



Identification of beer spoilage lactic acid bacteria

A comparison of FAMES & RAPD-PCR

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INTRODUCTION

- Lactic acid bacteria are the most frequently isolated beer spoilage bacteria.
 - Spoilage due to lactic acid, diacetyl, and haze. Can be resistant to hop iso- α -acids.
- Traditional methods of identifying lactic acid bacteria involve testing multiple (often >50) phenotypic characteristics.
 - Results may not be entirely reliable.
- Here we have evaluated two rapid methods to identify beer spoilage lactic acid bacteria.
 - Fatty Acid Methyl Esters (FAMES) profiles are based on the lipid composition of the cells.
 - Randomly Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) which differentiates based on DNA composition.

MATERIALS & METHODS

- FAMES (Fatty Acid Methyl Esters): Grow cultures → Prepare & Extract FAMES → Gas Chromatography (GC) → Sigma 6 coefficients**
 - Cultures were grown to stationary phase under identical conditions (microaerophilic, 25 °C) in MRS without Tween 80.
 - Whole cells were digested, and fatty acids were saponified and methylated. FAMES were extracted and base washed.
 - FAMES were injected into a Gas Chromatograph (GC) fitted with a BP1 column and a Flame Ionisation Detector (FID).
 - Sigma 6 coefficients were calculated as a measure of distance between strains (based on six largest peaks). A value > 3 suggests the strains belong to different species.
- RAPD-PCR (Randomly Amplified Polymorphic DNA – Polymerase Chain Reaction): Grow cultures → Extract DNA → RAPD-PCR → Analyse bands**
 - Cellular DNA was extracted and amplified by PCR using two primer sets; M13 and D11344.
 - Amplification products were separated by gel electrophoresis, stained with ethidium bromide, and scanned.
 - Cluster analysis of band profiles was performed using GelCompar. A dendrogram was produced, and species were allocated with the assistance of the in-house library of RAPD-PCR profiles maintained by the Biotechnology Group, Institute of Food Quality and Technologies, Thiene, Italy.
 - Type strains were included in the RAPD-PCR analysis, and representative isolates were sequenced (16S rRNA). Over three independent RAPD-PCR analyses, the type strain *Lb. brevis* clustered with 88% similarity.

RESULTS & DISCUSSION

IDENTIFICATION BY FAME PROFILES

- Example chromatograms are shown as Fig. 1.
- Top six peaks used to calculate Sigma 6 coefficients as a measure of distance. Two strains are tentatively allocated to a different species when the Sigma 6 coefficient is greater than three.
- Most species were effectively differentiated (Sigma 6 coefficient > 3).
- In some instances variation within species was more than variation between species (for *Lb. brevis* & *Lb. paracasei*)
- This technique is rapid and uses equipment found in many brewery laboratories (GC), however growth conditions must be standardised and an in-house library is required.
- Technique can be improved by using Pyrolysis mass spectrometry (PyMS)

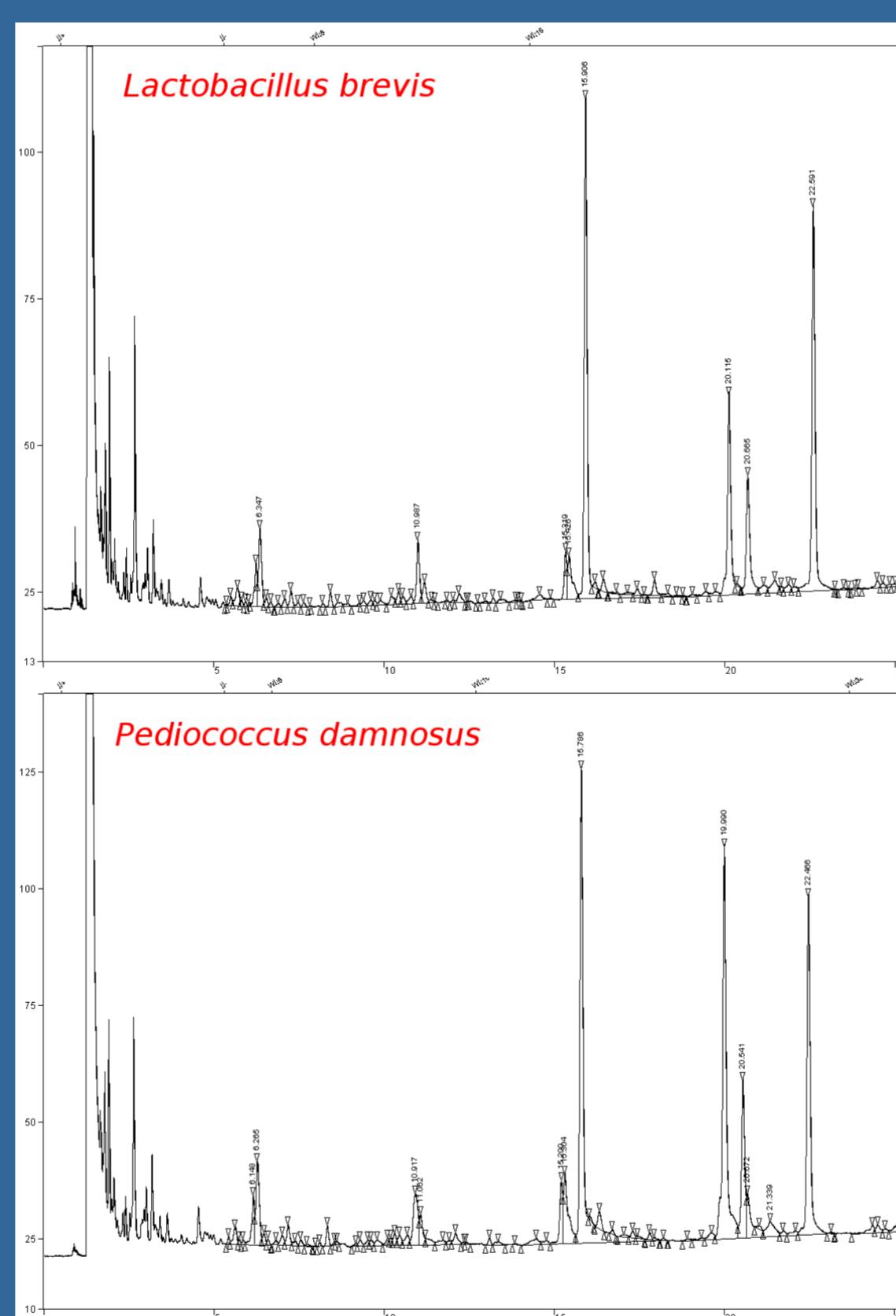


Fig. 1 – Fatty Acid Methyl Ester (FAME) Gas Chromatography (GC) profiles for a representative strain of each beer spoilage lactic acid bacteria. Note the ratios of the six largest peaks.

IDENTIFICATION BY RAPD-PCR

- Species correctly assigned (Fig. 2)
 - confirmed by 16S rRNA sequencing
- Strains could be clustered using the two primer sets
- Rapid, easy to analyse multiple samples simultaneously
- Preferred method over FAMES

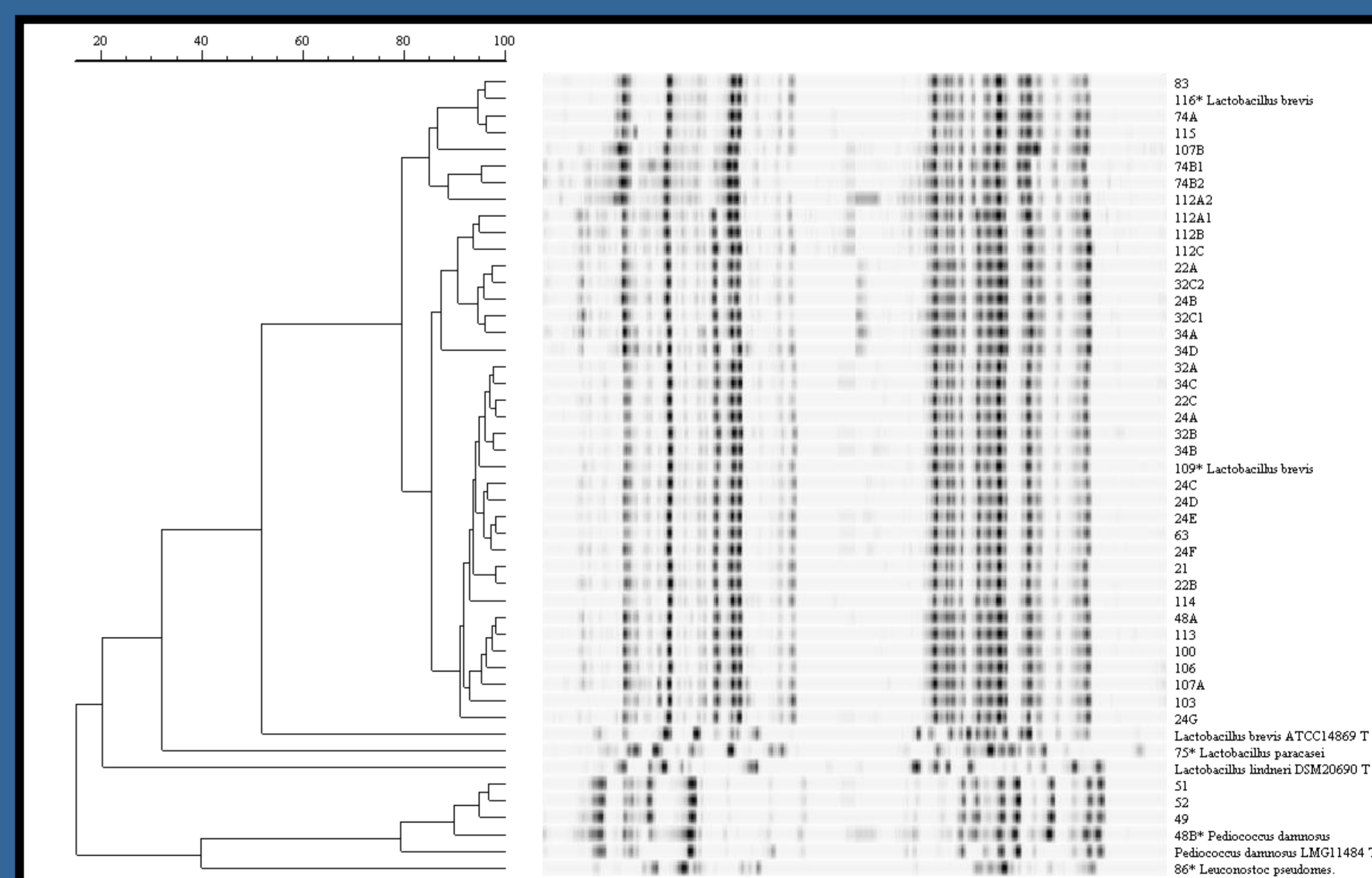


Fig. 2 – Cluster analysis of Randomly Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) patterns of beer spoilage lactic acid bacteria produced using with two primer sets. 'T' indicates type strain, and '*' indicates that the strain was sequenced (16S rRNA).

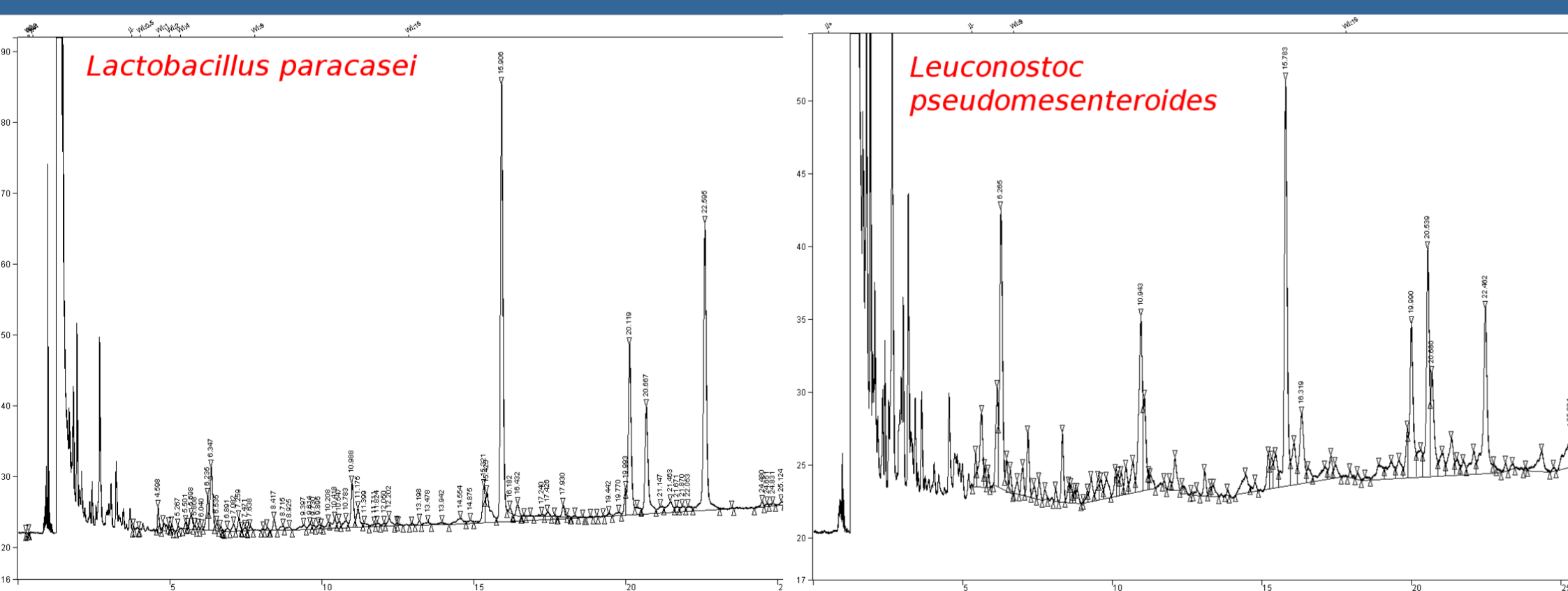


Table 1 – Sigma 6 coefficients calculated from the largest six peaks from the Fatty Acid Methyl Ester (FAME) Gas Chromatography (GC) profiles. A value greater than three suggests the compared strains belong to different species. *Listeria monocytogenes* (another G+ve rod) was included for comparison against the LAB. The maximum Sigma 6 value is 400.

	<i>Lb. brevis</i> 1	<i>Lb. brevis</i> 2	<i>Lb. brevis</i> 3	<i>P. damnosus</i>	<i>Lb. paracasei</i>	<i>Leuc. pseudo</i>	<i>List. mono.</i>
<i>Lb. brevis</i> 1							
<i>Lb. brevis</i> 2	8.0						
<i>Lb. brevis</i> 3	5.9	4.3					
<i>P. damnosus</i>	12.3	11.2	8.5				
<i>Lb. paracasei</i>	0.6	5.6	6.0	10.7			
<i>Leuc. pseudo</i>	46.2	39.7	52.7	59.7	45.7		
<i>List. mono.</i>	185.1	156.0	181.2	206.2	181.3	93.3	

CONCLUSIONS

- FAMES were useful for the identification of most of the isolates tested, although some species could not be reliably differentiated.
- RAPD-PCR was the most reliable method for identification, and this method also allowed for strain clustering.

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