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ORIGINAL ARTICLE

Methods for yeast characterization from industrial products

Luiz H. Gomes[1]*, Keila M.R. Duarte[1], Juan L. Argueso[2], Sergio Echeverrigaray[3] and Flavio C. A. Tavares[1]

This work compared the efficiency of four methods for the identification of industrial yeast strains and the establishment of a pattern for yeast characterization to be used during industrial fermentation processes, allowing the detection of yeast contaminants. Five strains of yeast currently used in the Brazilian fuel alcohol industry (about 99% of the yeast used for this purpose), and yeast strains isolated from the five major beer industries that represent 95% of the Brazilian beer market were evaluated for their growth and absorption of dyes on differential culture media, their total protein electrophoretic patterns (SDS–PAGE), CHEF chromosome separation patterns, and RAPD profiles. For the identification of brewing yeast, all tested methods were efficient, allowing the identification of at least two different species, one of which was Saccharomyces cerevisiae. *The strains used for the fuel alcohol industries were best characterized by SDS–PAGE and RAPD analysis. Those strains share high level of genetic similarity and they are all known as* S. cerevisiae *strains.*

Introduction

Yeast classification is traditionally based on morphological and physiological criteria. However, these phenotypic traits commonly fail to discriminate among strains within a single species. The most frequently used strains in ethanolic fermentation processes belong to the *Saccharomyces* group. The accurate identification of industrial strains constitutes an important challenge to yeast breeders and yeast producers, who seek to obtain a quality product with a predictable influence on the fermentation process and final manufacturing products. Several alternative methods, such as serology (Tsuchiya et al. 1965), bioluminescence (Miller and Galston 1989), trehalose content (Gutierrez 1990), properties of the fatty acids (Bendová et al. 1991), inhibition effects by different chemical compounds (Simpson et al. 1992), total proteins (Van Vuuren and Van Der Meer 1987) and isoenzymatic (Subden et al. 1982) electrophoretic patterns, pulse-field electrophoresis for karyotype comparisons (Degrée et al. 1989) and analysis of DNA polymorphisms (Pedersen 1986b, Degrée et al. 1989, Meaden 1990), have been used with variable success in the identification and characterization of yeast lines). To obtain a better and more reliable identification of industrial yeast, four

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[1]Department of Genetics, ESALQ/USP, Av. Padua Dias, 11, Cx. Postal 83, Piracicaba, SP Brazil, 13418-900
[2]Section of Genetics and Development, 457 Biotechnology Building, Cornell University, Ithaca, New York, USA, 14853
[3]Institute of Biotechnology-UCS, Rua Getulio vargas, 1130, Caxias do Sul, RS Brazil, 95070-560

*Corresponding author. Fax: 055 (019) 433-6706. E-mail: luhgomes@carpa.ciagri.usp.br

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methods were compared to characterize strains (Differential Culture Medium, RAPD, SDS–PAGE and CHEF).

Materials and Methods

Strains

All the strains used came from ethanol fermentation industry and from brewing industry. The strains were all maintained in solid YEPD (1% w/v yeast extract, 1% w/v peptone, 2% w/v dextrose, 2% w/v agar, supplemented with 100 ppm tetracycline and 100 ppm penicillin).

Ethanol fermentation strains

IZ-1904 (*Saccharomyces cerevisiae*, Department of Science and Agroindustrial Technology – ESALQ-USP) yeast Fleischmann (*S. cerevisiae*), yeast ITAIQUARA (*S. cerevisiae*); M300A-10 and M304-2C (*S. cerevisiae*, industrial strains obtained by the Laboratory of Yeast Genetics, Department of Genetics – ESALQ-USP).

Brewing strains

Yeast colonies were obtained by filtering 100 ml of commercial draught beer through Millipore filters (0·22 μm), transferring the membranes to Petri dishes containing solid YEPD. The strains isolated were represented as L6-SC; L7-AN; L8-KA; L9-BR and L10-SK.

Differential culture medium

The differential culture media used in this work were adapted from Lin (1975). The following dyes were added to a basal YEPD medium: Basic Fuchsin, Nile Blue, Bromocresol Purple, Aniline Blue-Black, Rose Bengal, and Acid Fuchsin (final concentration 100 $\mu g\ ml^{-1}$), Methyl Violet, Crystal Violet (final concentration 20 $\mu g\ ml^{-1}$), Brilliant Green (final concentration 50 $\mu g\ ml^{-1}$) (Sigma, St Louis, Missouri, USA). Yeast strains were streaked on the media and incubated at 30°C for 48 h.

SDS–PAGE electrophoretic analysis

Total protein electrophoretic profiles of each yeast were obtained by the method described by Laemmli (1970) using a lower gel with 10% (w/v) acrylamide and a molecular weight marker (LMW 17-0446-01, Pharmacia (Sao Paulo, Brazil)) was used. The gels were silver stained as described by Gomes (1995) and were scanned and analyzed in a Hoeffer GS-300 densitometer (Hoeffer Scientific Instruments San Francisco, USA).

Analysis of chromosomes

The analysis of chromosomes was performed by high resolution CHEF gel electrophoresis. The samples were prepared according to McCluskey et al. (1990) and Ibeas and Jimenez (1993). The CHEF electrophoresis was performed using a CHEF-DR II (Bio-Rad, Sao Paulo, Brazil) system. The gels (1% w/v agarose on 0·25 × TBE buffer) were run for 15 h with pulse of 60 s and for 8 h with pulse of 90 s, at 14°C and 200 V. Gels were stained with ethidium bromide and directly analysed under UV light.

RAPD analysis

For RAPD analysis, DNA was isolated according to Johnston (1988). The amplification reactions were performed according to Williams et al. (1990), in a final volume of 25 μl containing 20 mM Tris-HCl pH 8·0; 50 mM KCl; 3·75 mM $MgCl_2$; 100 μM dNTP (GIBCO-BRL, Sao Paulo, Brazil); 5 pmol primer (10bp); 40 ng of genomic DNA and 1·5 units Taq DNA Polymerase (Gibco-BRL). Amplification was performed in a thermal cycler PTC-100 (MJ Research Inc., Sao Paulo, Brazil) programmed for 40 cycles of 1 min at 92°C; 1 min at 37°C and 2 min at 72°C with a final extension of 3 min at 72°C. The amplification products were separated by electrophoresis in 1·4% w/v agarose gels and visualized by staining with ethidium bromide.

The following decamer primers from Operon Techn. Inc.: OPB-02 (TGATCCCTGG), OPB-10 (CTGCTGGGAC), OPB-11 (GTAGACCCGT), OPB-12 (CCTTGACGCA), OPB-13 (TTCCCCCGCT), OPB-17 (AGGGAACGAG) and OPB-20 (GGACCCTTAC) were used for the analysis.

Table 1. Absorption of dyes during the growth of yeasts on differential media. Strains listed are: L1 – IZ1904; L2 – M300A-10; L3 – 'Fleischmann'; L4 – 'Itaiquara'; L5 – M304-2C; L6 – SC; L7 – AN; L8 – KA; L9 – BR and L10 – SK

Strains	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Dyes[a]										
Basic fuchsin	+	+	+	+	+	0	+	0	+	+
Nile blue	0	0	0	0	0	0	0	0	0	+
Methyl violet	+	+	+	+	+	0	0	0	+	+
Bromocresol purple	+	+	+	+	+	0	0	0	0	+
Aniline blue black	0	0	0	0	0	0	0	0	0	+
Violet crystal	+	+	+	+	+	0	0	0	+	0
Brilliant green	0	0	0	0	0	+	0	+	0	+
Rose bengal	+	+	+	+	+	+	+	+	+	+
Acid fuchsin	+	+	+	+	+	0	+	0	+	+

[a](0) non-absorption and (+) absorption of the dye.

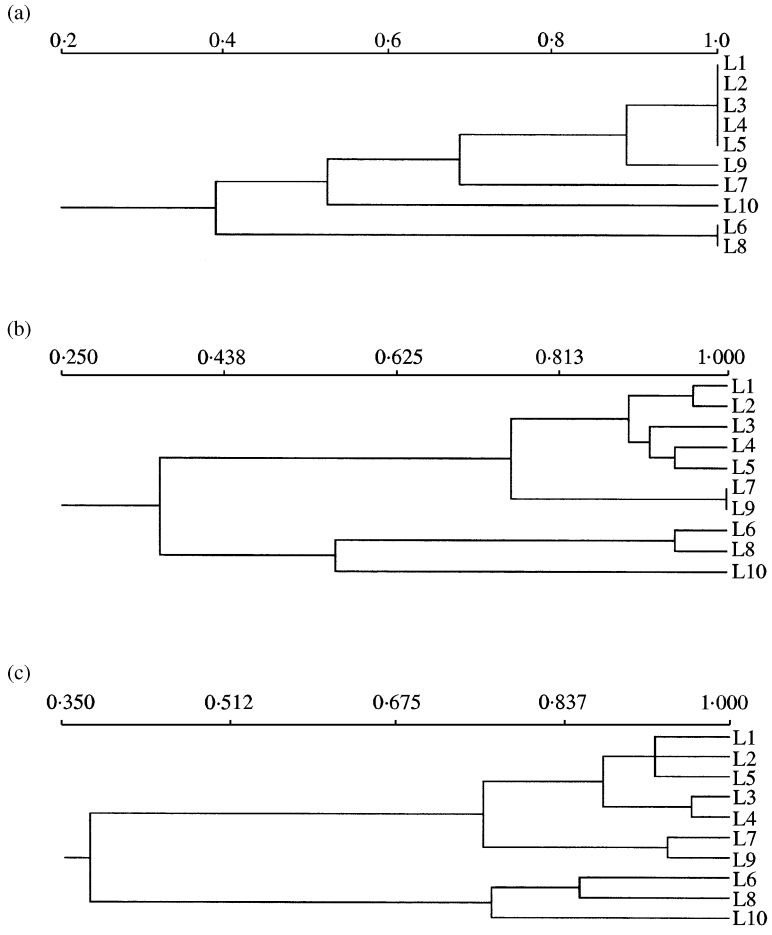

Figure 1. Similarity dendrograms among industrial yeasts as obtained by UPGMA using single mating coefficient comparing to (a) – differential media; (b) – SDS–PAGE and (c) – RAPD data. The yeasts strains used were: L1 – IZ1904; L2 – M300A-10; L3 – Bakery yeast 'Fleischmann'; L4 – Bakery yeast 'Itaiquara'; L5 – M304-2C; L6 – SC; L7 –AN; L8 – KA; L9 – BR and L10 – SK.

The similarity dendrograms were built using the UPGMA method with single mating coefficient (NTSYS-PC version 1·70) for RAPD, SDS–PAGE and differential culture medium data (Rohef, 1992).

Results and Discussion

Differential media

The results obtained for the observation of the nine dyes used for the 10 strains represented as L1-IZ1904; L2-M300A-10; L3-'Fleischmann'; L4-'Itaiquara'; L5-M304-2C; L6-SC; L7-AN; L8-KA; L9-BR and L10-SK (Table 1), revealed that this method was unable to detect any difference among the fuel alcohol industrial yeasts. However, it was able to identify three of the five beer isolates. The dendrogram obtained by the analysis of the data (Fig. 1) allows to distinguish a group formed by the five yeast strains from the alcohol industry with 100% similarity. The other groups were formed by the isolates from beer. Among these strains, L6 and L8 present 100% similarity between them. The other strains formed independent groups. These results obtained with the brewery strains are similar to those obtained by Lin (1975) who characterized five species of the genus *Saccharomyces* and another 15 species from different genera among the brewers yeasts but makes no mention of identification of strains belonging to the same species. The results obtained with this method for industrial alcohol strains, as previously demonstrated by Oliveira and Pagnocca (1988) has limited efficiency for the identification of the alcohol industry yeast.

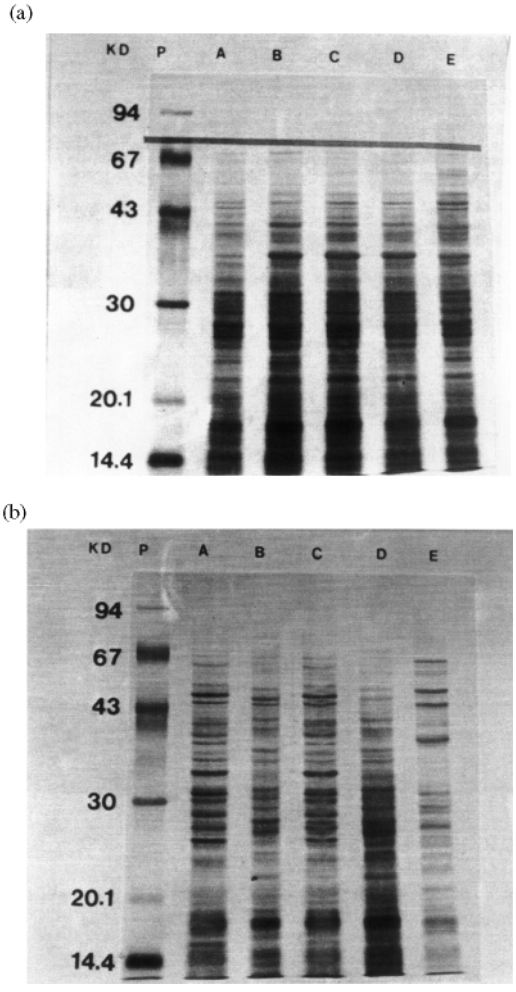

Figure 2. Band profiles from SDS–PAGE gels at 10% w/v, where the photo (a) is from alcoholic strains (A – IZ1904; V – M300A-10; C – 'Fleischmann'; D – 'Itaiquara' and E – M304-2C) and (b) is from brewers' yeast strains (A – SC; B – AN; C – KA; D – BR and E – SK).

SDS–PAGE electrophoretic analysis

The comparison between the protein profiles of the yeast strains obtained by SDS–PAGE analysis (Fig. 2) allowed the differentiation of the strains by the presence or absence of a band, except for strains 7 and 9. The variability among the strains from the alcohol industry was smaller than that observed among the brewing strains. As shown previously in Fig. 1(b), in which the maximum similarity calculated among the yeast from alcohol industry was 85%, and for the brewing strains reached up to 40%. Analysis of band profiles showed that the brewing strains 7 and 9 are similar to strains used in the alcohol industry belonging therefore, to the same species, *S. cerevisiae*. As already mentioned for wine yeast (Van Vurren and Van Der Meer 1987), brewers yeast (Van Vurren and Van Der Meer 1988) and fuel alcohol yeast (Tavares et al. 1992), the present results confirm the efficiency of SDS–PAGE analysis for identification of industrial yeast.

Analysis of chromosomes

The yeast karyotype analysis using CHEF gel electrophoresis allowed the identification of three groups: one formed by the five yeast strains from the alcohol industry and the brewers yeast strains 7 and 9, with 11–12 bands, a typical pattern of genus *Saccharomyces*. Another group was formed by strains 6 and 8, with only three bands and a third group was formed by the strain 10, with six bands (Fig. 3). As pointed out by several authors (Bidenne et al. 1992, Carle and Olson, 1985, Versavaud et al. 1995), yeast karyotyping allows the identification of yeast species. However, the methods of yeast chromosomal separation are not efficient enough for the identification of strains within a species (Pedersen 1986a, b, Meaden 1990).

Analysis of random amplified fragments (RAPD)

The RAPD analysis was able to differentiate all the strains studied (Fig. 4). The profiles exhibited 12–19 bands depending on the primer, producing a total of 83 scorable bands. The variability among the brewers strains was larger than observed among the strains from the alcohol industry. In the dendrogram of Fig. 1(c), two groups were distinguished. One group was formed by the yeast from the alcohol industry and the brewers yeast strains 7 and 9. The other group was formed by strains 6, 8 and 10. PCR and RAPD analysis have been used with success in the identification and characterization of plants, animals and micro-organisms (Palumbi 1996). In the case of industrial yeast these methods have been able to identify isolated strains at inter- and intra-species levels (Meaden 1990, Lavallée et al. 1994, Quesada and Cenis 1995, Lopes et al. 1996).

Comparison of the methods

Several important aspects should be considered in adopting an industrial routine analytical methods, such as its efficiency, reproducibility, simplicity and low cost. In this sense, the use of differential media is simple and affordable. However, it is time consuming, as the yeast should has to be grown for at least 48 h, and its efficiency is very poor. Karyotype analysis is a difficult, expensive and time consuming method with limited efficiency for the identification of strains within a species in addition, this method, among the four used, presented low reproducibility. In this sense, Sheehan and Weiss (1991) considered that the high cost, the low number of variables and the

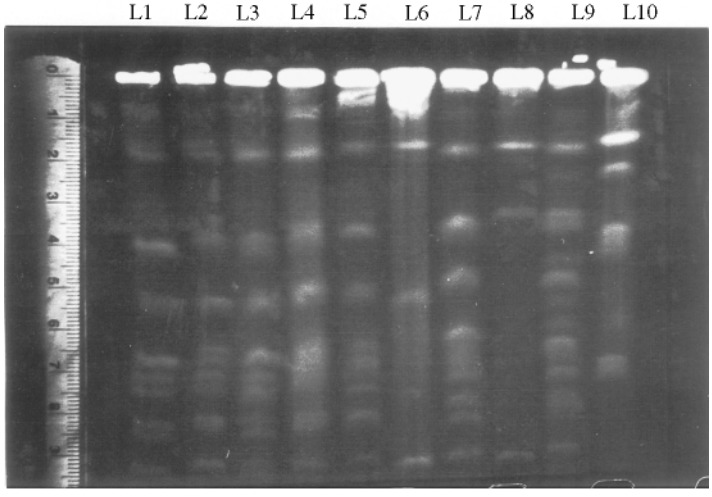

Figure 3. Karyotype profiles in CHEF-electrophoresis, where from the left to the right can be seen the chromosomes bands of L1 – IZ1904; L2 – M300A-10; L3 – 'Fleischmann'; L4 – 'Itaiquara'; L5 – M304-2C; L6 – SC; L7 – AN; L8 – KA; L9 – BR and L10 – SK.

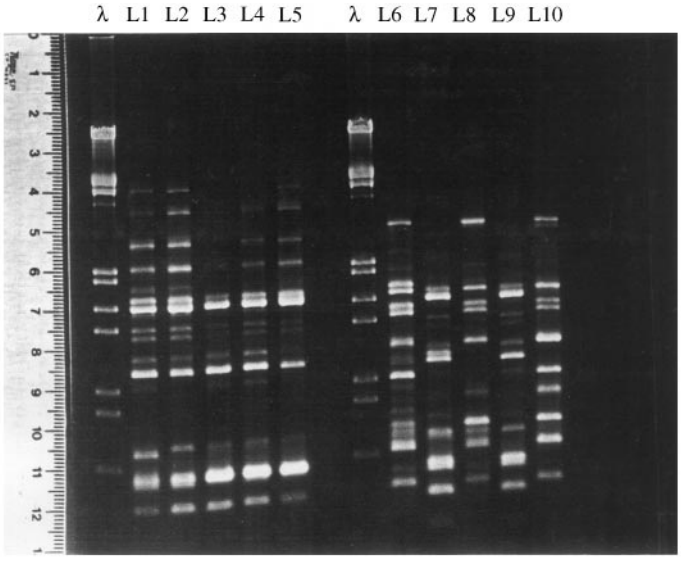

Figure 4. DNA band profiles from RAPD using primer OPB-11. From left to right '*λ*'-*λ Eco*RI/*Hind*III standard, L1 – IZ1904; L2 – M300A-10; L3 – 'Fleischmann'; L4 – 'Itaiquara'; L5 – M304-2C; '*λ*'-*λ Eco*RI/*Hind*III standard; L6 – SC; L7 – AN; L8 – KA; L9 – BR and L10 – SK.

great number of factors that influence the result make this method unsuitable for routine analysis.

SDS–PAGE and RAPD analysis were the most efficient methods for the identification of yeast industrial strains. SDS–PAGE is a rapid and inexpensive method. However, since it analyses genetic products, a rigorous standardization should be adopted to reduce the influence of the environment on gene expression. The large number of bands and the small distance between them makes the analysis of the SDS–PAGE gels difficult, demanding technical expertise.

The RAPD analysis was considered the most appropriate method for yeast identification due to the low cost of the analysis, the high number of analysable and polymorphic variables and the absence of environmental effects. In methodological terms, RAPD is simple, but its reproducibility demands special care during the whole process (Palumbi, 1996).

We conclude that the best system for analysis of yeast from the alcohol industry and breweries should involve an initial screen using differential media followed by SDS–PAGE and/or RAPD being capable of giving the highest number of analysable variables.

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