Staircase electrophoresis profiles of stable low-molecular-weight RNA – a new technique for yeast fingerprinting

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INTRODUCTION

Yeasts are a group of eukaryotic micro-organisms included in the kingdom Fungi. They are generally unicellular and reproduce asexually by budding or fission. Some species are known to reproduce sexually via asci or basidia. The identification of these micro-organisms is currently based on physiological and morphological characters (Barnett et al., 1990; Kreger-van Rij, 1984), although the process is long and complex owing to the large numbers of such characters described (Barnett et al., 1990; Yarrow, 1998). Furthermore, these characters (morphological and physiological) were considered to be important in yeast classification (Barnett et al., 1990; Kreger-van Rij, 1984) and have been used for identification and characterization of genera and species (Barnett et al., 1990; Yarrow, 1998). The drawbacks in using this type of character lie in the difficulty involved in interpreting certain results derived from morphological tests and in the large number of strains of a species that can be found in nature and whose assignation to an already described species is difficult due to the high variability that many species show concerning their physiological characteristics (Barnett et al., 1990).

The complexity of the taxonomy of this group of micro-organisms may be seen in nearly all the species admitted, which over time have been reclassified and renamed many times. Indeed, in the case of *Saccharomyces cerevisiae*, Barnett et al. (1990) have collected up to 130 synonyms, and most species of this genus recognized as different species up to a few decades ago have been reclassified as *Saccharomyces cerevisiae* according to DNA relatedness (Vaughan-Martini & Kurtzman, 1985, 1988; Vaughan-Martini & Martini, 1987; Vaughan-Martini & Pollaci, 1996). This background makes it necessary to have available molecular techniques able to shed light on the taxonomy of these micro-organisms. Recently, molecular techniques involving rRNA and rDNA sequencing have become available for conducting phylogenetic and classification studies (Boekhout et al., 1994; Cai et al., 1996; James et al., 1997; Kurtzman, 1994), and RFLP and RAPD have been applied to identification (Kurtzman, 1994). For example, the sequencing of D1/D2 domains allows the rapid identification of ascomycetous yeast (Fell, 1993).

Abbreviations: LMW RNA, low-molecular-weight RNA; SCE, staircase electrophoresis.
There is a general trend to seek new methods for the identification and classification of yeast instead of the conventional fermentations and assimilations of different compounds and in particular the use of morphological characters.

Stable low-molecular-weight RNAs (LMW RNAs) are molecules whose interest for taxonomic purposes lies in (i) their presence in all cells from the very beginning of evolution and the fact that they play the same role (protein synthesis, an indispensable function for cell growth), and (ii) their ubiquitous distribution in living organisms. Recently, a new one-dimensional electrophoretic technique in polyacrylamide gels—staircase electrophoresis (SCE)—has permitted optimum separation of these molecules (Cruz-Sánchez et al., 1997), allowing its application in the identification of prokaryotes. This technique has already been applied to genera and species of the family *Rhizobiaceae*, obtaining different profiles for each bacterial species assayed (Velázquez et al., 1998a). These results have been validated for other bacterial groups as diverse as *Frankia* (Velázquez et al., 1998b) and *Clavibacter* (J. L. Palomo, E. Velázquez, P. F. Mateos, P. García-Benavides, M. López & E. Martínez-Molina, unpublished data).

To study the pattern of the SCE LMW RNA profile in eukaryotes, a group with a complex taxonomy, i.e. yeasts, was used as a model in this study to determine the possible use of LMW RNAs in eukaryote identification.

**METHODS**

**Strains.** The strains used in this study were from Central Bureau voor Schimmelcultures (CBS) and the Spanish Type Culture Collection (CECT): *Schizosaccharomyces pombe* CBS 356, CECT 1378 (ATCC 24751) and CECT 1379 (ATCC 26760), *Zygosaccharomyces rouxii* CBS 732 (neotype of *Zygosaccharomyces rouxii*) and CECT 1129 (NCYC 381), *Pichia fermentans* CBS 187, *Hanseniaspora uvarum* CBS 314 (type of *Kloeckera* *uvarum*) and CECT 1444 (CBS 314), *Yarrowia lipolytica* CBS 599 (neotype of *Mycoderma lipolytica*), CECT 1468 and CECT 1469 (ATCC 32338), *Deckeria bruxellensis* CBS 74 (type of *Mycoderma bruxellensis*) and CECT 1452 (CBS 4914), *Kluyveromyces marxianus* CBS 712 (type of *Saccharomyces marxianus*), CECT 1018 (CBS 607), *Kluyveromyces lactis* CBS 683 (neotype of *Saccharomyces lactis*), CBS 2103 and CECT 1121, *Saccharomyces cerevisiae* CBS 1171 (neotype), CECT 1318 (CBS 2258), CECT 1319 (ATCC 26602), CECT 1170 (DCL 740), CECT 1171 (CBS 1320), CECT 1324 (CBS 679), CECT 1325 (ATCC 3602) and CECT 1894, *Candida zeylanoides* CBS 619 (neotype of *Monilia zeylanoides*) and CECT 1441 (ATCC 36275), *Candida parapsilosis* CBS 1954 and CECT 1449 (CBS 604), *Candida tropicalis* CBS 1920, CECT 1427 (CBS 5701) and CECT 1440 (CBS 94), *Trichosporon cutaneum* CBS 2466 (neotype of *Oidium cutaneum*), *Cryptococcus laurentii* CBS 318 (type of *Torula aurea*), *Rhodotorula mucilaginosus* CBS 17 (neotype of *Rhodotorula rubra*) and *Rhodotorula glutinis* CBS 324 and CECT 1137 (NCYC 162).

**LMW RNA extraction.** LMW RNA extraction was accomplished following the technique described by Höflé (1988).

**RESULTS AND DISCUSSION**

In this study, for the first time by using SCE, we have established the type of molecule that makes up the LMW RNA profile in a eukaryotic micro-organism. To do so, we used SCE in minigels; this enabled us to separate three zones in the LMW RNA profile of *Saccharomyces cerevisiae* (Fig. 1a). These zones were identified using specific probes for 5S rRNA, 5S rRNA and tRNA<sup>3</sup> respectively. Fig. 1(b) shows the hybridization signals obtained with the probes used. Hybridizations were performed sequentially using an oligonucleotide that specifically hybridizes with 5S rRNA as first probe, a specific oligonucleotide that hybridizes with 5S rRNA as second probe and, finally, a specific oligonucleotide that hybridizes with tRNA<sup>3</sup>. The results show that SCE LMW RNA profiles of yeasts have an additional molecule to those forming the SCE LMW RNA of prokaryotes (Cruz-Sánchez et al., 1997; Höflé, 1988; Velázquez et al., 1998a, b), namely 5S rRNA (see Fig. 1).
Application of SCE in gels of 40 × 360 × 0.4 mm (Cruz-Sánchez et al., 1997) for the separation of yeast LMW RNA molecules affords the same resolution as that observed for the SCE LMW RNA profiles of prokaryotes. In yeast (see Figs 2–4), four zones are seen in the profiles (from bottom to top): class 1 tRNA [77 nt for tRNA\textsuperscript{val} from \textit{Escherichia coli} (Sprinzl et al., 1985)], class 2 tRNA [85 nt for tRNA\textsuperscript{tyr} from \textit{Escherichia coli} (Sprinzl et al., 1985)], 5S rRNA [115–120 nt for 5S rRNA from \textit{Escherichia coli} MRE 600 (Bidle & Fletcher, 1995)] and 5–8S rRNA [158 nt for 5–8S rRNA from \textit{Saccharomyces cerevisiae} (Skryabin et al., 1984); considered as the reference in this study (Fig. 2)]. We also observed the same four zones in the SCE LMW RNA profiles of other eukaryotes, including filamentous fungi and plants (data not shown).

The SCE LMW RNA profiles obtained for the yeast species included in this study are shown in Figs 2–4. The different RNA classes in the SCE LMW RNA profiles represent 5–8S rRNA, 5S rRNA, class 2 tRNA and class 1 tRNA.

Analysis of the SCE LMW RNA profiles of \textit{Saccharomyces cerevisiae} CECT 1319 (Fig. 2) through the successive phases of the cell cycle revealed that, as in the case of prokaryotes (Höfte, 1988), they are stable and show no qualitative differences at different growth stages.

All strains of \textit{Saccharomyces cerevisiae} used in this study (see Methods) show identical SCE LMW RNA profiles (Fig. 3, lanes 1–8). These results are confirmed in other yeast, a characteristic and unique SCE LMW RNA profile being obtained for all the strains belonging to the same species. Fig. 4 shows the characteristic profile of each of the species tested.

We have analysed the SCE LMW RNA profiles of several type species of yeasts belonging to diverse genera from collection cultures, including budding yeasts (\textit{Saccharomyces}, \textit{Kluveromyces}, \textit{Zygosaccharomyces}, \textit{Hanseniaspora}, \textit{Pichia}, \textit{Candida}, \textit{Yarrowia}, \textit{Rhodotorula}, \textit{Cryptococcus}, \textit{Trichosporon}) and fission yeasts (\textit{Schizosaccharomyces}, \textit{Trichosporon}), those that exhibit sexual reproduction and those that do not (\textit{Candida}), and ascomycetous yeasts (\textit{Saccharomyces}, \textit{Kluveromyces}, \textit{Zygosaccharomyces}, \textit{Hanseniaspora},...
Pichia, Candida, Yarrowia) and basidiomycetous yeasts (Rhodotorula, Cryptococcus, Trichosporon). We included a number of species from each of the genera Kluyveromyces, Candida and Rhodotorula, and, as mentioned above, several strains of each of the following species: Saccharomyces cerevisiae, Yarrowia lipolytica, Kluyveromyces lactis, Kluyveromyces marxianus, Zygosaccharomyces rouxii, Hanseniaspora uvarum, Dekkera bruxellensis, Candida zeylanoides, Candida parapsilosis, Candida tropicalis, Rhodotorula glutinis and Schizosaccharomyces pombe.

Fig. 4 shows that the different genera included in the study differ in their 5–8S and 5S rRNA zones. 5–8S rRNA displays greater variation in size than 5S rRNA zone. According to these data, 5–8S rRNA is better suited for use for generic differentiation among yeasts than 5S rRNA.

The differences at species level are seen in the class 2 tRNA zone. Thus, the species of Candida, Rhodotorula and Kluyveromyces tested in this study can be differentiated at the level of class 2 of tRNA.

Therefore, each of the species of each genus included in this study showed a characteristic and unique SCE LMW RNA profile that can be used as a new molecular tool for yeast fingerprinting. In some groups of bacteria the SCE LMW RNA profiles can be used to construct dendrograms that coincide with those obtained from sequencing the 16S rRNA in such bacteria (Velázquez et al., 1998a, b).

To compile a database on yeast SCE LMW RNA profiles, we coded the bands present in the SCE LMW RNA profiles in binary form and applied Jaccard’s similarity coefficient to obtain the distance matrix. Using this matrix we constructed the corresponding dendrogram using the UPGMA method. The results are shown in Fig. 5 and reveal that the yeasts studied can be divided into four groups. The first includes basidiomycetous yeasts, the second includes Yarrowia lipolytica, the third includes Schizosaccharomyces pombe and the fourth includes ascomycetous yeasts together with yeasts that do not exhibit sexual reproduction such as Candida. These results are essentially in agreement with those reported by other authors using rDNA and rRNA nucleotide sequences (Cai et al., 1996; James et al., 1997; Kurtzman, 1994).

Regarding the first group (basidiomycetous yeasts), Trichosporon is associated with species of the genus

![Fig. 3. SCE LMW RNA profiles of Saccharomyces cerevisiae strains. Lanes: 1, CBS 1171 (neotype); 2, CECT 1318 (CBS 2358); 3, CECT 1319 (ATCC 26602); 4, CECT 1170 (DCL 740); 5, CECT 1171 (CBS 1320); 6, CECT 1324 (CBS 679); 7, CECT 1325 (ATCC 13602); 8, CECT 1894; MW, RNA size markers (tRNAval, 77 nt; tRNA tyr, 85 nt; 5S rRNA, 115–120 nt).]
Cryptococcus. The two species of Rhodotorula cluster together and are included in the first group. Zygosaccharomyces rouxii, Saccharomyces cerevisiae and the two species of Kluyveromyces (Kluyveromyces marxianus and Kluyveromyces lactis) lie within the same group. Pichia and Dekkera belong to different groups. These observations are consistent with the results reported by other authors (Cai et al., 1996; James et al., 1997; Kurtzman, 1994) derived from analyses of small or large rRNA subunit sequences. Also, according to our results (Fig. 5), Hanseniaspora, Dekkera and Saccharomyces belong to different groups, in agreement with the findings of other authors using rDNA and rRNA nucleotide sequences (Boekhout et al., 1994; Cai et al., 1996; Kurtzman, 1994). Within the fourth subgroup, Candida species cluster together. All these results are essentially in agreement with the current classification of yeasts based on molecular characteristics (Kurtzman & Blanz, 1998).

All the foregoing data point to the usefulness of this technique with regard to yeast taxonomy, since yeast identification is currently a long and complex process owing to the taxonomic complexity of this group of micro-organisms. An idea of this can be gained from the high number of species admitted from 1985 (Barnett et al., 1985) to 1990 (Barnett et al., 1990), which raised the number of species from 469 to 590: most of them have been reclassified several times. In many instances, this reclassification has not consisted merely of a change in species within a given genus, but rather of changes in species between different genera (Barnett et al., 1985, 1990). In the future, SCE LMW RNA profiles should allow more reliable and faster identification of any isolate to a species already described or to detect new species and new genera.

We have shown that: (i) it is possible to analyse yeast SCE LMW RNA profiles; (ii) these profiles show a new zone corresponding to 5S rRNA, useful in studies of yeast identification and classification, that is absent in bacterial SCE LMW RNA profiles; (iii) the rest of the zones (5S rRNA, class 1 tRNA and class 2

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**Fig. 4.** SCE LMW RNA profiles of reference yeast strains used in this study. (a) Lanes: 1, Dekkera bruxellensis CBS 747; 2, Zygosaccharomyces rouxii CBS 732T; 3, Kluyveromyces marxianus CBS 712T; 4, Kluyveromyces lactis CBS 683T; 5, Saccharomyces cerevisiae CBS 1171T. (b) Lanes: 6, Hanseniaspora uvarum CBS 314T; 7, Pichia fermentans CBS 187T; 8, Candida parapsilosis CBS 1954T; 9, Candida zeillii CBS 619T; 10, Candida tropicalis CBS 1920T. (c) Lanes: 11, Rhodotorula mucilaginosa CBS 17T; 12, Rhodotorula glutinis CBS 324T; 13, Yarrowia lipolytica CBS 599T; 14, Trichosporon cutaneum CBS 2466T; 15, Cryptococcus laurentii CBS 318T; 16 Schizosaccharomyces pombe CBS 356T. Lanes MW, RNA size markers (tRNAval, 77 nt; tRNAtyr, 85 nt; 5S rRNA, 115–120 nt).
Rhodotorula mucilaginosa CBS17
Rhodotorula glutinis CBS324
Trichosporon cutaneum CBS2466
Cryptococcus laurentii CBS139
Yarrowia lipolytica CBS599
Schizosaccharomyces pombe CBS356
Dekkera bruxellensis CBS74
Hanseniaspora uvarum CBS314
Pichia fermentans CBS187
Zygosaccharomyces rouxii CBS732
Kluyveromyces marxianus CBS712
Kluyveromyces lactis CBS5683
Saccharomyces cerevisiae CBS1171
Candida parapsilosis CBS1954
Candida zeylanoides CBS619
Candida tropicalis CBS1920

**Fig. 5.** UPGMA dendrogram based on Jaccard’s coefficient derived from SCE LMW RNA profile characteristics for the reference strains of yeast species included in this study.

- tRNA) have a similar distribution to that seen in prokaryotes; (iv) this new profile has been shown to be general among yeasts; (v) no qualitative variation in SCE LMW RNA profiles is seen during the growth of a yeast strain; (vi) yeast strains belonging to the same species display identical SCE LMW RNA profiles; (vii) different species of yeasts have different tRNA profiles; (viii) different genera show differences at the rRNA level (5S and 5.8S); and (ix) SCE LMW RNA profiles may therefore be used as tools of fingerprinting yeasts in the same way as in bacteria.

In conclusion, SCE LMW RNA profiles in yeast offer a new and fast method for identification. SCE LMW RNA profiles have proved to be useful in the identification of Gram-negative (Velázquez et al., 1998a) and Gram-positive bacteria (Velázquez et al., 1998b) and, in view of the results obtained here, also yeasts. We are currently studying the potential of the application of these profiles on taxonomy of yeasts and other microbial groups.

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**REFERENCES**


