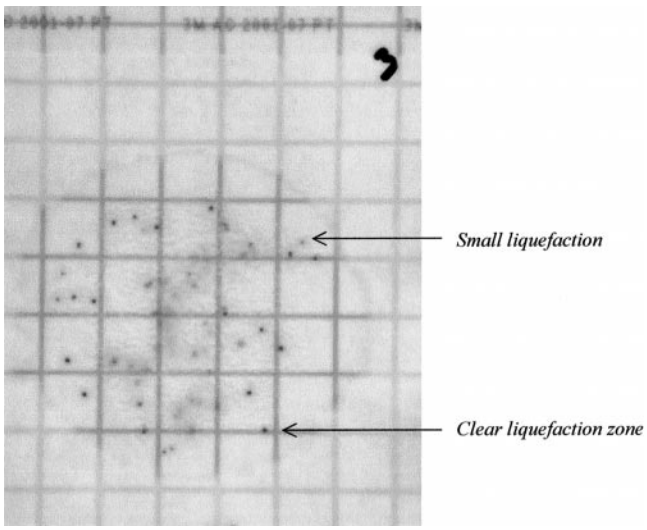

FIGURE 1. Streptococcus *(group D) liquefaction on Petrifilm.*

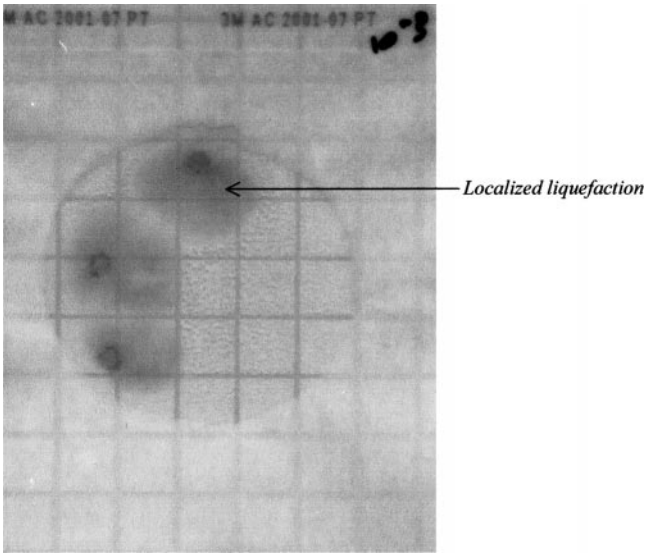

FIGURE 2. Bacillus subtilis *liquefaction on Petrifilm.*

TABLE 4. *Incidence of liquefaction organisms in sample groups grown on Petrifilm plates*

Organism	No. (%) of plates with organism					
	Poultry	Beef	Fish	Ice cream	Pasteurized liquid milk	Powdered milk
Bacillus subtilis	3 (2.0)	3 (2.2)	0	15 (10.5)	1 (1.3)	8 (6.7)
B. licheniformis	0	0	0	28 (19.6)	1 (1.3)	70 (58.8)
Streptococcus group D	5 (3.3)	0	0	0	0	0

licheniformis (1). One or both enzymes are secreted by the group D streptococci.

Streptococcus (Fig. 1) produces clear liquefaction zones, whereas *B. subtilis* (Fig. 2) and *B. licheniformis* (Fig. 3) produce colored (red or pink) liquefaction zones. These color differences were due to the movement of *B. subtilis* and *B. licheniformis* cells through the medium, creating large diffused colonies. *Streptococcus* is nonmotile and so produces distinct confined colonies surrounded by an area of liquefaction. This bacterium can also create large diffused colonies if the liquefaction zone meets other motile organisms on the plate, allowing them to spread throughout the liquefaction zone.

The size of the liquefaction zone produced by each organism is an indication of the amount of mannanase or hemicellulase being secreted. *B. subtilis* produced the largest zones of liquefaction, and *B. licheniformis* produced the smallest. This finding supports that of Araujo and Ward *(1),* who showed that *B. subtilis* produces significantly more mannanase than does *B. licheniformis* and that *B. subtilis* is an industrial producer of β-mannanase and hemicellulase *(8–10, 15, 31).*

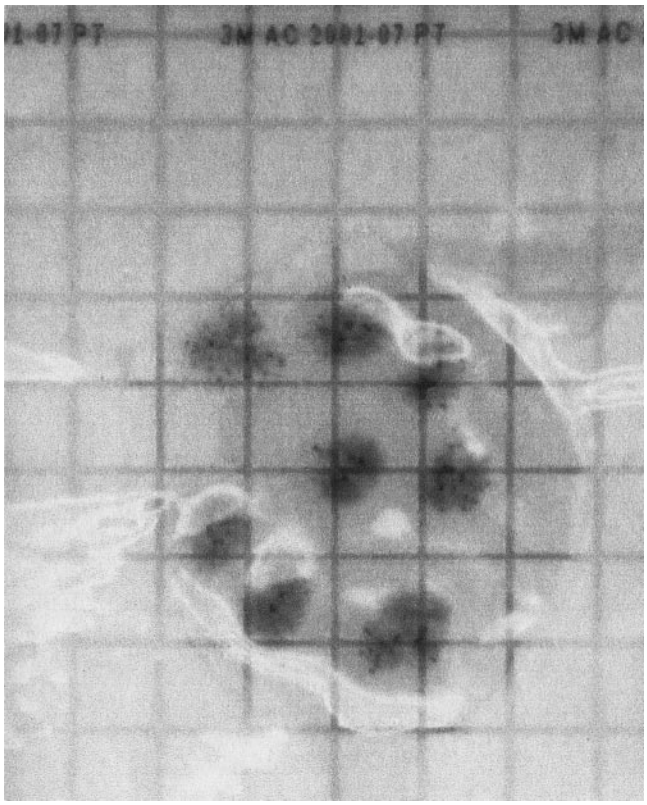

FIGURE 3. Bacillus licheniformis *liquefaction on Petrifilm.*

Pure cultures of *B. subtilis* on Petrifilm plates produced liquefaction areas of 1.205 ± 1.469 cm^2. *B. licheniformis* and *Streptococcus* group D produced liquefaction areas of 0.154 ± 0.142 cm^2 and 0.355 ± 0.187 cm^2, respectively. Petrifilm aerobic count plates with an area of 20 cm^2 would theoretically be completely liquefied by 17 *B. subtilis* colonies, 130 *B. licheniformis* colonies, or 56 colonies of group D streptococci. However, the results of the present study indicate that a much smaller number of colonies would prevent enumeration, because not all the plate surface area has to be liquefied for enumeration to be rendered impossible (Table 5).

B. subtilis and *B. licheniformis* are endospore-producing soil microorganisms. The spores are resistant to environmental stress and can contaminate foods from direct contact with soil or airborne dust *(28).* As a result these organisms appear in foods as natural contaminants or as a result of improper processing and/or storage. *Streptococcus* group D is associated with the lower gut of animals and can contaminate animal products during processing. *B. subtilis, B. licheniformis,* and group D *Streptococcus* are not the only organisms capable of secreting galactomannanose-digesting enzymes *(11, 13, 14, 17, 22).* Other food contaminating organisms, which were not present in the samples tested, may also be able to liquefy Petrifilm. Before a transition can to be made to Petrifilm, more information is needed concerning the relative frequency at which these problematic organisms occur in the products to be tested routinely and hence the feasibility of Petrifilm use for quick and accurate counts.

One organism, *Streptococcus viridans,* did not produce a red color on Petrifilm within the recommended incubation period. Colonies of this organism appeared white on milk agar but could not be detected on Petrifilm after the recommended incubation time; however, with prolonged in-

TABLE 5. *Average liquefaction area of different microorganisms grown on Petrifilm plates*

Organism	Mean ± SE liquefaction area (cm^2)	Theoretical no. of colonies for total liquefaction
Bacillus subtilis	1.205 ± 0.158	17
B. licheniformis	0.154 ± 0.033	130
Streptococcus group D	0.355 ± 0.024	56

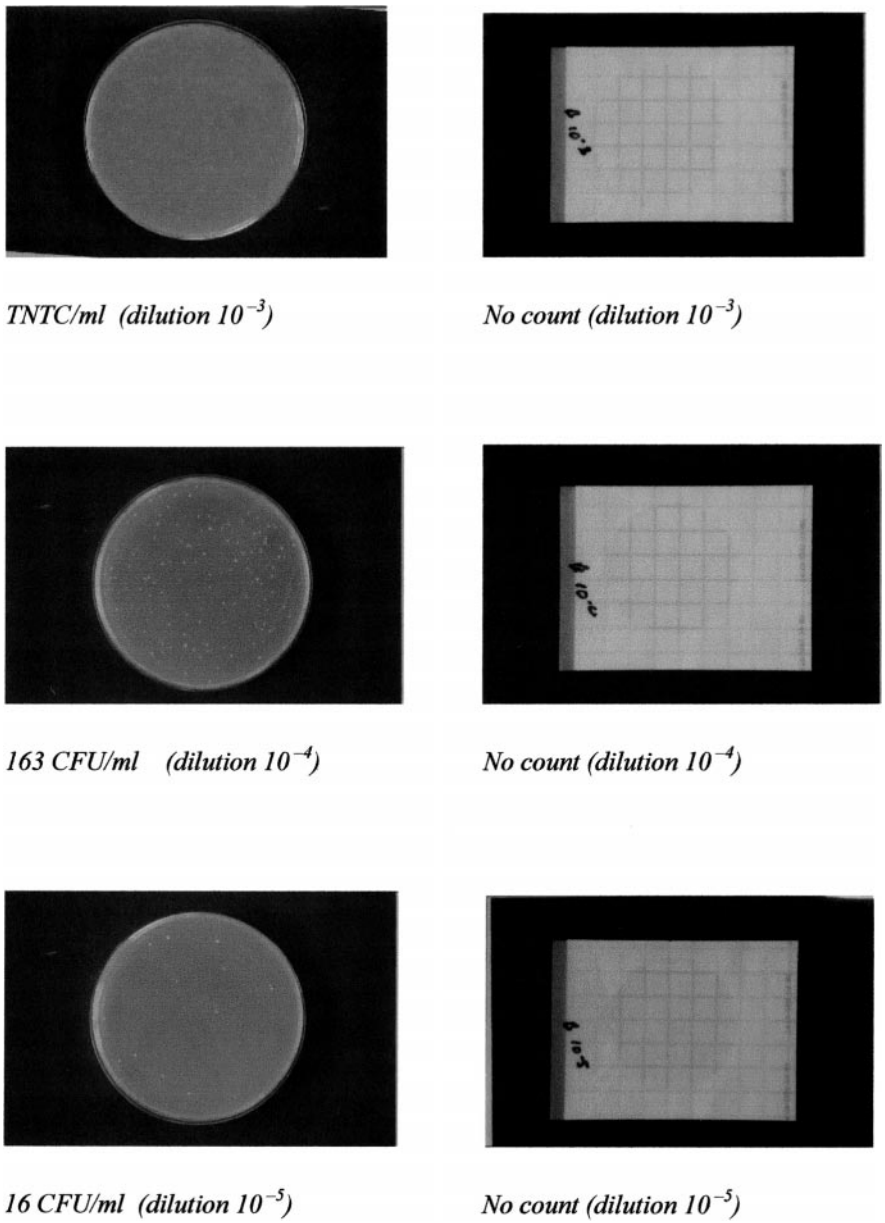

FIGURE 4. *Corresponding Petrifilm (ACP) and pour plates (PCA) with a pure culture of* Streptococcus viridans *after incubation for 48 h.*

cubation (5 days) a faint color developed (Fig. 4). *S. viridans* was isolated from ice cream samples derived from a single company. This organism is not a usual contaminant of ice cream, and its presence is indicative of contamination within the processing facility. This finding underscores the need to be able to detect a wide range of organisms, including usual contaminants and those that occur in the event of a breakdown of proper processing procedures. Although the incidence of the organism was very low (0.3%), changes in its abundance could have serious implications for Petrifilm use.

Petrifilm aerobic count plates are in general a suitable alternative to standard plates for the enumeration of aerobic bacteria in dairy products and meats because of the low incidence of problematic organisms in these products. However, Petrifilm aerobic count plates would be unsuitable for samples with high numbers of *B. subtilis*, *B. licheniformis*, group D *Streptococcus*, other mannanase- or hemicellulase-secreting organisms, and *S. viridans.*

REFERENCES

1. Araujo, A., and O. P. Ward. 1990. Hemicellulases of *Bacillus* species: preliminary comparative studies on production and properties of mannanases and galactanases. *J. Appl. Bacteriol.* 68:253.
2. Blackburn, C. D. W., C. L. Baylis, and S. B. Petitt. 1996. Evaluation of Petrifilm™ methods for enumeration of aerobic flora and coliforms in a wide range of foods. *Lett. Appl. Microbiol.* 22:137–140.
3. Byrne, R. D., and J. R. Bishop. 1991. Evaluation of a dry medium culture plate (3M Petrifilm AC) for laboratory pasteurized counts. *J. Food Prot.* 54:308–309.
4. Chain, V. S., and D. Y. C Fung. 1991. Comparison of redigel, Petrifilm™, spiral plate system, isogrid, and aerobic plate count for determining the numbers of aerobic bacteria in selected foods. *J. Food Prot.* 54:208–211.
5. Cormier, A., S. Chiasson, and A. Leger. 1993. Comparison of maceration and enumeration procedures for aerobic count in selected seafoods by standard method, Petrifilm, Redigel and Isogrid. *J. Food Prot.* 56:249–251.
6. Curiale, M. S., P. Fahey, T. L. Fox, and J. S. McAllister. 1989. Dry rehydratable film for enumeration of coliforms and aerobic bacteria in dairy products: collaborative study. *J. Assoc. Off. Anal. Chem.* 72: 312–318.

7. Curiale, M. S., T. Sons, J. S. McAllister, B. Halsey, and T. L. Fox. 1990. Dry rehydratable film for enumeration of total aerobic bacteria in foods: collaborative study. *J. Assoc. Off. Anal. Chem.* 73:242–248.
8. Feng, Y., Z. He, S. L. Ong, J. Hu, Z. Zhang, and W. J. Ng. 2003. Optimization of agitation, aeration, and temperature conditions for maximum β-mannanase production. *Enzyme Microb. Technol.* 32: 282–289.
9. Feng, Y. Y., Z. M. He, L. F. Song, S. L. Ong, J. Y. Hu, Z. G. Zhang, and W. J. Ng. 2003. Kinetics of β-mannanase fermentation by *Bacillus licheniformis. Biotechnol. Lett.* 25:1143–1146.
10. Fodge, D. W., D.M. Anderson, and T. M. Pettey. 1997. Hemicellulase active at pH and temperature extremes. *Biotechnol. Adv.* 15:96.
11. Fondevila, M., and B. A. Dehority. 1994. Degradation and utilization of forage hemicellulose by rumen bacteria, singly in coculture or added sequentially. *J. Appl. Bacteriol.* 77:541–548.
12. Ginn, R. E., V. S. Packard, and T. L. Fox. 1984. Evaluation of the 3M dry medium culture plate (Petrifilm™ SM) method for detecting numbers of bacteria in raw milk. *J. Food Prot.* 47:753–755.
13. Hamlyn, P. F. 1998. Fungal biotechnology. Available at: http://fungus.org.uk/nwfg/fungbiot.htm. Accessed 1 July 2004.
14. Johnson, D. G., M. C. Silva, C. R. Mackenzie, H. Schneider, and J. D. Fontana. 1989. Microbial degradation of hemicellulosic materials. *Appl. Biochem. Biotechnol.* 20:245–258.
15. Jun, Z., H. Zhimin, and H. Kun. 2001. Preparation of beta-mannanase from *Bacillus licheniformis. Food Ferm. Ind.* 27:5–7.
16. McAllister, J. S., M. P. Stadtherr, and T. L. Fox. 1988. Evaluation of the 3M Petrifilm™ culture plate method for enumerating aerobic flora and coliforms in poultry processing facilities. *J. Food Prot.* 51: 658–659.
17. Osborne, J. M., and B. A. Dehority. 1989. Synergism in degradation and utilization of intact forage cellulose, hemicellulose, and pectin by three pure cultures of ruminal bacteria. *Appl. Environ. Microbiol.* 55:2247–2250.
18. Park, H. P., K. S. Seo, J. S. Ahm, H. S. Yoo, and S. P. Kim. 2001. Evaluation of the Petrifilm plate method for the enumeration of aerobic microorganisms and coliforms in retailed meet samples. *J. Food Prot.* 64:1841–1843.
19. Patel, M. B., and J. McGinnis. 1985. The effect of autoclaving and enzyme supplementation of guar meal on the performance of broiler chicks and laying hens. *Poult. Sci.* 64:1148–1156.
20. Robert, T. M. (ed.). 1992. Standard methods for the examination of dairy products. American Public Health Association, Washington, D.C.
21. Shingo, M., and H. Kodaka. 2000. Evaluation of dry sheet medium culture plate (compact TC) method for determining numbers of bacteria in food samples. *J. Food Prot.* 63:665–667.
22. Smith, D. C., and C. W. Forsberg. 1991. β-glucuronidase and other hemicellulase activities of *Fibrobacter succinogenes* s85 grown on crystalline cellulose or ball-milled barley straw. *Appl. Environ. Microbiol.* 57:3552–3557.
23. Smith, L. B., T. L. Fox, and F. F. Busta. 1985. Comparison of a dry culture plate (Petrifilm™ SM plates) method to the aerobic plate count method for enumeration of mesophilic aerobic colony-forming units in fresh ground beef. *J. Food Prot.* 48:1044–1045.
24. Steven, I. C., and M. W. Moody. 1990. Enumeration of aerobic plate count and *E. coli* during blue crab processing by standard methods, Petrifilm, and redigel. *J. Food Prot.* 53:423–424.
25. Swanson, K. M. J., F. F. Busta, E. H. Peterson, and M. G. Johnson. 1992. Colony count methods, p. 75–95. *In* Compendium of methods for the microbiological examination of food. American Public Health Association, Washington, D.C.
26. Talbot, G., and J. Sygusch. 1990. Purification and characterization of thermostable beta-mannanase and alpha-galactosidase from *Bacillus stearothermophilus. Appl. Environ. Microbiol.* 56:3505–3510.
27. 3M Microbiology. 2004. Petrifilm plate approvals, recognitions and certificates. Available at: http://solutions.3m.com/wps/portal/_s.155/84505/_s.155/92829. Accessed 27 February 2004.
28. Todar, K. 2003. Todar's online textbook of bacteriology. Available at: http://textbookofbacteriology.net/Bacillus.html. Accessed 1 July 2004.
29. Vanderzant, C., and D. Splittstoesser (ed.). 1992. Compendium of methods for the microbiological examination of food. American Public Health Association, Washington, D.C.
30. Vicki, C. S., and D. Y. C. Fung. 1991. Comparison of redigel, Petrifilm, spiral plate system, isogrid, and aerobic plate count for determining the numbers of aerobic bacteria in selected foods. *J. Food Prot.* 54:208–211.
31. Yim, J. S., J. W. Jung, J. S. Lee, D. K. Kang, and H. K. Kim. 2003. Optimization of beta-mannanase production from *Bacillus subtilis* JS-1. *Korean J. Microbiol. Biotechnol.* 31:57–62.