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A NEW WAY OF IDENTIFICATION OF YEASTS: USE OF NIR TECHNIQUE

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Abstract

Four strains of Saccharomyces genera were investigated: *S. pastorianus* CBS 1503, *S. cerevisiae* CBS 1395, *S. cerevisiae* CB 67 and *S. cerevisiae* CB 89. The aim of our work was to test the suitability of NIR technique for rapid identification of different yeasts. Yeasts were grown in batch culture and samples were taken from the exponential and stationary growth stages, later ones were divided into parts and one of them was heat-shocked.

The four yeasts tested were morphologically different, the CBS signed showed oval, the CB signed ones had round shaped cells.

Optimum growth temperatures were all in the range of 30–35 °C, and at 30 °C growth rates of mixed cultures did not differ significantly from those of monocultures.

The SDS-PAGE protein prints of the four strains were different both in the number of subfractions and their molecular mass distributions. This method is suitable to characterize and differentiate the investigated yeasts . Quantitative evaluation of the SDS-PAGE patterns enables us to detect infections in the pure culture.

NIR spectra of the investigated strains were significantly different. The growth stage or heat-shock treatment had serious effect on the spectrum. Mixtures of two pure cultures (0, 10, 20... 90% 'infection') showed NIR spectra in between those of the pure culture and even 10% contamination caused a significant difference.

Keywords: yeast identification, SDS-PAGE, NIR technique.

Introduction

The classification of yeasts has a long history, the main characteristics which are used to differentiate are:

- the microscopical appearance of the cells [1]
- the mode of sexual reproduction [2]
- certain physiological activities and
- certain biochemical features [3, 4, 5, 6].

All of these methods are highly time and material consuming or need expensive equipment. GORIN and SPENCER [7] applied proton magnetic

resonance spectroscopy in the identification and chemotaxonomy of yeasts, HALÁSZ and MÁTRAI [8] reported on the successful application of SDS-PAGE in yeast identification.

Near infrared reflectance spectroscopy (NIR) is widely used as a rapid, economical, nondestructive and simple technique for analytical purposes [9, 10, 11]. However, the NIR–technique has not been mentioned as a method of identification of yeasts until now.

The aim of our work was to investigate the capabilities of NIR spectroscopy in the differentiation of yeast strains and in the detection of infections in pure cultures.

Materials and Methods

Investigated yeast strains: *S. pastorianus* CBS 1503, *S. cerevisiae* CBS 1395, *S. cerevisiae* CB 67 and *S. cerevisiae* CB 89.

Yeasts were subcultured in synthetic medium on a shaker at 30 °C. Samples for analysis were taken from the exponential and stationary growth phases, harvested by centrifugation.

For heat shock treatment exponentially growing cells were harvested, washed twice with distilled water and cell suspensions in 0.6 mol/l KCl buffer were either pre-incubated at 30 °C for 30 min (control sample) or subjected to heat shock at 55 °C for 10 min and then incubated at 50 °C for 30 min (experimental sample). Heat-treated cells were cooled to 30 °C and harvested.

Near infrared analysis of yeast samples was done with a NIR system 6250, monochromator: grating; detector PbS reflection type; measurement range 1100–2500 nm; step 2 nm; operation: manual; software: NSAS 2–31/USA; mode detector: reflection; classification: algorithm is PCA (principle component analysis).

Morphological observation of the yeast was performed directly under phase contrast microscope.

Growth rates were calculated from growth curves determined at different temperatures.

Progress of growth was studied by changes in optical density.

SDS-PAGE protein print of yeast cultures was prepared from the water-salt soluble protein fraction of the cells. Electrophoresis was carried out according to LAEMMLI [12].

Results and Discussion

Phase contrast observations of the investigated yeast strains exhibited morphological differences in the shape of the cells: CB 67 and CB 89 strains had round shaped cells while the CBS 1503 and CBS 1395 strains showed oval ones.

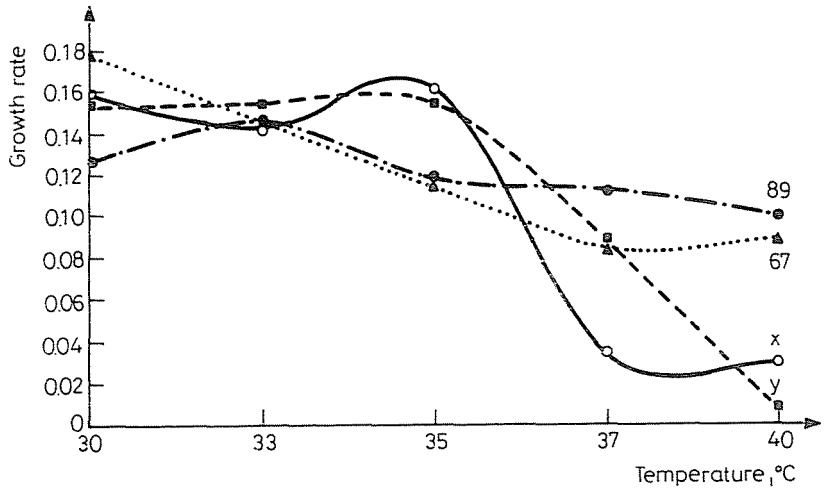

Fig. 1. Growth rate of the investigated strains at different temperatures
X – *S. pastorianus* CBS 1503 Y – *S. cerevisiae* CBS 1395
67 – *S. cerevisiae* CB 67 89 – *S. cerevisiae* CB 89

The optimum growth temperatures of the tested strains were calculated from the growth rates ($\Delta OD/dt$) taken of the growth curves determined for selected temperatures. The observed growth optima for *S. pastorianus* CBS 1503, and *S. cerevisiae* CBS 1395, were 35 °C and 30–35 °C, respectively, and for *S. cerevisiae* CB 67 and *S. cerevisiae* CB 89 < 30 °C and 33 °C, respectively (*Fig. 1*).

The water-salt soluble protein fraction separated by SDS-PAGE showed different prints in the number of subfractions and their mobilities, too (*Table 1*).

Quantitative evaluation of the electrophoregram by Biotect-Fisher densitometer showed that subfractions which could be used for differentiation were present only in small quantities, which means that the presence of a contaminating yeast strain might be detected only above a concentration of 20–30%.

NIR spectra were determined for each strain, samples were taken from exponential and stationary growth phases and after heat shock treatment.

Table 1
Heat shock protein for the tested yeast strains

Yeast strain	Labatt code	Number of subfractions	App.molecular weights of HSPS (KDa)
S. pastorianus	CBS 1503	7	123, 62, 47, 41, 34, 27, 5
S. cerevisiae	CBS 1395	3	45, 36, 30
S. cerevisiae	CB 67	9	93, 58, 49, 41, 39, 33, 28, 21, 16
S. cerevisiae	CB 89	10	54, 45, 41, 37, 35, 32, 28, 20, 16, 11

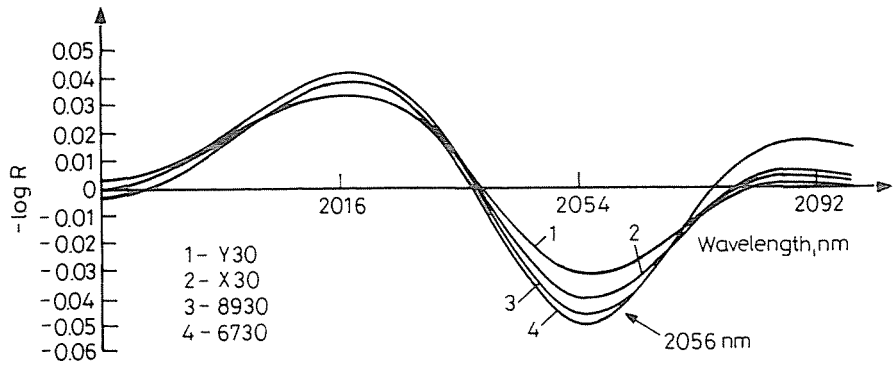

Fig. 2. 2OFD spectra of the investigated strains
X 30 – *S. pastorianus* CBS 1503 Y 30 – *S. cerevisiae* CBS 1395
67 30 – *S. cerevisiae* CB 67 89 30 – *S. cerevisiae* CB 89

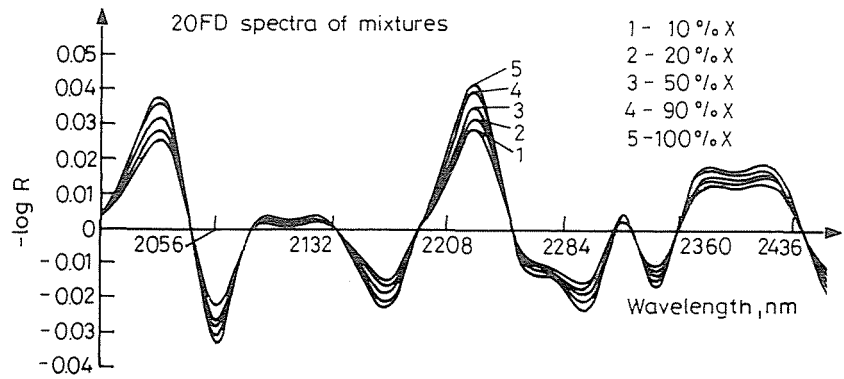

Fig. 3. NIR spectrum of the mixture of *S. pastorianus* (X) and *S. cerevisiae* (Y)

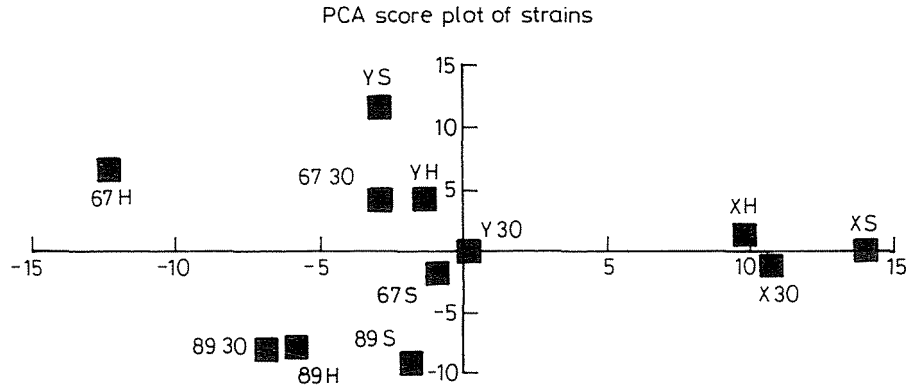

Fig. 4. PCA diagram of the yeast strains (H–heat shock treated) (30 – not treated) (S – from stationary phase)

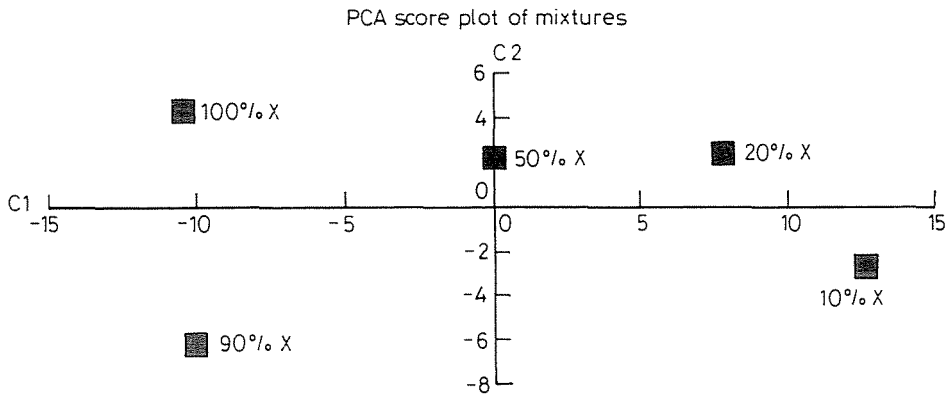

Fig. 5. PCA diagram of yeast mixtures (X – amount of *S. pastorianus* in the mixture)

The 2OFD spectra of the investigated yeasts showed significant differences (*Fig. 2*). Mixtures prepared from *S. pastorianus* (X) and *S. cerevisiae* (Y) showed different peak values in all cases (*Fig. 3*).

PCA score plots of the four yeast strains show that the NIR technique enables us to differentiate between growth phases and heat shocked and untreated yeasts, respectively, of the same strain and between strains also (*Figs. 4, 5*).

This method is also suitable to detect the presence of 'contamination' even at a concentration of 10%.

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