Yeast identification

The isolates were identified according to the current methodology used at the German Collection of Microorganisms and Cell Cultures (DSMZ Braunschweig; HOFFMANN pers. comm.).

This methodology makes use of the API50 CH test kits (Biomérieux), supplemented by a fermentation test (glucose), a nitrate utilisation test, temperature tolerance tests (if necessary) and microscopic morphology (cell shape, pseudomycelium formation, sexual reproduction).

Figure 1: Yeast on potato-glucose-agar

For the physiological tests the yeast isolate was grown on potato-glucose-agar and one colony (2-3 mm Ø) suspended in 1 ml sterile distilled water.

API50 CH test

For the API50 test strips, a drop of the yeast suspension was given to a warm solution of 0.3% (w/v) agar with 10 % (w/v) Yeast Nitrogen Base concentrate and distributed evenly before filling the test strips and incubation at 25 °C for 3 to 4 weeks.

A first visual evaluation was done after 3 to 5 days and again in the second and third week.

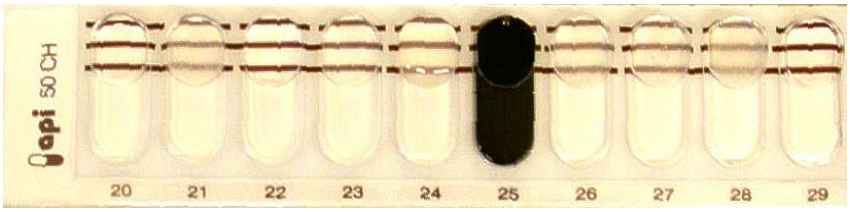

Figure 2: API50 test strip

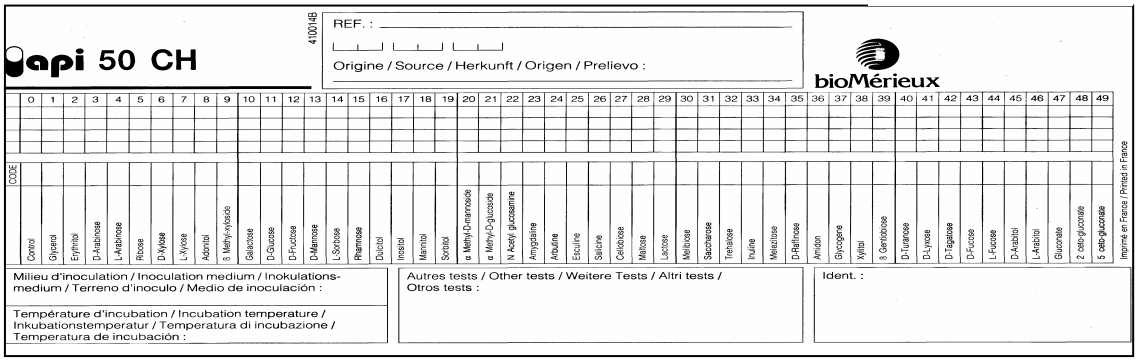

Figure 3: Evaluation of the API50 test strip

Fermentation test

For the fermentation test, a drop of the yeast suspension was given to 5 ml of a 2 % (w/v) glucose solution with 10 % (w/v) Yeast Nitrogen Base. The liquid was sealed with a paraffin mixture solid at room temperature and kept at 25°C. Gas formation under the paraffin was regarded as a positive reaction.

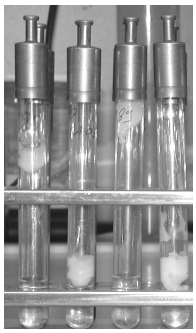

Figure 4: Fermentation test

Nitrate test

Nitrate medium:

1.4 g KNO_3, 1.6 g Yeast Carbon Base, 0.12 g bromothymol blue, 1000 ml distilled water, 15 g agar; pH 5.9-6.0 (yellow colour).

A drop of the yeast suspension was placed on nitrate medium on slant cultures. A colour change from yellow to blue marks a positive reaction.

Figure 5: Nitrate test

Temperature tolerance

For this the yeast suspension was placed on potato-glucose-agar slants and incubated at 30, 35, 37 and 40°C exact temperature. If growth was observed they were regarded as tolerant to the particular temperature.

Morphology

To observe the morphology by light microscopy the yeast was inoculated on corn meal agar as a point and a line and covered with two coverslips. The plate was incubated at 25 °C and first evaluated after one week for a duration of 4 weeks in total.

Identification

The assessment was done with the help of the software ‘Yeasts of the World’ (BOEKHOUT et al., ETI Amsterdam 2002) with the additional aid of The Yeasts – A Taxonomic Study (KURTZMANN and FELL, 1998).

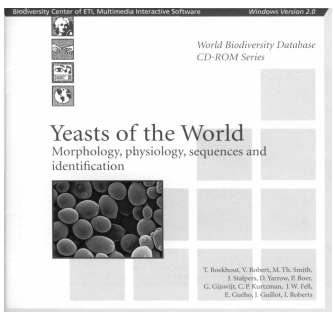

Figure 6: Identification software