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Whole-organism Fingerprinting of the Genus *Carnobacterium* using Fourier Transform Infrared Spectroscopy (FT-IR)

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Summary

Sixty seven strains of *Carnobacterium*, atypical *Lactobacillus*, *Enterococcus durans*, *Lactobacillus maltaromicus* and *Vagacoccus salmoninarum* were examined by Fourier transform infrared (FT-IR) spectroscopy. The effects of culture age and reproducibility over a six month period were also investigated. The results were analysed by multivariate statistics and compared with those from a previous numerical phenetic study, a pyrolysis mass spectrometry (PyMS) study and with investigations which used DNA-DNA and 16S rRNA sequencing homologies. Taxonomic correlations were observed between the FT-IR data and these studies. Culture age was observed to have little effect on the spectra obtained. The reproducibility study indicated that there was correlation between spectra produced on two occasions over the six month period. It was concluded that FTIR is a reliable method for investigating carnobacterial classification, and may have further potential as a rapid method for use in *Carnobacterium* identification.

Key words: *Carnobacterium* – Taxonomy – Fourier transform infrared spectroscopy

Introduction

The genus *Carnobacterium* currently consists of seven species with isolates from a range of habitats including meat and meat products, fish, dairy products and seawater. The original four *Carnobacterium* species, *C. divergens*, *C. gallinarum*, *C. mobile* and *C. piscicola*, were first isolated by Thornley and Sharpe [27] from irradiated chicken carcasses and assigned to the genus *Carnobacterium* by Collins *et al.* [3]. Isolates from Antarctic lakes have been named as *C. alterfunditum* and *C. funditum* [6], and the most recently described species, *C. inhibens*, was isolated from the natural microflora of the Atlantic salmon *(Salmo salar)* intestine [9].

Methods used in the classification of carnobacteria include physiological and biochemical tests [3, 5, 13], 16S rDNA sequence analysis [30], nucleic acid hybridisation [2, 14, 22], and pyrolysis mass spectrometry [18]. However, although these methods are helping to provide a clearer picture of carnobacterial systematics not all are suitable for use in large scale studies as they can be very labour intensive and time consuming.

Fourier transform infrared (FT-IR) spectroscopy [4, 8, 25] is a methodology used for whole-cell analysis and its use in systematics has been revolutionised by developments in advanced chemometric techniques to extract (bio) chemical information from hyperspectral data [7, 19, 20].

In the current study, 67 *Carnobacterium* and related strains were analysed by FT-IR to evaluate the classification of the strains and to determine the potential of FT-IR with multivariate statistical analyses for *Carnobacterium* systematics.

Materials and Methods

Strains and cultivation

Sixty seven strains (Table 1) were subcultured twice on casein-peptone soymeal-peptone agar (CASO broth (Merck), 1.2% (w/v) Lab M No. 1 agar) for 3 days at 25 °C. Ten strains were examined in duplicate to assess the reproducibility of the system.

Effect of culture age

Type strains of *C. divergens* (NCFB 2763^T), *C. gallinarum* (MT44^T), *C. mobile* (NFCB 2765^T) and *C. piscicola* (NCFB 2762^T) were cultivated on CASO agar for 2, 3, 4 and 5 days at 25 °C.

Reproducibility over six months

The reproducibility of the method was examined using 15 randomly selected *Carnobacterium* strains (Table 1). The strains were grown on two occasions separated by a period of six months as previously described on CASO agar at 25 °C for 3 days.

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Table 1. Designation and cluster assignment of *Carnobacterium* and related strains using Euclidean distance and UPGMA analysis.

Strain	Received as	Reference
	Strains assigned to cluster 1	
NCFB 2777[T]	*Vagacoccus salmoninarum*	
MT12	Atypical *Lactobacillus*	[3]
	Single member cluster	
MT13	Atypical *Lactobacillus*	[3]
	Strains assigned to cluster 2	
DSMZ 5973[T]	*Carnobacterium alterfunditum*	
NCFB 2855[†],	*C. divergens*	
C741, C836, C855,	*C. divergens*	[24]
MT22, MT47	*C. divergens*	[3]
DSMZ 5971[T]	*C. funditum*	
MT44[2,3,4,5]	*C. gallinarum*	[3]
NCFB 1230, NCFB 2764, NCFB 2935, 2, 213, 501[†]	*C. piscicola*	
CP7[†], CP14, CP25, CP27	*C. piscicola*	[21]
GN	*C. piscicola*	[16]
MT2, MT3, MT29, MT31, MT32, MT59	*C. piscicola*	[3]
PC4C12	*C. piscicola*	Toole unpublished
NCFB 2382[T]	*Lactobacillus maltaromicus*	
	Strains assigned to cluster 3	
NCFB 2855[†], NCFB 2857	*C. divergens*	
554	*C. divergens*	[22]
C881	*C. divergens*	[24]
MT23, MT46, MT49[†], MT50,		
MT51, MT53, MT56	*C. divergens*	[3]
MT44	*C. gallinarum*	[3]
CPISCDX, DX	*C. piscicola*	[16]
	Strains assigned to cluster 4	
508	*C. divergens*	[22]
NCFB 2762[T,2,3,4,5,] NCFB 2934	*C. piscicola*	
CP17,	*C. piscicola*	[21]
ACP7C12, PN519, PC5C6, PC4C7	*C. piscicola*	Toole, unpublished
	Strains assigned to cluster 5	
NCFB 2763[T,2,3,4,5]	*C. divergens*	
327, 185[†], 506, 544	*C. divergens*	[22]
ANP7C33	*C. divergens*	Toole, unpublished
C718[†]	*C. divergens*	[24]
MT4	*C. divergens*	
545[†]	*C. piscicola*	[22]
CP17	*C. piscicola*	
MT6[†]	Atypical *Lactobacillus* strain	[3]
	Strains assigned to cluster 6	
506	*C. divergens*	[22]
C758	*C. divergens*	[24]
543[†]	*C. piscicola*	[22]
	Strains in cluster 7	
NCFB 2307	*C. mobile*	
MT34	*C. mobile*	[3]
NCFB 596[T]	*Enterococcus durans*	
	Strains in cluster 8	
NCFB 2308, NCFB 2765[T,2,3,4,5]	*C. mobile*	
	Strains in cluster 9	
NCFB 3003[T]	*C. alterfunditum*	
NCFB 3002[T]	*C. funditum*	
41	*C. piscicola*	[22]
	Single member clusters	
694	*C. divergens*	[22]
C749	*C. divergens*	[24]

[T]Type strain: DSMZ; Deutsche Sammlung von Mikroorganismen und Zelkulturen GmbH, NCFB; National Collection of Food Bacteria, NCIMB; The National Collections of Industrial and Marine Bacteria Ltd. [†] Duplicated strains. [2,3,4,5] Strains used to study effect of incubation period – [2], growth for 48 hours; [3], growth for 72 hours; [4], growth for 96 hours; [5], growth for 120 hours.

Sample preparation for diffuse reflectance-absorbance FT-IR

Biomass from a CASO agar plate was homogenised in 1 ml of sterile physiological saline (0.9% NaCl) solution using a sterile disposable plastic loop. The final bacterial concentration was adjusted to 20–30 mg dry wt. ml^{-1}.

Diffuse reflectance-absorbance FT-IR

Five-microlitre aliquots of the bacterial and external reference samples were evenly applied, in triplicate, to wells in an aluminium plate. Prior to analysis, samples were oven-dried at 50 °C for 30 min. Fourier transform infrared spectroscopy was performed using a Bruker IFS 28 spectrometer (Bruker Spectrospin Ltd., Banner Lane, Coventry, UK.). Full operational procedures and analytical conditions are described by Timmins et al. [28].

Spectra were collected over the wavenumber range of 4000 cm^{-1} to 600 cm^{-1} under the control of an IBM-compatible personal computer using OPUS 2.1 software running under the IBM OS/2 Warp operating system. Spectra were acquired at a rate of 20 s^{-1} and at a spectral resolution of 4 cm^{-1}. The signal-to-noise ratio was improved by summing 256 spectra and averaging the total from each sample. Spectra (see Fig. 1 for examples) were displayed in terms of absorbance (as calculated from the reflectance-absorbance spectra using the dedicated Opus software) as a function of the wavenumber (cm^{-1}).

Data analysis

As detailed elsewhere [7, 28], the cluster analysis method of PC-DFA was employed to analyse the FT-IR data using Matlab version 5 (The MathWorks, Natick, MA, USA). Briefly, principal components analysis (PCA; [10]) was used to reduce the dimensionality of the FT-IR data from 882 to 30 PCs (which represented >99% of the total explained variance). Next discriminant function analysis (DFA; [17]) then discriminated between groups on the basis of these retained PCs and the *a priori* knowledge of which spectra were replicates, and thus this process does not bias the analysis in any way. Finally, the Euclidean distance between a priori group centres in DFA space (using the first 15 DFs) was used to construct a similarity measurement. These distance measures were processed using the unweighted pair group method with arithmetic averages (UPGMA) clustering algorithm to generate a dendrogram [17].

Results and Discussion

Typical FTIR spectra for two of the *Carnobacterium* type strains studied are shown in Fig. 1. The spectra have complex and broad contours with little qualitative difference, although on closer inspection quantitative difference between these two spectra, and the others collected (data not shown), can be observed. This demonstrates the requirement to use multivariate statistical methods for the analyses of these data.

In the ordination plot (Fig. 2), in which the first two discriminant functions accounted for 64.4% of the total variance, the majority of the strains were recovered in a single group with seven outlier strains. The outliers were *Vagacoccus salmoninarum* NCFB2777^T, the atypical *Lactobacillus* strains MT12 and MT13, *C. alterfunditum* NCFB 3003^T, *C. funditum* NCFB 3002^T, *C. divergens* 694 and *C. piscicola* 41. This distribution is reflected in the dendrogram (Fig. 3) where the outliers were recovered either into cluster 1, cluster 9 or as single member

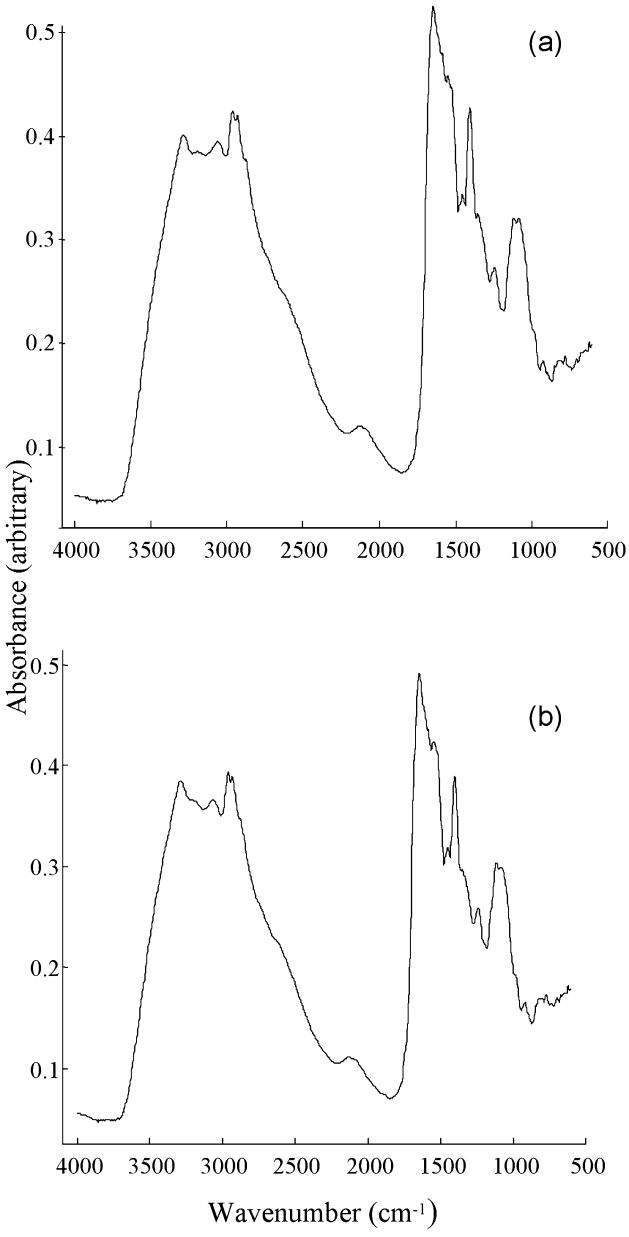

Fig. 1. FT-IR diffuse reflectance-absorbance spectra of (**a**) *C. divergens* NCFB 2763^T and (**b**) *C. piscicola* NCFB 2762^T.

clusters (Table 1). In the dendrogram the large group of strains was recovered into seven clusters with three or more members. In addition, duplicated strains were recovered in the same cluster highlighting the good reproducibility of FT-IR.

It is encouraging to note that some of the clusters correspond to groupings recognised in the numerical phenetic work of Lai and Manchester [13] and the PyMS work of Manchester et al. [18]. All of the strains in cluster 2, except *C. piscicola* 1230 and 213, and all the strains in cluster 4 were recovered into the cluster-group which was defined as the species *C. piscicola* Collins et al. 1987 in

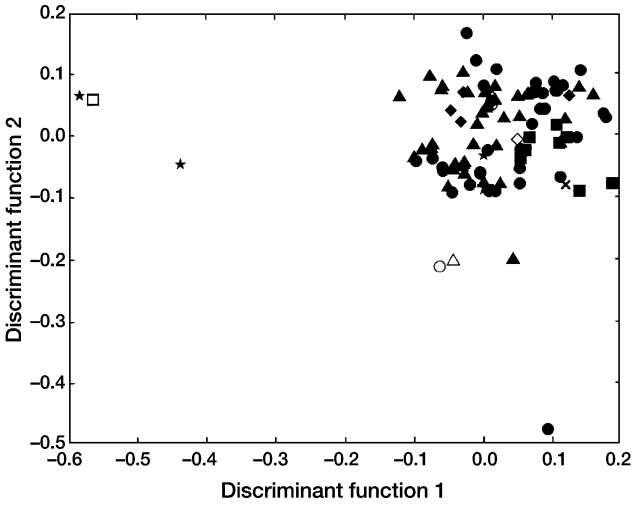

Fig. 2. DFA ordination plots based on FT-IR spectroscopy data showing the relationship between the *Carnobacterium* spp., atypical *Lactobacillus, Enterococcus durans, Lactobacillus maltaromicus* and *Vagacoccus salmoninarum*.
△, *Carnobacterium alterfunditum*; ●, *C. divergens*; ○, *C. funditum*; ◆, *C. gallinarum*; ■, *C. mobile*; ▲, *C. piscicola*; △, *Enterococcus durans*; ★; atypical *Lactobacillus*; ×, *L. maltaromicus*; □, *Vagacoccus salmoninarum*.

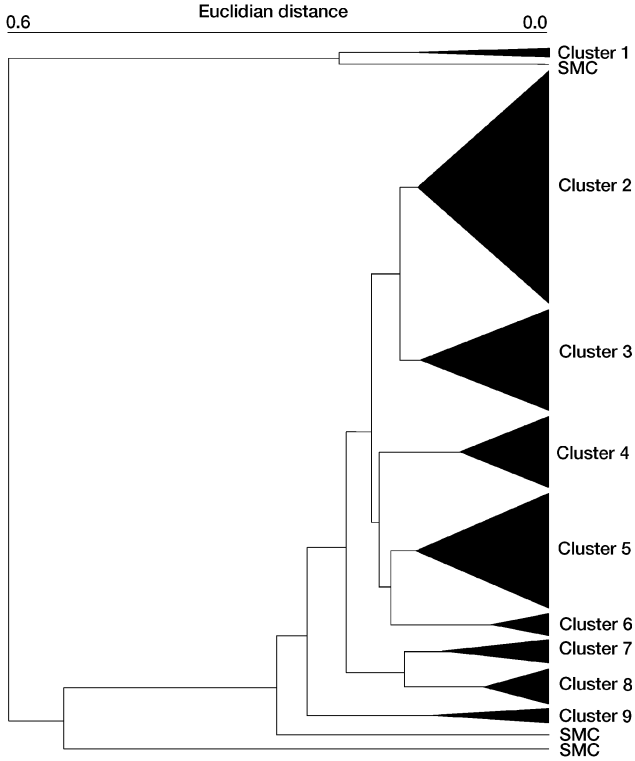

Fig. 3. Dendrogram representing the relationships between *Carnobacterium* spp., atypical *Lactobacillus, Enterococcus durans, Lactobacillus maltaromicus* and *Vagacoccus salmoninarum* based on Fourier Transform Infrared spectroscopy data analysed by MATLAB.

the numerical phenetic study of Lai and Manchester [13]. The recovery of these strains in two major groupings adds weight to the suggestion that there is recognisable subspecies variation within *C. piscicola*. Corresponding clusters for a more limited number of strains were recognisable in the PyMS study. It has previously been suggested that *Lactobacillus maltaromicus* and *C. piscicola* are objective synonyms. The recovery of *L. maltaromicus* NCFB 2382^T in this study mirrors its recovery in the numerical phenetic work and further supports this suggestion.

Cluster 3, 5 and 6 contained strains which were recovered into the cluster-group defined as the species *C. divergens* Collins et al. 1987 in the numerical taxonomic study of Lai and Manchester [13]. Cluster 5 contained strains (NCFB 2763^T, 327, 185, 506, 544, ANP7C33, MT4) which were assigned to a corresponding pyrogroup in the work of Mancehster et al. [18], as did some of the strains recovered into cluster 3 (*C. divergens* 554, MT49, MT51, MT53). There is little correlation between the findings from DNA:DNA relatedness data [3, 22].

It has been suggested that the atypical *Lactobacillus* strain MT6 is a bone fide member of *Carnobacterium divergens* [3, 13]. Its recovery in cluster 5, which contains the type strains of *C. divergens*, NCFB 2763^T, supports this suggestion.

The recovery of the *C. mobile* strains in two closely related clusters, cluster 7 and 8, separated from the majority of the *C. divergens*, *C. gallinarum* and *C. piscicola* strains is very encouraging. Previous work based upon 16S rRNA sequencing techniques [30] has shown that *C. mobile* is not as closely related to these species as they are to each other.

Unfortunately there are few representative strains of *C. gallinarum*, *C. funditum* and *C. alterfunditum* available and it is difficult to draw conclusions regarding the taxonomic status of these species. The close relationship between *C. gallinarum* and *C. piscicola* strains as shown here has been observed in other studies, *e.g.* [1, 5, 13, 30].

It is interesting to note the relationship between *C. alterfunditum* NCFB 3003, *C. funditum* NCFB 3002 and the *C. mobile* strains. Franzmann et al. [6] and Jöburn et al. [9] have demonstrated that these species form a distinct phylogenetic group from *C. divergens, C. gallinarum* and *C. piscicola*. The recovery of *C. alterfunditum* DSMZ 5973, and *C. funditum* DSMZ 5971, with *C. piscicola* strains corresponds to their observed relationship of these particular strains in numerical taxonomy work. This suggests that the identity of these strains should be re-examined.

Vagacoccus salmoninarum and the atypical *Lactobacillus* strains MT12 and MT13 were recovered separately to the main body of the *Carnobacterium* strains and this indicates that this technique may prove useful in distinguishing *Carnobacterium* isolates from others which inhabit the same types of environment. The recovery of *Enterococcus durans* in cluster 7 is unexpected, although previous work [13, 30] has shown that the genera *Carnobacterium* and *Enterococcus* are closely related.

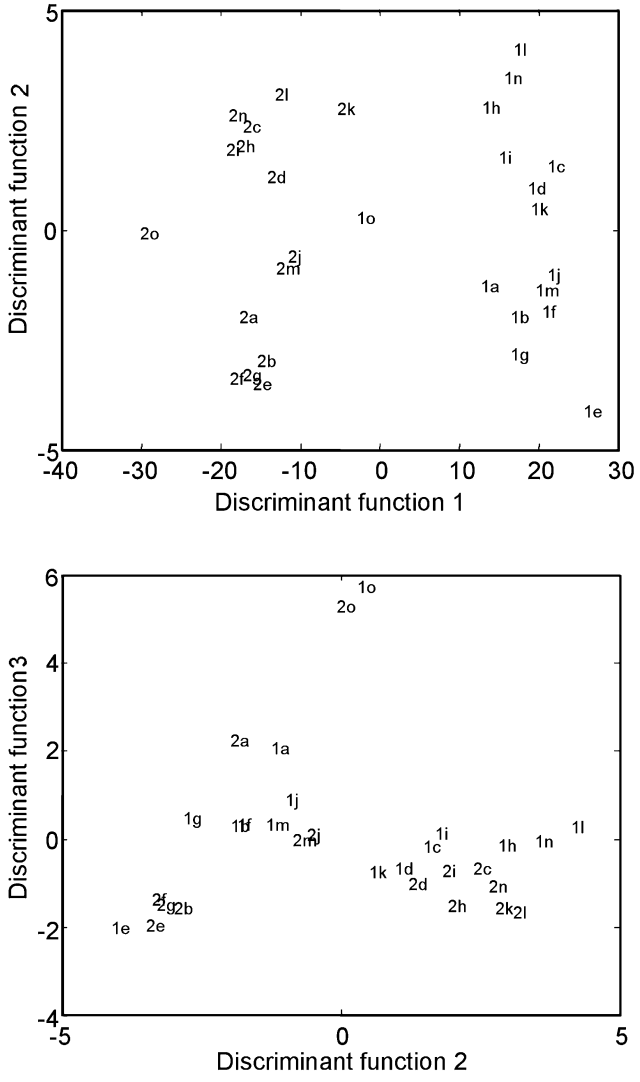

Fig. 4. DFA ordination plots based on FT-IR spectroscopy data analysed by MATLAB showing the relationship between 15 *Carnobacterium* spp cultivated and analysed on two separate occasions, 1 and 2, over a period of six months. a, *C. mobile* NCFB 2307; b, *C. divergens* 185; c, *C. piscicola* DX; d, *C. divergens* NFCB 2855; e, *C. divergens* C718; f, *C. divergens* 185; g, *C. piscicola* 545; h, *C. piscicola* MT29; i, *C. piscicola* MT2; j, *C. piscicola* CP7; k, *C. piscicola* PC4C7; l, *C. piscicola* NCFB 2764; m, *C. divergens* C836; n, *C. piscicola* NCFB 1230; o, Atypical *Lactobacillus* MT13.

Culture age was not observed to affect the recovery of strains as those grown for different lengths of times were always recovered into the same cluster. This implies that there is very little phenotypic change in these strains over 2–5 days growth at 25 °C.

Figure 4 presents the ordination plots obtained when 15 randomly selected *Carnobacterium* strains were studied over a period of six months. When the first and second discriminant function were used (Fig. 4a) two distinct groups of strains were observed which correspond to two strain analyses separated by six months. Some of the variation reflected in this plot may have originated from instrument drift, *e.g.* variations in reflectance-absorbance signal detection introduced by batch difference between the aluminium sample-carrier. The effect of this variation can be minimised by using the second and third discriminant functions. Figure 4b presents such an ordination plot in which corresponding strains from the two analyses were, in most cases, recovered together. It is, therefore, likely that the carnobacterial FT-IR spectra are stable but further work is required to ensure the robustness of this technique before it is applied to other areas of carnobacterial systematics, *e.g.* identification. It has been suggested by Lefier et al. [15] that the reproducibility of spectral data is equally dependant upon the kinds of strains being examined and the bacterial sampling parameters; it is therefore encouraging that for carnobacteria culture age differences appear to be negligible.

The application of different taxonomic techniques to a single group of organisms inevitably invites comparisons between the resultant classifications. However, one problem that was encountered for some of the *Carnobacterium* species, *e.g. C. gallinarum, C. funditum* and *C. alterfunditum,* was the lack of available examples of these species. Until more strains are available it will always be difficult to draw reliable conclusions on the taxonomic status of such taxospecies in any study. However, the observed congruencies between the FT-IR, numerical taxonomic and 16S rRNA data for the genus *Carnobacterium,* were very encouraging; a finding that has been observed by workers in bacteria and yeast [11, 29]. Consistent groupings of *C. piscicola* and *C. divergens* strains can be recognised, and this indicates good taxon homogeneity and separation of these taxa. Further work will include the identification of characteristic vibrational peaks for these groupings, a technique which has been used successfully for improving the reproducibility of species-level bacterial identification and discrimination [23, 26]. These together with the use of artificial neural networks (ANNs) could provide a means to identify objectively *Carnobacterium* isolates and help to further elucidate the taxonomic relationships of members of the genus *Carnobacterium*.

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References

1. Brooks, J. L., Moore, A. S., Patchett, R. A., Collins, M. D., Kroll, R. G.: Use of the polymerase chain reaction and oligonucleotide probes for the rapid detection and identification of *Carnobacterium* species from meat. J. Appl. Bact. 72, 294–301 (1992).
2. Champomier, M.-C., Montel, M.-C., Talon, R.: Nucleic acid relatedness studies on the genus *Carnobacterium* and related taxa. J. Gen. Microbiol. 135, 1391–1394 (1989).

3. Collins, M. D., Farrow, J. A. E., Phillips, B. A., Feresu, S., Jones, D.: Classification of *Lactobacillus divergens, Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. Int. J. Syst. Bacteriol. 37, 310–316 (1987).
4. Colthup, N. B., Daly, L. H., Wiberley, S. E.: Introduction to Infrared and Raman Spectroscopy. 3rd ed., Academic Press 1990.
5. Feresu, S. B., Jones, D.: Taxonomic studies on *Brochothrix, Erysipelothrix, Listeria* and atypical lactobacilli. J. Gen. Microbiol. 134, 1165–1183 (1988).
6. Franzmann, P. D., Höpfl, P., Weiss, N., Tindall, B. J.: Psychrotrophic, lactic acid-producing bacteria from anoxic waters in Ace Lake, Antarctica; *Carnobacterium funditum* sp. nov. and *Carnobacterium alterfunditum* sp. nov. Arch. Microbiol. 156, 255–262 (1991).
7. Goodacre, R., Timmins, É. M., Burton, R., Kaderbhai, N., Woodward, A.M., Kell, D. B., Rooney, P.J.: Rapid identification of urinary tract infection bacteria using hyperspectral, whole organisms fingerprinting and artificial neural networks. Microbiol. 144, 1157 – 1170 (1998).
8. Griffiths, P. R., de Haseth, J. A.: Fourier Transform Infrared Spectrometry. John Wiley and Sons (1986).
9. Jöborn, A., Dorsch, M., Olsson, J. C., Westerdahl, A., Kjelleberg, S.: *Carnobacterium inhibens* sp. nov., isolated from the intestine of Atlantic Salmon *(Salmo salar)*. Int. J. Syst. Bacteriol 49, 1891–1898 (1999).
10. Jolliffe, I. T.: Principal Component Analysis. Springer-Verlag (1986).
11. Kirschner, C., Maquelin, K., Pina, P., Ngo Thi, N. A., Choo-Smith, L.-P., Sockalingum, G. D., Sandt, C., Ami, D., Orsini, F., Doglia, S. M., Allouch, P., Mainfait, M., Puppels, G. J., Naumann, D. Classification and identification of enterococci: a comparative phenotypic, genotypic, and vibrational spectroscopic study. J. Clin. Microbiol. 39, 1763–1770 (2001).
12. Kümmerle, M., Scherer, S., Seiler, H.: Rapid and reliable identification of food-borne yeasts by Fourier-transform infrared spectroscopy. Appl. Environ. Microbiol. 64, 2207–2214 (1998).
13. Lai, S., Manchester, L. N.: A numerical phenotypic classification of the genus *Carnobacterium*. Antonie Van Leeuwenhoek 78, 73–85 (2000).
14. Lai, S., Shojaei, H., Manchester, L. N.: The differentiation of *Carnobacterium divergens* using the random amplification of polymorphic DNA polymerase chain reaction technique. Lett. Appl. Microbiol. 30, 448–452 (2000).
15. Lefier, D., Hirst, D., Holt, C., Williams, A. G.: Effect of sampling procedure and strain variation in *Listeria monocytogenes* on the discrimination of species in the genus *Listeria* by Fourier transform infrared spectroscopy and canonical variates analysis. FEMS Microbiol. Lett. 147, 45–50 (1997).
16. Lewus C.B., Kaiser A., Montville T.J.: Inhibition of foodborne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57, 1683–1688 (1991).
17. Manly, B.F.J. Multivariate Statistical Methods: A Primer. pp. 215 Chapman & Hall, London (1994).
18. Manchester, L. N., Toole, A., Goodacre, R.: Characterization of *Carnobacterium* species by pyrolysis mass spectrometry. J. Appl. Bact. 78, 88–96 (1995).
19. Mariey, L., Signolle, J.P., Amiel, C., Travert, J. Discrimination, classification, identification of microorganisms using FT-IR spectroscopy and chemometrics. Vibr. Spectrosc. 26, 151–159 (2001).
20. Maquelin, K., Kirschner, C., Choo-Smith, L.-P., Van Den Braak, N., Endtz, H.P., Naumann, D., Puppels, G.J.: Identification of medically relevant microorganisms by vibrational spectroscopy. J. Microbiol. Meth. 51, 255–271 (2002).
21. Milliére J.B., Michel M., Mathieu F., Lefebvre G.: Presence of *Carnobacterium* spp. in French surface mould-ripened soft-cheese. J. Appl. Bact. 76, 264–269 (1994).
22. Montel, M.-C., Talon, R., Fournaud, J., Champomier, M.-C.: A simplified key for identifying homofermentative *Lactobacillus* and *Carnobacterium* spp. from meat. J. Appl. Bact. 70, 469–472 (1991).
23. Oberreuter, H., Seiler, H., Scherer, S.: Identification of coryneform bacteria and related taxa by Fourier-transform infrared (FT-IR) spectroscopy. Int. J. Syst. Bacteriol. 52, 91–100 (2002).
24. Schillinger, U., and Lücke, F.-K.: Identification of lactobacilli from meat and meat products. Food Microbiol. 4, 199–208 (1987).
25. Stuart, N.: Biological Applications of Infrared Spectroscopy. John Wiley & Sons. Chichester (1997).
26. Taylor, J., Goodacre, R., Wade, W. G., Rowland, J. J. Kell, D. B.: The deconvolution of pyrolysis mass spectra using genetic programming: application to the identification of some *Eubacterium* species. FEMS Microbiol. Lett. 160, 237–246 (1998).
27. Thornley, M.: Observations on the microflora of minced chicken meat irradiated with 4 MeV cathode rays. J. Appl. Bact. 20, 286–298 (1957).
28. Timmins, É., M., Howell, S. A., Alsberg, B. K., Noble, W. C., Goodacre, R.: Rapid differentiation of closely related *Candida* species and strains by pyrolysis-mass spectrometry and Fourier transform-infrared spectroscopy. J. Clin. Microbiol. 36, 367–374 (1998).
29. Tintelnot, K., Haase, G., Seibold, M., Bergmann, F., Staemmler, M., Franz, T. and Naumann, D.: Evaluation of phenotypic markers for selection and identification of *Candida dubliniensis*. J. Clin. Microbiol. 38, 1599–1608 (2000).
30. Wallbanks, S., Martinez-Murcia, A. J., Fryer, J. L., Phillips, B. A., Collins, M. D.: 16S rRNA sequence determination for members of the genus *Carnobacterium* and related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. Int. J. Syst. Bacteriol 40, 224–230 (1990)

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