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Differentiation of Brewing Yeast Strains by Pyrolysis Mass Spectrometry and Fourier Transform Infrared Spectroscopy

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Two rapid spectroscopic approaches for whole-organism fingerprinting—pyrolysis mass spectrometry (PyMS) and Fourier transform infrared spectroscopy (FT-IR)—were used to analyse 22 production brewery *Saccharomyces cerevisiae* strains. Multivariate discriminant analysis of the spectral data was then performed to observe relationships between the 22 isolates. Upon visual inspection of the cluster analyses, similar differentiation of the strains was observed for both approaches. Moreover, these phenetic classifications were found to be very similar to those previously obtained using genotypic studies of the same brewing yeasts. Both spectroscopic techniques are rapid (typically 2 min for PyMS and 10 s for FT-IR) and were shown to be capable of the successful discrimination of both ale and lager yeasts. We believe that these whole-organism fingerprinting methods could find application in brewery quality control laboratories. © 1998 John Wiley & Sons, Ltd.

KEY WORDS — pyrolysis mass spectrometry; Fourier transform infrared spectroscopy; chemometrics, quality assurance

INTRODUCTION

Within the brewing industry, pure yeast cultures are of critical importance for product quality and consistency. Despite the best efforts however, yeast handling and management systems are often the cause of cross-contamination of pitching yeast by other production yeast strains. Also, work has shown that brewing yeasts may undergo genetic changes which can cause a switch in yeast flocculence (Oakley-Gutowski *et al*., 1992; Quain, 1995), or atypical fermentation performance and beer flavour (Morrison and Sugget, 1983; Quain, 1995). Strain quality assurance then, is essential in ensuring a consistently good quality product.

Using traditional strain QA procedures, identification and differentiation of brewing yeasts is often very difficult. These methods, which can be lengthy and non-reproducible, are based on properties such as flocculation, colony morphology, sugar fermentation and resistance or sensitivity to some antibiotics (Quain, 1986). The ideal method to replace these labour-intensive processes would have minimum sample preparation, would analyse samples directly (i.e. would not require reagents), would be rapid, automated, and (at least relatively) inexpensive. With recent developments in analytical instrumentation, these requirements are being fulfilled by physico-chemical spectroscopic methods, often referred to as 'whole-organism fingerprinting' (Magee, 1993). The most common such methods are pyrolysis mass spectrometry (PyMS; Goodacre and Kell, 1996), Fourier transform-infrared spectroscopy (FT-IR; Naumann *et al*., 1991) and UV resonance Raman spectroscopy (Nelson *et al*., 1992).

PyMS and FT-IR are physico-chemical methods which measure predominantly the bond strengths of molecules and the vibrations of bonds within

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functional groups respectively (Colthup *et al*., 1990; Griffiths and de Haseth, 1986; Meuzelaar *et al*., 1982). Therefore, they are in essence, techniques which give quantitative information about the total biochemical composition of a sample. For taxonomic purposes they measure the phenotype of an organism which is a 'snap shot' (albeit a limited one) of its expressed genotype. This is unlike genotypic methods of DNA fingerprinting which can distinguish yeast strains on the basis of DNA restriction fragment length polymorphisms (RFLPs) or chromosome size and ploidy.

The aims of this study were to differentiate 22 brewery yeast strains by the phenotypic approaches of PyMS and FT-IR, and to compare these results with those from previous genotypic investigations of the same isolates by Schofield *et al*. (1995) and Wightman *et al*. (1996). An additional aim was to determine if growing the yeasts on different media types would cause a phenotypic change which would lead to an appreciable change in their PyMS spectra.

MATERIALS AND METHODS

Strains and cultivation

Twenty-two Bass Brewers (BB) *Saccharomyces cerevisiae* strains, comprising 15 ale strains and seven lager strains (see Table 1 for BB strain numbers) were studied. The ale strains BB12, BB13 and BB14 had previously been separately isolated, on the basis of flocculation tests, from a mixed yeast strain (Hough, 1957). Strains BB21, BB22 and BB23 were also pure strains isolated from a mixed strain on the basis of flocculation tests while ale strain BB24 had been selected as a strain with improved fermentation performance after production trials in another brewery with BB3.

All strains were aerobically grown in both liquid and solid media. Liquid culturing involved growing the strains overnight at 30°C in 10-ml aliquots of static Yeast Peptone Dextrose (YPD) medium followed by the addition of 200 µl of culture to 500 ml of pre-warmed YPD and growing at 30°C (with agitation) for 72 h. For solid culturing, the strains were grown on Sabouraud–1% dextrose–1% maltose agar (SDMA) medium at 22°C for 72 h. The biomass was then carefully collected using sterile plastic loops and suspended in 1-ml aliquots of sterile physiological saline (0·9% NaCl).

Table 1. Bass Brewers (BB) strain numbers for the 22 *Saccharomyces cerevisiae* strains together with their source.

Strain number	Source
BB1	Ale
BB2	Ale
BB3	Ale
BB6	Lager
BB9	Lager
BB10	Lager
BB11	Lager
BB12	Ale
BB13	Ale
BB14	Ale
BB15	Ale
BB16	Ale
BB17	Lager
BB18	Ale
BB19	Ale
BB20	Ale
BB21	Ale
BB22	Ale
BB23	Ale
BB24	Ale
BB26	Lager
BB27	Lager

Pyrolysis mass spectrometry

Five microlitres of the above yeast samples were evenly applied to clean iron-nickel foils which had been partially inserted into clean pyrolysis tubes. Samples were run in triplicate. Prior to pyrolysis the samples were oven-dried at 50°C for 30 min and the foils were then pushed into the tubes using a stainless steel depth gauge so as to lie 10 mm from the mouth of the tube. Viton O-rings were next placed approximately 1 mm from the mouth of each tube.

Pyrolysis mass spectrometry was then performed on a Horizon Instrument PyMS-200X (Horizon Instruments Ltd, Heathfield, UK). For full operational procedures see Goodacre *et al*. (1993, 1994a, b) and Timmins and Goodacre (1997). Conditions used for each experiment involved heating the sample to 100°C for 5 s followed by Curie-point pyrolysis at 530°C for 3 s with a temperature rise time of 0·5 s.

PyMS data may be displayed as quantitative pyrolysis mass spectra (e.g., as in Figure 1). The abscissa represents the 150 *m/z* ratios, while the ordinate contains information on ion count for

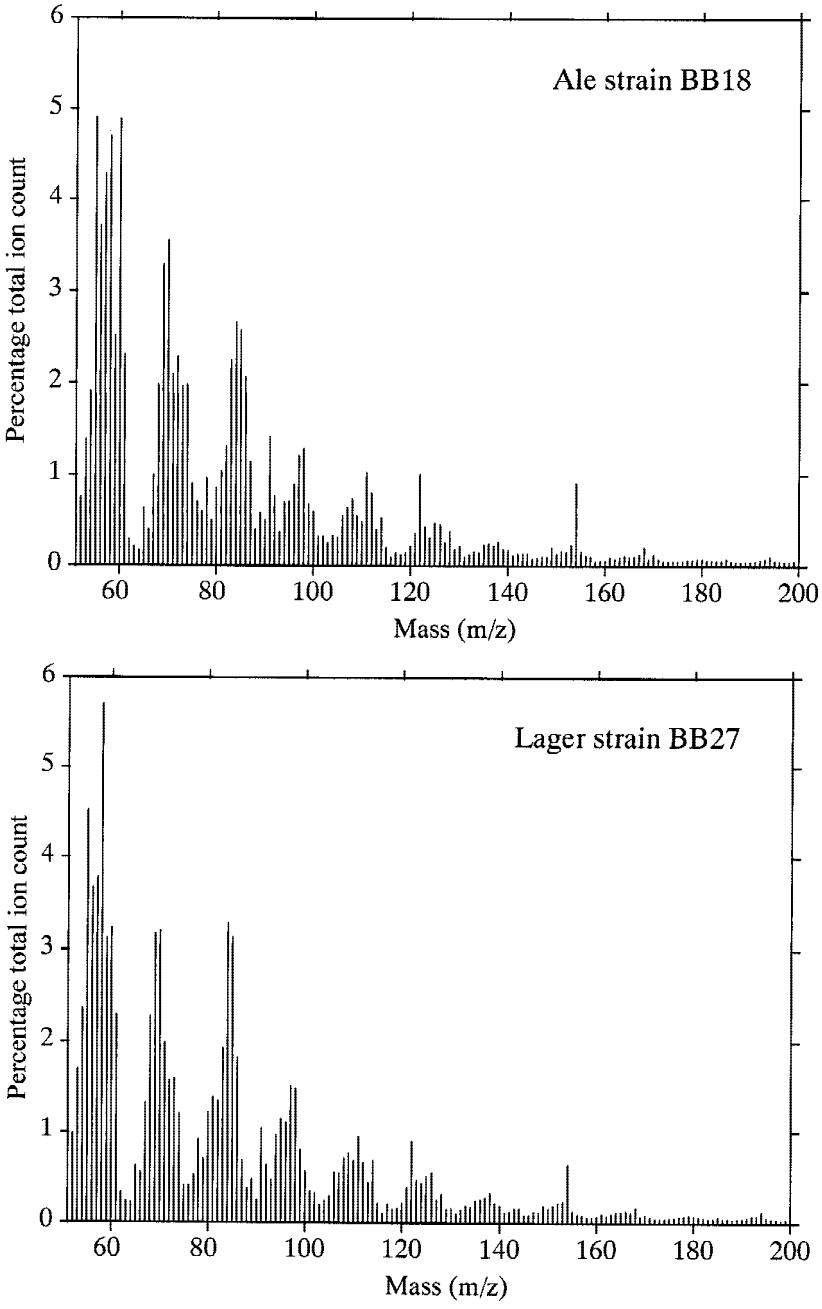

Figure 1. Normalized pyrolysis mass spectra of *S. cerevisiae* ale strain BB18 and *S. cerevisiae* lager strain BB27.

any particular *m/z* value ranging from 51 to 200. To remove the influence of sample size *per se* data were normalized as a percentage of the total ion count.

Diffuse reflectance-absorbance Fourier transform infrared spectroscopy

Ten microlitres of the above yeast samples were evenly applied onto a sand-blasted aluminium

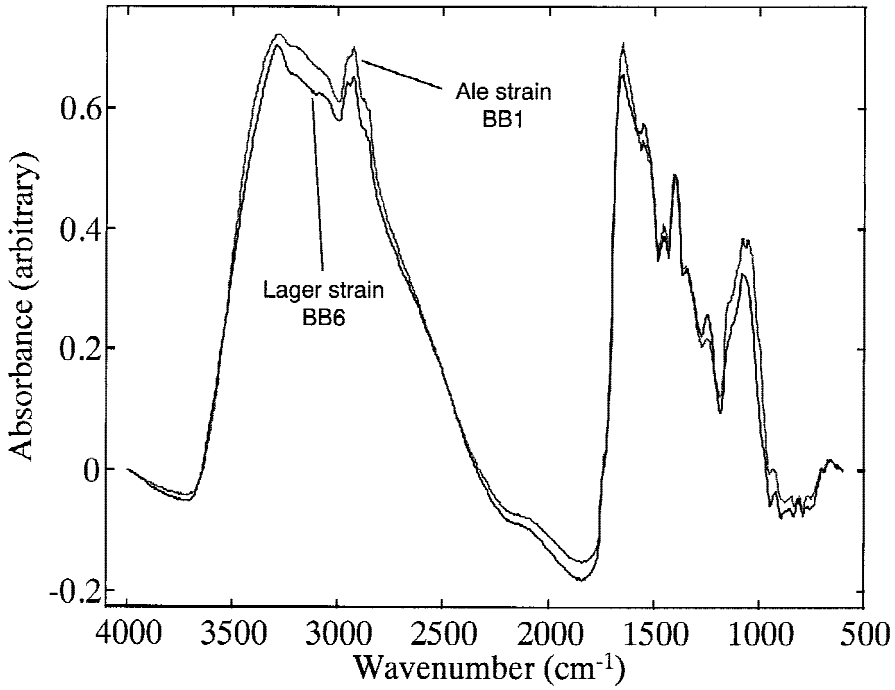

Figure 2. FT-IR diffuse reflectance-absorbance spectra of *S. cerevisiae* ale strain BB1 and *S. cerevisiae* lager strain BB6.

plate. Prior to analysis the samples were oven-dried at 50°C for 30 min. Samples were run in triplicate. The FT-IR instrument used was the Bruker IFS28 FT-IR spectrometer (Bruker Spectrospin Ltd, Banner Lane, Coventry, UK) equipped with a mercury-cadmium-telluride detector cooled with liquid N_2. The aluminium plate was then loaded onto the motorized stage of a reflectance TLC accessory (Bouffard *et al.*, 1994; Goodacre *et al.*, 1996; Timmins *et al.*, 1997; Winson *et al.*, 1997).

The IBM-compatible PC used to control the IFS28 was also programmed (using OPUS version 2.1 software running under IBM O/S2 Warp provided by the manufacturers) to collect spectra over the wavenumber range 4000 cm^{-1} to 600 cm^{-1}. Spectra were acquired at a rate of 20 s^{-1}. The spectral resolution used was 4 cm^{-1}. To improve the signal-to-noise ratio, 256 spectra were co-added and averaged. Each sample was thus represented by a spectrum containing 882 points and spectra were displayed in terms of absorbance as calculated from the reflectance-absorbance spectra using the Opus software. Typical FT-IR spectra are shown in Figure 2.

ASCII data were exported from the Opus software used to control the FT-IR instrument and imported into Matlab version 4.2c. l (The MathWorks, Inc., 24 Prime Par Way, Natick, MA, USA), which runs under Microsoft Windows NT on an IBM-compatible PC. To minimize problems arising from baseline shifts the following procedure was implemented: (i) the spectra were first normalized so that the smallest absorbance was set to 0 and the highest to +1 for each spectrum, (ii) next these normalized spectra were detrended by subtracting a linearly increasing baseline from 4000 cm^{-1} to 600 cm^{-1}, (iii) finally the smoothed first derivative of these normalized and detrended spectra using the Savitzky-Golay algorithm (Savitzky and Golay, 1964) using 5-point smoothing were calculated.

Cluster analysis

The initial stage involved the reduction of the dimensionality of the PyMS and FT-IR data by principal components analysis (PCA; Causton, 1987; Jolliffe, 1986). PCA is a well-known technique for reducing the dimensionality of

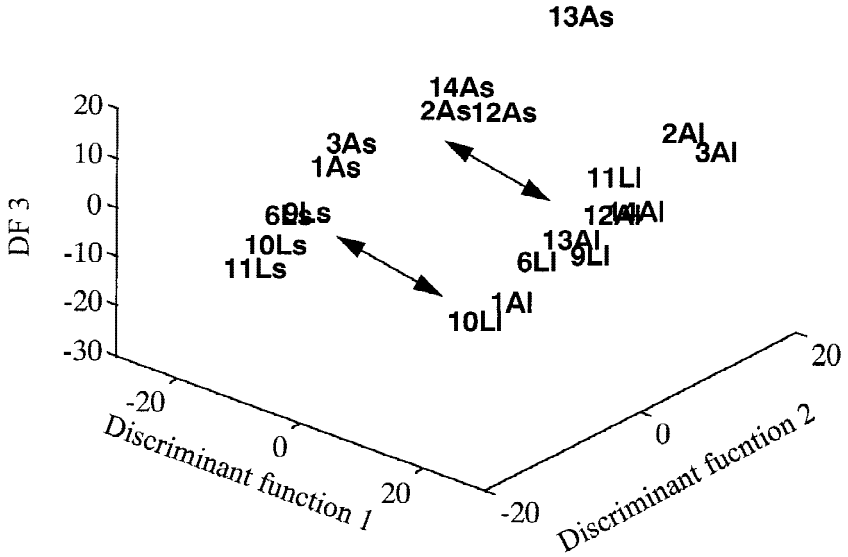

Figure 3. Pseudo-3-D discriminant function (DF) analysis plot based on PyMS data showing the comparison between 10 yeast strains grown on nutrient agar (*s*) and in liquid media (*l*). The first three ordinates are displayed and they account for 37·4, 31·1 and 12·8% (81·3% total) of the total variation respectively. The numbers refer to the Bass Brewers strain number and whether ale (A) or lager (L). The double-headed arrow indicates that the first DF contains information of whether samples were grown on solid or in liquid media.

multivariate data whilst preserving most of the variance, and Matlab was employed to perform PCA according to the NIPALS algorithm (Wold, 1966). Discriminant function analysis (DFA) then discriminated between groups on the basis of the retained PCs and the *a priori* knowledge of which spectra were replicates (MacFie *et al*., 1978; Windig *et al*., 1983), and thus this process does not bias the analysis in any way. DFA was programmed according to Manly's principles (Manly, 1994). Finally, the Euclidean distance between *a priori* group centres in DFA space was used to construct a similarity measure, with the Gower similarity coefficient S_G (Gower, 1966), and these distance measures were then processed by an agglomerative clustering algorithm to construct a dendrogram (Manly, 1994).

RESULTS AND DISCUSSION

Typical PyMS and FT-IR spectra for ale and lager *S. cerevisiae* strains are shown in Figures 1 and 2 respectively. The two PyMS spectra look very similar to each other as do the FT-IR spectra, although, on closer inspection, small quantitative differences may be observed. Such spectra readily illustrate the need to employ multivariate statistical techniques in the analysis of both PyMS and FT-IR data.

To observe any phenotypic differences caused by cultivating on different media, 10 selected strains (BB1, BB2, BB3, BB6, BB9, BB11, BB12, BB13 and BB14) were grown in liquid and solid media, as detailed above, and subjected to PyMS. The resulting DFA plot after cluster analyses is shown in Figure 3. It can be seen that cultivating on different media does indeed cause a change in their mass spectra and the double-headed arrow in this figure indicates that the first discriminant function (DF1) contains information on the cultivation method used. This is significant because DF1 is extracted by the DFA algorithm to contain the majority of the variance (and hence difference) between the samples (Manly, 1994). However, DF1 will also contain, although to a lesser extent, information regarding machine drift since these data were collected 70 days apart.

In addition, it can be seen (Figure 3) that the groupings seen in the two clusters do not mirror one another sufficiently well; indeed, in further studies which analysed these clusters separately (data not shown) dendrograms showed that these 10 yeasts were grouped very differently. These

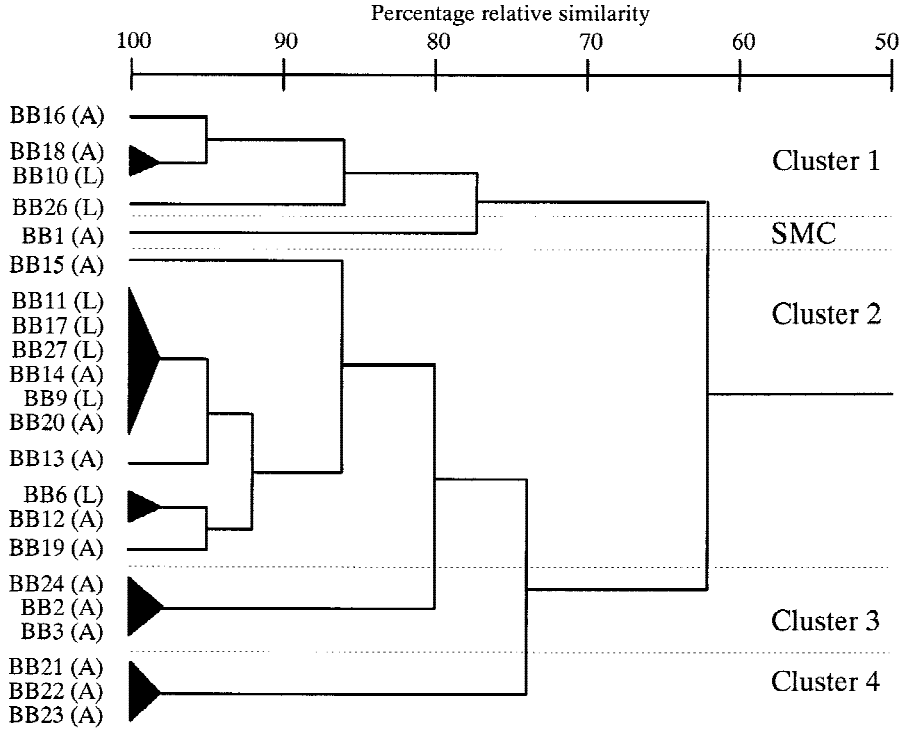

Figure 4. Dendrogram based on PyMS data showing the relationship between the 22 strains of *S. cerevisiae*. The numbers refer to the Bass Brewers strain number and whether ale (A) or lager (L)

results would suggest that the yeasts' phenotypes were different depending on the growth media and temperature used, and this is hardly surprising since this is a well-known problem with using 'whole organism fingerprinting' (Magee, 1993) which measure the *biochemistry* of the sample under investigation. Moreover, that the clustering observed in the dendrograms from yeasts grown in liquid culture showed more congruence with those from DNA studies (data not shown) than dendrograms based on yeasts grown on solid media, suggests that the most reliable phenotype is displayed when these organisms were grown in liquid culture. Indeed, this is hardly surprising when one considers that these brewing yeasts have been specifically selected for their performance in batch fermentations.

The next stage was therefore to analyse all 22 strains grown in liquid media by PyMS and FT-IR. The dendrogram from the PyMS data (Figure 4) shows four main clusters and a single member cluster (SMC) comprising the ale strain BB1 which clustered closest to cluster 1. Clusters 1 and 2 comprise a heterogeneous mixture of ale and lager strains. The other two clusters contain groups of closely related ale strains only; cluster 3 comprises strains BB24, BB2 and BB3 while cluster 4 comprises BB21, BB22 and BB23. It can also be seen from Figure 4 that the lager strains are more similar to each other while the ale strains are more diverse. This is to be expected because lager yeasts represent a comparatively homogeneous group of yeast strains (Casey, 1996; Pederson, 1983, 1985). Overall, this dendrogram shows good differentiation of the yeasts, although there is no clear separation between the ale and lager strains.

The history of some of these *S. cerevisiae* strains is known. In particular, BB21, BB22 and BB23 were pure strains originally isolated from a mixed strain on the basis of flocculation tests. It was therefore encouraging that these were recovered together in cluster 4. Likewise, the ale strain BB24 had been selected to have improved performance after production trials with BB3, and both strains were found together with the closely related BB2 in cluster 3. Furthermore, BB12, BB13 and BB14 were originally isolated from a mixed yeast culture,

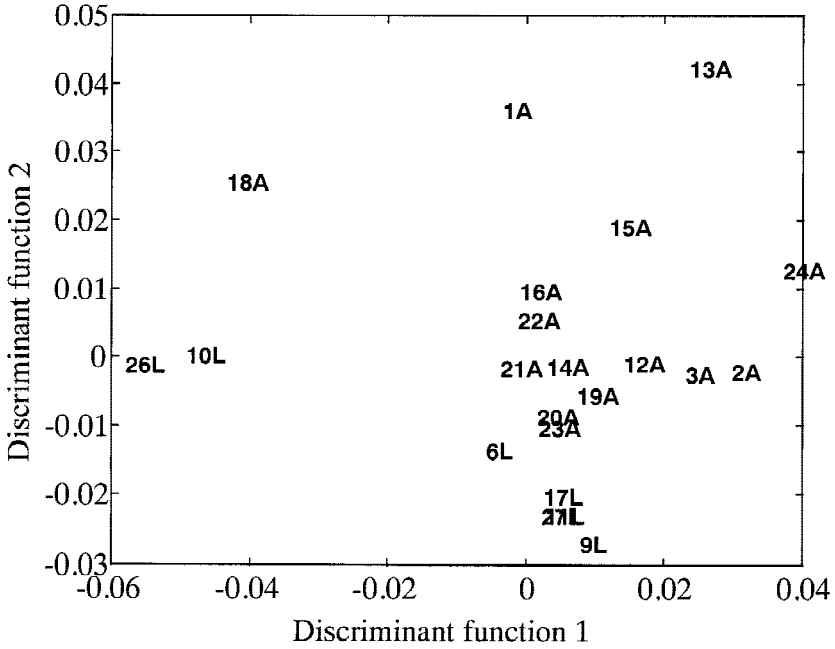

Figure 5. Discriminant analysis biplot based on FT-IR data showing the relationship between the 22 strains of *S. cerevisiae*. The numbers refer to the Bass Brewers strain number and whether ale (A) or lager (L)

and although these are recovered in cluster 2 it is obvious that these can be differentiated between.

The above groups, as judged by PyMS, were also seen in the analysis of these yeasts by FT-IR. The DFA plot from the infrared data (Figure 5) also shows good discrimination between the strains, and the ale strains again show more diversity than the lager strains. The seven lager yeast were recovered in two groups; BB10 and BB26 clustered together and away from the other lager strains BB6, BB9, BB11, BB17 and BB27. This was encouraging because this was also seen in the dendrogram from the PyMS spectra where the same two groups were recovered in cluster 1 and cluster 2 respectively (Figure 4). The DFA plot (Figure 5) also shows BB2, BB3 and BB24 (cluster 3 from PyMS dendrogram) to be recovered together and separately from the other yeast strains. However, although BB21, BB22 and BB23 cluster together, unlike the PyMS analysis (cluster 4; Figure 3) they are found to group with the other ale yeasts.

When the above results were compared to previous differentiation by DNA fingerprinting (Schofield *et al.*, 1995), similarities were seen between the DNA homologies and these two phenotypic approaches. Schofield *et al.* (1995) used a combination of restriction endonuclease *Hin*dIII and Ty1–2 probe, and were able to differentiate between BB1, BB2, BB3, BB6, BB9, BB10, BB11, BB12, BB13 and BB14, although the banding patterns were rather complex and there was no obvious generalized pattern for either ale or lager strains. Like both PyMS and FT-IR, this genotypic work showed strains BB2 and 3 to be similar. Schofield *et al.* (1995) also found BB6, BB10 and BB11 to have a very high degree of relationship as judged by sharing DNA polymorphisms on a RFLP gel. In contrast, these strains were easily differentiated by PyMS and FT-IR, although, Figures 4 and 5 both show strains BB6 and BB11 to be loosely clustered together. Schofield and co-workers (Schofield *et al.*, 1995) also found a strong DNA polymorphism relationship between strains BB12, BB13 and BB14. In these phenotypic studies, however, these strains are clearly differentiated, although Figure 4 does show them in the same main cluster 2 while Figure 5 shows BB12 and BB14 in the same group away from BB13.

Similarities were also observed when our results were compared to genotypic results from Wightman *et al.* (1996) who differentiated between

the strains BB3, BB6, BB9, BB10, BB11, BB12, BB13, BB14, BB21, BB22, BB23, BB24 and BB27 by DNA fingerprinting using different restriction enzymes and the Ty1–15 transposon probe. The ability to differentiate readily between strains was very dependent on the restriction enzyme used, and no enzyme was successful in causing obvious banding for differentiating lager strains from ale strains. This genotypic analysis showed a relationship between strains BB9 and BB27. Both PyMS and FT-IR also show these strains to be closely related. Finally, Wightman *et al.* (1996) also showed similarities between BB12, BB13 and BB14, and between B3 and BB24, and also between BB21, BB22 and BB23, which were mirrored in the present phenotypic studies.

It is clear that the application of PyMS and FT-IR is undoubtedly useful in the discrimination between these *S. cerevisiae* strains, and that these phenetic approaches mirror the known genotype (and brewing phenotype) of these organisms. In practice, either of these techniques could be used in tandem with other procedures to confirm that the correct strain is being used by the brewery. Both techniques have the major advantages of speed, sensitivity and ability to analyse many hundreds of samples per day. We therefore conclude that such whole-organism fingerprinting methods could find 'real time' application in yeast strain quality assurance procedures (e.g., Quain, 1995), in-process strain tracking or troubleshooting.

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