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DIFFERENTIATION OF REFERENCE AND INDUSTRIAL BAKER'S YEAST STRAINS BY CLASSICAL BIOCHEMICAL TESTS AND PCR-RAPD METHOD

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Key words: baker's yeast, *Saccharomyces cerevisiae*, PCR-RAPD, discrimination

The purpose of the study was to apply traditional and genetic methods for discrimination of baker's yeasts strains. The analysed strains were subjected to preliminary morphological characterization by microscopic and physiological observations by means of API 20 C AUX tests. Their DNA analyses were then carried out using the RAPD technique with the following primers: AB1-12 (5'CCTTGACGCA 3'), primer 21 (5'GCTCGTCGCT 3'), and primer 24 (5'GCGTGACTTG 3'). The analysis of assimilation and genetics profiles showed both similarity and differences between the investigated strains. As a result, the following groups of strains of baker's yeast have been discriminated: I group: 2200, 2200 – 3PJ, 2200-4PJ; II group: I1, I2, AFr; III group: IRHS, 102, D1; and IV group: HH.

The strain ATCC 2366 showed different profiles in both methods regarding to the investigated strains of bakers yeasts, which proved the usefulness of these methods in strains discrimination within the *S. cerevisiae* species.

INTRODUCTION

Traditional methods of identification are based on diversified morphological and physiological properties of yeast. In such methods, even tens of various tests need to be performed, which in turn is time- and labour-consuming. Rapid physiological tests in the form of a strip with mounted wells filled with already prepared media are somewhat convenient. Another simplification is the automation of read outs and analysis of results. Still, the above-mentioned methods may yield unreliable results and may not be suitable for the identification or discrimination of strains [Török & King, 1991; Arias *et al.*, 2002].

The broadening of knowledge in the field of molecular biology enabled the elaboration of new methods for the identification and differentiation of yeast. Predominant among such techniques are the techniques based on the PCR reaction that makes it possible to investigate differences and similarities at the level of nucleic acids.

The PCR has a number of variations. One of them is a RAPD technique based on the application of only one primer containing 5–15 nucleotides. Moreover, the starter is arbitrary, *i.e.* with any optional sequence, thus its construction does not require the knowledge of, for instance, a matrix fragment of DNA. In the course of reaction, the primer attaches to the matrix at many complementary sites and, once the distance and orientation between two subsequent starters are appropriate, products of reactions are formed. Differences in DNA occurring between organisms result in variations in the number and size of the products formed for each species or strain. Comparison of electrophoretic profiles of RAPD reaction products is a basis for identifying either similarity or difference between the analysed organisms [Williams *et al.*, 1990].

The above method has been successively used by Foschino *et al.* [2004]. They examined a yeast starter mix from eight Italian bakeries for diversifying their yeast flora and on that basis identified two species of *S. cerevisiae* and *Candida holmii*.

In turn, in a study by Vernocchi *et al*. [2004], the analysis of RAPD profiles of cells collected at a few sites of a production line of a traditional Italian bakery product called Cordoba enabled the identification of two groups of isolates: *Candida milleri* (1. group) and *S. cerevisiae* (2. group).

Fadda *et al.* [2004] investigated yeast flora during the ripening of Fiore Sardo cheese produced using traditional methods in a cheese-manufacturing unit in Sardinia. They noted the occurrence of 18 yeast species whose presence and percentage in cheese changed with time. DNA of isolates of five prevailing species and of standard strains were used in the RAPD reaction, which was proved to be effective in the identification process. The authors suggest that RAPD may be an alternative to conventional identification methods.

The literature provides a number of examples that indicate the high popularity of the above technique. Successful experiments using RAPD are improved in order to undertake other trials, yet caution should be exercised due to some restrictions linked with the above-mentioned method. The first obstacle is changeable repeatability of reaction results. Different conditions of PCR as well as various composition of a reaction

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mixture result in the formation of different products. Similar results may be evoked by: application of enzymes from various sources and application of different protocols or thermocyclers. To facilitate the comparison of the results between laboratories, it would be advisable to combine the applied procedures, reagents, and equipment [Penner *et al.,* 1993]. However, as reported by Tyler *et al.* [1997], even after unification and optimization of reaction conditions, results are not always repeatable, both between different research centers and within them.

According to Josepa *et al.* [2000], in the analysis of yeast differentiation using the RAPD method, the selection of starter is very significant. Appropriate oligonucleotides make it possible to obtain profiles which, in turn, enable easy identification of similarities and differences between the examined organisms. The number of products should not be high as it would render the analysis difficult. Researchers postulate checking some number of primers before the selection of a proper one for a given organism. It is also advisable to carry out several reactions with different starters and then to analyse their joint results or to compile RAPD with other methods, which has been the subject of the reported study.

In the production of baker's yeast, use is made of strains belonging to the *S. cerevisiae* species selected for their technological usability. Yeast factories possessing a number of strains (originating from various sources) not always observe distinct diversification of their technological characteristics in the production process. It is likely that strains of different origin are the same strains. Thus, an attempt was undertaken to differentiate strains of baker's yeast in terms of their morphology, physiology and analysis of DNA isolated from their cells.

MATERIAL AND METHODS

Organisms and growth conditions

Experiments were carried out with the use of 10 strains of baker's *Saccharomyces cerevisiae* yeast as follows: I1, I2, AFr, IRHS, 102 originating from the Museum of Pure Cultures of Biotechnology and Microbiology of Food of ULS-SGGW in Warsaw, and *S. cerevisiae* 2200, *S. cerevisiae* 2200-3P-J, *S. cerevisiae* 2200-4P-J, *S. cerevisiae* HH, *S. cerevisiae* D1 originating from the Yeast Factory culture collection, and *S. cerevisiae* ATCC 2366 for comparative purposes.

Yeasts were stored at a temperature of 4°C on YPD medium slants with the following composition (g/L): glucose 20, peptone 20, and yeast extract 10.

For analyses of yeast cell morphology and for culture DNA isolation, the biological material was suspended in 50 mL of liquid YPD medium (pH 5.0). The cultures were run at a temperature of 28°C for 18-24 h, on a laboratory shaker (ROSI 1000, Thermolyne, USA) at 200 rpm.

In turn, for physiological tests the yeasts were inoculated on Sabouraud agar with chloramphenicol (bioMerieux, L'Ecole, France) (BHL, Poland) and incubated for 18-24 h at a temperature of 28°C.

Light microscope measurements

Cell morphology of the strains examined was observed under a light microscope (Zeiss Axiostar Plus, Germany) coupled with a camera (Sony DSC-S85). Pictures were saved in Zeiss LSM Image Browser software that was used for measurements of 100 yeast cells selected at random from each strain.

Statistical analysis

The results obtained in measurements of the length and width of 100 cells were analysed statistically using Analysis of Variance and Tukey's Multiple Range Test at a significance level of $\alpha=0.05$ (Statgraphics Plus 5.1).

Investigation of physiological properties of yeast

The cultures grown on the Sabouraud agar medium with chloramphenicol (bioMerieux, L'Ecole, France) (BHL, Poland) were inoculated onto API 20C AUX tests (bioMerieux, L'Ecole, France) in order to observe strain capacity for assimilation of 19 different chemical compounds. Homogeneous yeast suspensions with a turbidity equivalent to 2 McFarland were prepared and introduced into microtubes of the incubation boxes. Results were read after 48-72-h incubation at 30°C.

DNA isolation

Isolation of DNA was carried out by means of Wizard® Genomic DNA Purification Kit (A1120, Promega). To determine the effectiveness of isolation, the obtained DNA was loaded on 1.2% agarose gel (prepared from agarose [Sigma, #A-9539] and 1x TAE buffer [Fermentas, #B49]) and subjected to 10-min electrophoresis in a Mini-Sub® Cell GT apparatus (Biorad) under voltage of 70 V in 1xTAE buffer medium. The gel was visualized in UV light in a Gel Doc 2000 device (Biorad).

Approximate concentration of the isolated DNA was analysed spectrophotometrically (a SmartSpec 3000 Biorad spectrophotometer) at a wavelength of $\lambda=260$ nm. A dilution factor of a=50 and an extinction factor of $1A_{260}=50$ μg/mL dsDNA were applied to the device, and the results were expressed in μg/mL. The isolated DNA was stored at a temperature of -20°C.

PCR reaction

The following components were used in the PCR reaction: primers (provided by Laboratory of DNA Sequencing and Oligonucleaotides Synthesis, Institute of Biochemistry and Biophysics of Polish Academy of Sciences): primer 21 (5' GCTCGTCGCT 3') [Tornai-Lehoczki & Dlauchy, 2000], primer 24 (5' GCGTGACTTG 3') [Baleiras-Couto *et al*., 1996], AB1-12 (5' CCTTGACGCA 3') [Xufre *et al.*, 2000]; dNTP Mix, 10 mmol/L each (#R0192, Fermentas); Taq DNA Polymerase (native, without BSA), 5 U/μL, (#EP0281, Fermentas) provided together with 10x Taq buffer with KCl (100 mmol/L Tris–HCl, pH 8.8; 500 mmol/L KCl; 0.8% Nonidet P40) and with 25 mmol/L $MgCl_2$; water (#R0582, Fermentas); and isolated yeast DNA. The reaction mixture applied was composed of: primer 0.5 μmol/L, dNTPs 0.2 mmol/L, Taq polymerase 1 U, DNA 50 ng, Tris–HCl 10 mmol/L, KCl 50 mmol/L, $MgCl_2$ 2.5 mmol/L, and water up to 20 μL.

PCR reactions were carried out in a Mastercycler® gradient thermocycler (Eppendorf) under the following conditions:

preliminary denaturation 95°C/3 min, 35 cycles: denaturation 95°C/1 min, annealing: 36°C/1 min – for primer 24 and AB1--12, 38°C/1 min – for primer 21, extension 72°C/2 min, and final extension 72°C/3 min.

Electrophoretic separation of products and visualization of gel

PCR products were separated electrophoretically on 1.2% agarose gel prepared from agarose (Sigma, #A-9539) and 1x TAE buffer (Fermentas, #B49) with the addition of ethidium bromide (Sigma, #E1510) at a concentration of 1 μg/1 mL gel. O'GeneRuler™ 100 bp DNA Ladder Plus (Fermentas, #SM1153) was used as a size marker.

Electrophoresis was conducted on a Mini-Sub® Cell GT (Biorad) apparatus under voltage of 75 V in 1× TAE buffer medium. The gel was visualized in UV light in a Gel Doc 2000 device (Biorad). Analyses of gels were carried out using Quantity One software (Biorad).

Construction of a dendrogram

TREECON software [Van de Peer & De Wachter, 1994] was used to construct a dendrogram. The presence (denoted as "1") or a lack (denoted as "0") of the analysed products of three RAPD reactions using various primers enabled the construction of a matrix that constituted an input file of the software. Genetic distance between the examined strains was computed using Nei and Li algorithm provided in the software. A dendrogram was constructed using a method of UPGMA (unweighted pair group with arithmetic mean).

RESULTS AND DISCUSSION

Evaluation of morphological traits of *S. cerevisiae* yeast

The size and shape of baker's yeast cells is their significant characteristics in the industrial practice. Therefore, strains characterised by large sizes and oval shape are desired in the yeast industry.

Morphology of yeast cells was evaluated based on microscopic observations of preparations made of 18–24-h cultures in the YPD medium. The length and width of 100 cells selected at random from each strains were measured and the obtained mean values were presented in Figures 1 and 2. They were found to fit within ranges described by Vaughan-Martini & Martini [1998], wherein the width was 3–8 μm and the length was 5–10 μm.

The least mean length of the cells (7.49 μm) was reported for I1 strain, whereas the greatest one (8.15 μm) for 2200-4P--J strain. The statistical analysis demonstrated that mean values of cell length of the examined strains were not statistically different (honestly significant difference HSD=0.75).

Mean width of cells had the lowest value (5.66 μm) in the case of ATCC 2366 strain and the highest value (6.23 μm) in the case of 2200-4P-J strain. The statistical analysis of results, at HSD=0.45, demonstrated that 2200-4P-J strain possessed significantly wider cells than did the other strains. Mean values of cell width of the other strains analysed did not differ significantly between one another and ranged from 7.49 to 7.74 μm.

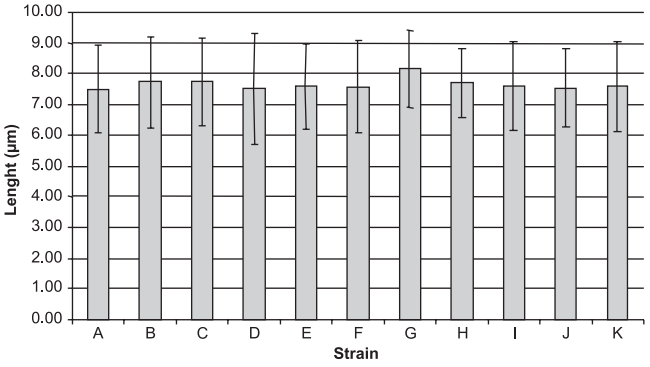

FIGURE 1. Mean length of yeast cells from 24-h cultures in YPD medium (A – K – analysed strains of *S. cerevisiae*: A – I1, B – AFr, C – 2200-3P-J, D – ATCC 2366, E – IRHS, F – I2, G – 2200-4P-J, H – 102, I – 2200, J – HH, K – D1).

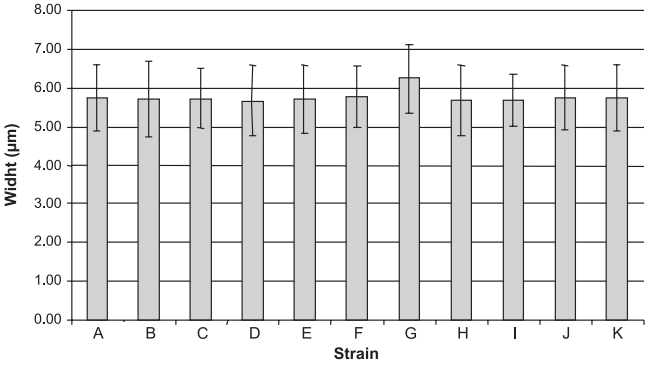

FIGURE 2. Mean width of yeast cells from 24-h cultures in YPD medium (A – K – analysed strains of *S. cerevisiae*: A – I1, B – AFr, C – 2200-3P-J, D – ATCC 2366, E – IRHS, F – I2, G – 2200-4P-J, H – 102, I – 2200, J – HH, K – D1).

Evaluation of physiological properties of *S. cerevisiae* yeast

Taking into account physiological characteristics, the yeast industry seeks for strains characterised by a wide spectrum of utilization of carbon and nitrogen compounds.

The baker's yeast were evaluated for their capacity to assimilate 19 different chemical compounds (Table 1). The results confirmed their affiliation to the species *S. cerevisiae*. It was observed that all strains assimilated glucose, galactose, maltose, and saccharose, whereas did not assimilate lactose, which is consistent with data reported by Vaughan-Martini & Martini [1998].

The results of physiological tests enabled the division of the yeast into three groups that differed in assimilation profiles. The diversity referred to the capacity to assimilate methyl-α-D-glucopyranoside, trehalose, and melezitose. Strain ATCC 2366 assimilated methyl-α-D-glucopyranoside, whereas did not assimilate trehalose and melezitose. Strains: 102, 2200, 2200-3P-J, 2200-4P-J, D1, and IRHS were observed to assimilate all the above-mentioned compounds, whereas strains: I1, I2, HH, and AFr did not assimilate any of them. Thus, it could be concluded that the assimilation capacity of those compounds depends on the strain of *S. cerevisiae* yeast. It has previously been confirmed by Succi *et al*. [2003] and is consistent with findings of Vaughan-Martini & Martini [1998].

TABLE 1. Assimilation profiles of the analysed strains of *S. cerevisiae* yeasts.

	glucose	glycerol	2KG	arabinose	xylose	adonitol	xylitol	galactose	inositol	sorbitol	MDG	NAG	cellobiose	lactose	maltose	saccharose	trehalose	melezitose	raffinose
ATCC 2366	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	+
I1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+
I2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+
AFr	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+
HH	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+
IRHS	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+
2200	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+
2200-3P-J	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+
2200-4P-J	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+
102	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+
D1	+	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+

"+" – positive result (assimilation of a given compound), "-" – negative result (lack of assimilation). Abbreviations used in the table: 2KG calcium 2-keto-gluconate; MDG – methyl-α-D-glucopyranoside; NAG – *N*-acetyl-glucosamine.

Low usability of simple biochemical tests for differentiation of strains within the species *S. cerevisiae* has been earlier confirmed by Hayford & Jespersen [1999] as well as by Foschino *et al.* [2004]. Simultaneously, those authors suggest that the molecular methods can be used for the differentiation.

Genotyping of *S. cerevisiae* yeast strains

Due to small morphological and physiological diversity of the examined strains of baker's yeast, they were identified for their genetic diversification. The identification was conducted with the use of the RAPD method and three primers described as effective in differentiation of some other strains of *S. cerevisiae* [Robak *et al.,* 2005].

The following electrophoretic picture was obtained using primer AB1-12 (Figure 3). Six to eight PCR products were obtained with the RAPD reaction using primer AB1-12. The PCR fragments with sizes of *ca*. 2200 bp, 1700 bp, 1600 bp, 1100 bp, 850 bp, and 650 bp were obtained in the case of each strain. A product with the size of *ca.* 1150 bp was obtained for all strains except for the HH strain. In turn, the line with the size of *ca.* 700 bp was typical only of the ATCC 2366 strain.

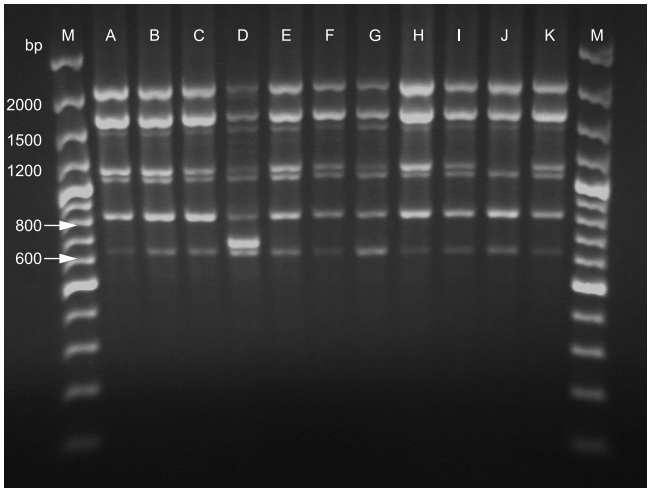

FIGURE 3. Image of electrophoretic separation of RAPD products using primer AB1-12 (M – Gene Ruler 100bp DNA Ladder Plus (Fermentas); A – K – analysed strains of *S. cerevisiae*: A – I1, B – AFr, C – 2200-3P-J, D – ATCC 2366, E – IRHS, F – I2, G – 2200-4P-J, H – 102, I – 2200, J – HH, K – D1).

Primer AB1-12 did not demonstrate high discrimination capacity toward the analysed strains. It enabled discrimination of only HH and ATCC 2366 strains, which constitute two separate groups in terms of an electrophoretic profile. The other strains constituted the third group. Those results indicate small diversity among the strains of *S. cerevisiae* used in bakery. PCR products common for all strains may be typical of species or genus, which may be used in the identification of unknown isolates. In order to confirm that, it would be advisable to carry out additional tests, using AB1-12 primer, covering a greater number of strains and species of yeast.

Experiments using the same primer were conducted by Xufre *et al.* [2000]. They have managed to demonstrate differences between the analysed strains of wine yeast *S. cerevisiae*. Reactions yielded from 5 to 7 bands, yet only one of them was common for all strains. Each of the six yeast strains displayed a different RAPD profile. On the basis of the results of reactions with four primers, the authors observed similarity between the strains that ranged from *ca.* 45% to *ca.* 80%.

Echeverrigaray *et al.* [2000] used a primer called OPB--12 (its sequence is identical to that of AB1-12) and different primers for genetic differentiation of 16 wine strains. In the reaction, the discussed starter yielded 12 various products, including 3 polymorphic ones, which enabled dividing the yeast into groups. However, no profile characteristics for individual strains were obtained in the study. Complete differentiation of the strains was possible only based on several reactions with various primers.

An image as shown in Figure 4 was obtained in the RAPD reaction using primer 24. The RAPD reaction using primer 24 resulted in PCR products ranging from 4 to 9. However,

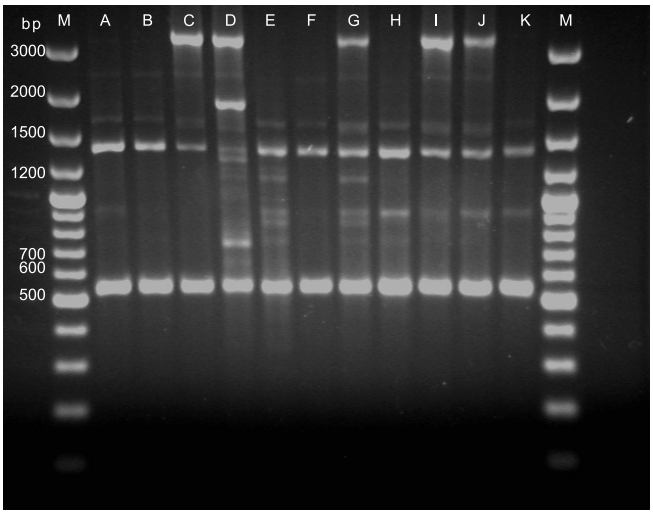

FIGURE 4. Image of electrophoretic separation of RAPD products using primer 24 (M – Gene Ruler 100bp DNA Ladder Plus (Fermentas); A – K – analysed strains of *S. cerevisiae*: A – I1, B – AFr, C – 2200-3P-J, D – ATCC 2366, E – IRHS, F – I2, G – 2200-4P-J, H – 102, I – 2200, J – HH, K – D1).

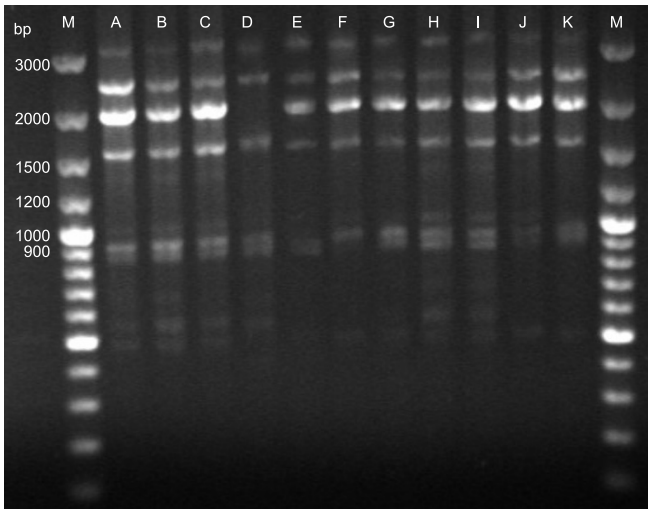

FIGURE 5. Image of electrophoretic separation of RAPD products using primer 21 (M – Gene Ruler 100bp DNA Ladder Plus (Fermentas); A – K – analysed strains of *S. cerevisiae*: A – I1, B – AFr, C – 2200-3P-J, D – ATCC 2366, E – IRHS, F – I2, G – 2200-4P-J, H – 102, I – 2200, J – HH, K – D1).

it should be emphasized that certain bands could not be visualized properly and were not obtained when the reaction was repeated more than 10 times. Thus, analyses were carried out only for products that were obtained each time and could be claimed as fully specific. Lines with the size of *ca.* 1750 bp, 1440 bp, and 555 bp were obtained for all strains. A product with the size of *ca.* 3450 bp was typical of the strains ATCC 2366, 2200, 2200-3P-J, 2200-4PJ, and HH. Lines with the sizes of *ca.* 1980 bp, 1350 bp, and 770 bp appeared only in the case of the strain ATCC 2366.

Either presence or absence of the PCR products described enabled the division of the analysed strains into three groups. The first included strain ATCC 2366, the second – strains 2200-3P-J, 2200-4P-J, 2200, and HH, and the third – the other strains. Differentiation of the strains of baker's yeast using primer 24 is, therefore, not complete, which is likely to be due to relatively high homogeneity of the examined group.

Baleiras-Couto *et al.* [1996], using the RAPD method with three primers, analysed diversity of *S. cerevisiae* isolates contaminating beers and wines. They observed that the strains originating from wine differed in profiles from beer isolates, although they also demonstrated some similarity between the groups. The use of primer 24 allowed the researchers to divide 16 isolates that were examined into 6 groups in terms of different electrophoretic profiles. Among products obtained for all strains, there was one with the size of *ca.* 1440 bp, hence identical with one product common for all strains analysed in our study. The results of investigations seem to confirm a thesis that products occurring in the case of all strains might be claimed markers of species or genus.

Figure 5 shows an electrophoretic picture of products obtained using primer 21. The RAPD reaction using primer 21 yielded 5–6 PCR products. All strains yielded bands with the sizes of *ca.* 3250 bp, 2600 bp, 1650 bp, 950 bp, and 900 bp. A line of 2100 bp was obtained for all strains except for ATCC 2366. Likewise in the case of the previous primer, low-specific products were sometimes obtained in the study, and they were not analysed further.

Primer 21 allowed for differentiation of the strain ATCC 2366 only. Its electrophoretic profile differed from that of the other strains in that it lacked one PCR fragments. The results indicate low usability of the primer used for differentiation of baker's yeast as well as high similarity of the strains examined.

Higher usability of primer 21 for differentiation of *S. cerevisiae* isolates contaminating beers and wines has been confirmed by [Baleiras-Couto *et al.,* 1994]. The above primer generated 6 different profiles, which enabled some differentiation of the strains. In addition, Baleiras-Couto *et al.* [1994] demonstrated that using the primer, it is possible to differentiate effectively yeasts of the species *S. cerevisiae*, *Zygosaccharomyces rouxii,* and *Z. bailii*.

In turn, using the RAPD reaction with primer 21, Barszczewski & Robak [2004] divided 27 strains isolated from beer, from a production line of a brewery, and reference strains into 16 groups. On the basis of electrophoretic profiles, they identified the production strain and demonstrated the presence of diversified contaminating yeast microflora. The authors suggest that the RAPD method combined with PCR-RFLP may serve for routine identification of yeast.

The same authors [Barszczewski & Robak, 2006] investigated genetic diversity of brewing yeast applied to laboratory and industrial practices in Poland. Again, primer 21 displayed heterogeneity for the analysed group of strains – 21 strains were divided into 16 groups. The correlation of those results with the results of the RAPD reaction with different primers allowed for complete differentiation of the strains. A comparison of the above-mentioned results with those obtained in our study demonstrated considerably lower diversity of baker's strains as compared with the strains that acted as contaminants in the brewery and wine-making industry.

Tornai-Lehoczki & Dlauchy [2000], using primer 21 for RAPD, differentiated brewing yeast of "ale" type from those

of "lager" type. In addition, they demonstrated high similarity of "ale" yeast profiles to reference strains of *S. cerevisiae*, as well as similarity of "lager" yeast to reference strains of *S. pastorianus*. They showed relatively high diversity in the "ale" group and high homogeneity in the "lager" group.

A combined analysis of RAPD profiles obtained by means of the three primers made it possible to construct a dendrogram (Figure 6) depicting relative similarity between the strains.

The analysed strains were divided into four groups. The first included strains I1, AFr, IRHS, I2, 102, and D1 that demonstrated 100% similarity. The second group included strain HH. The third group comprised strains 2200, 2200-3P--J, and 2200-4P-J that did not show any differences between one another. In turn, strain ATCC 2366 constituted the fourth group, differing from the others by *ca.* 15%.

The results obtained indicate small differences between the strains. Some of the strains show 100% similarity with one another; therefore, application of even three examined primers does not allow for their differentiation. Only strains ATCC 2366 and HH form separate single-element groups, which enables their identification by means of the RAPD reaction. Such a result necessitates testing other primers in order to find those that would make it possible to completely differentiate strains of baker's yeast.

It was observed that morphological diversity of strain 2200-4PJ (considerably greater sizes of cells as compared with other strains) was not reflected in its genetic properties. On the basis of the RAPD reaction, it was classified into the same group with 2200 and 2200-3P-J strains whose cells had standard sizes. The three above-described strains (together with several others) were also classified under one group due to their assimilation profile. Taking this into account as well as considering their similar names, it may be assumed that they originate from one source and differ only to a negligible extent or even that they are the same strains.

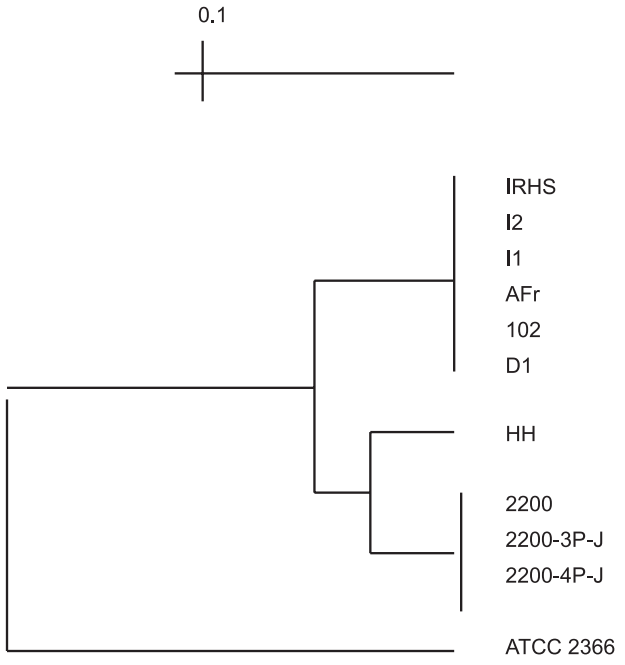

FIGURE 6. Dendrogram of baker's *S. cerevisiae* yeast.

Division into groups obtained based on assimilation of various sources of carbon is not reflected (with one exception) in the division due to the RAPD profile. Only the ATCC 2366 strain, which constituted a separate group in assimilation tests, showed specificity for the RAPD profiles in the case of each primer.

SUMMARY

The analysis of assimilation and genetic profiles showed both similarity and differences between the investigated strains. As a result, the following groups of strains of baker's yeast have been selected: I group: 2200, 2200 – 3PJ, 2200-4PJ; II group: I1, I2, AFr; III group: IRHS, 102, D1; and IV group: HH.

The strain ATCC 2366 showed different profiles in both methods regarding to the investigated strains of bakers yeasts, which proved the usefulness of these methods in strains discrimination within the *S. cerevisiae* species.

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