Growth of Hansenula holstii on Cadavers

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Growth of a yeast was observed on prosected cadavers used for demonstration purposes in a medical school. An asporogenous yeast was isolated and identified as an atypical form of Hansenula holstii by analysis of the extracellular polysaccharide. The isolate showed resistance to embalming fluid but was eventually eradicated by addition of piclodoxine digluconate to the fluid.

Cadavers acquired by the Department of Anatomy, University of Glasgow, are preserved by infusing 8 to 12 liters of a preservative solution (eight parts methanol, and one part each of formaldehyde, phenol, glycerol, and water) under pressure into the femoral artery. Certain cadavers are prosected for demonstration purposes and stored "dry" in polyethylene bags at room temperature. They are moistened periodically by spraying with a 50% solution of the preservative and generally remain usable for up to 2 years. It was on such specimens that the growth of a yeast was noted, and about 15% were affected during several years in succession. The growth, manifested by foul-smelling, slimy, white patches, occurred on any part of the cadavers. It spread on and between the tissues, often extending quite deeply, and the affected cadavers rapidly became unfit for use. Other prosected cadavers kept for examination purposes at 4 C were never affected. This report concerns the identification of an unusual strain of Hansenula holstii Wickerham as the yeast involved and the methods used for preventing its development on cadavers.

MATERIALS AND METHODS

Isolation and identification of the yeast. The yeast was isolated on a number of occasions from two of the affected cadavers on 4% malt extract (ME) agar (9), and a representative isolate, NRRL Y-7178, was selected for study. The culture techniques used for identification were described by Wickerham (9, 12). Later the composition of the extracellular polysaccharide was determined by the procedures for purification and analysis as described by Slodki et al. (7).

Tolerance to embalming fluid and piclodoxine digluconate. The tolerance of NRRL Y-7178 to embalming fluid was compared with five other yeast species, Cryptococcus neoformans (Sanfelice) Vuillemin, C. albicans (Saito) Skinner var. diffluens (Zach) Pfaff et Fell, Torulopsis glabratra (Anderson) Lodder et de Vries, Rhodotorula rubra (Demmke) Lodder, and Candida albicans (Robin) Berkhout. Tolerance was determined by inoculating glucose-peptide broth containing 0.65 to 5% concentrations of embalming fluid.

The sensitivity of the cadaver yeast to the preservative piclodoxine digluconate (Resiguard, Nicholas Laboratories, Ltd., Bucks, England), was determined by inoculating glucose-peptide broth containing this compound at concentrations from 1:100 to 1:5,000.

All cultures were incubated at 24 C, checked for visual growth, and subcultured after 3 days.

RESULTS

Colonies of NRRL Y-7178 were white to greyish-white and quite mucoid. Budding was multilateral and the cells measured 2.0 by 2.5 µm to 3.5 by 5.0 µm. Neither hyphae nor pseudohyphae were produced on Dalmau plates (9) of yeast morphology agar or corn meal agar. There was no sporulation at 15 or 25 C on ME, yeast-malt (YM), V-8 (9), corn meal, or Gorodkowa agars (8), or on carrot, cucumber, and gyspum blocks. Addition of 2, 5, 10, and 20% glucose or sodium chloride to YM and V-8 agars to increase osmotic pressure did not induce sporulation.

The strain assimilated nitrate and the following carbon compounds: d-glucose, maltose, sucrose, cellobiose, trehalose, melezitose, d-xylene, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine-hydrochloride, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, 2-methyl-D-glucitol, salicin, potassium D-gluconate, pyruvic acid (weak), succinic acid, and citric acid (weak). Compounds not assimilated were d-galactose, l-sorbose, lactose, melibiose, raffinose, inulin, soluble
starch, erythritol, galactitol, calcium 2-keto-D-glucarate, potassium 5-keto-D-glucarate, potassium sodium saccharate, lactic acid, and inositol. D-Glucose was fermented but not D-galactose, maltose, sucrose, lactose, and raffinose. This particular set of characteristics made the strain unlike previously described species of imperfect yeasts.

Analysis of the extracellular phosphomannan, however, was quite revealing. As shown in Table 1, the polymer formed from glucose (5 g/100 ml) was indistinguishable from phosphomannans elaborated by Hansenula holstii strains, especially the haploid strain NRRL Y-2154 (7). D-Mannose and D-mannose 6-phosphate were the sole products of vigorous acid hydrolysis (2 N HCl, 100 C, 1 hr). Autohydrolysis, i.e., conversion to the phosphomonoester form by heating decationized phosphomannan (pH 2.5, 100 C, 30 min) gave rise to a mixture of phosphorylated oligosaccharides and a monosaccharide phosphorylated mannan fragment. The high- and low-molecular-weight components were separated by gel chromatography (M. E. Slodki et al., Proc. 4th Int. Ferment. Symp., in press). The phosphorylated mannan fragment gave a strong precipitin reaction with concanavalin A, whereas the intact phosphomannan gave a weak reaction. As judged by paper electrophoresis in 0.05 M barbital, the oligosaccharide phosphates were a mixture of monosubstituted esters apparently containing 4 to 6 mannose units. The pentasaccharide phosphate ester was the predominant component. All these results are consistent with previous findings on the phosphomannans of H. holstii (4). As with most other phosphomannan-producing yeasts, an extracellular neutral mannan was alternatively formed when orthophosphate was omitted from the fermentation medium (5). The neutral mannan gave the same pattern of enzymatic degradation (M. E. Slodki et al., in press) observed when other H. holstii mannans were incubated with Arthrobacter α-mannosidase.

Attempts to mate NRRL Y-7178 with the mating types of H. holstii NRRL Y-2154 and Y-2155 were carried out on ME agar according to Wickerham (10), and on the restricted growth (RG) medium of Herman (2). There was no evidence of conjugation or sporulation on either of these media.

The cadaver yeast was significantly more tolerant to the embalming fluid than the other yeasts tested. It grew well in broth containing 2% preservative, a fungistatic effect was seen at 3%, and above 3% concentration the embalming fluid was fungicidal. Four out of the five test yeasts were killed by a concentration of 0.5% preservative and the fifth (C. albicans) by 1%; none showed growth above 0.2% concentration.

Picloxidine digluconate was fungicidal to the cadaver yeast at concentrations as low as 1:5,000. The compound was successfully employed as a preservative of cadavers at a 1:100 concentration in the presence of 0.5% embalming fluid.

**DISCUSSION**

Peterson (3) was apparently the first to report the isolation of a yeast from cadavers. His isolate, extremely tolerant to embalming fluid, was described eventually as the new species Hansenula petersonii Wickerham (11). Both the fermentation and assimilation patterns of this species differ from those of H. holstii (12).

A common habitat of H. holstii is the frass of coniferous trees and gums of fruit trees (10, 12). Strain Y-7178 differs from the usual isolates not only in habitat, but by its failure to produce hyphae or pseudohyphae and by its

**Table 1. Characterization of phosphomannans and mannans from Hansenula holstii NRRL Y-2154 and strain NRRL Y-7178**

| Strain     | Phosphomannan | Mannan       |
|------------|---------------|--------------|
|            | Yield (g/100 ml) | [α]_D | Molar ratio (M : P)* | Mild hydrolysis products (%) | Yield (g/100 ml) | [α]_D |
|            |               |      |                        | Phosphorylated mannan | Oligosaccharide-P* |       |      |
| NRRL Y-2154 | 3.0           | +93° | 5.2                     | 9.8                   | 90.2             | 3.2    | +89° |
| NRRL Y-7178 | 1.7           | +93° | 5.0                     | 11.0                  | 89.0             | 1.5    | +85° |

* Mannose-phosphorous.
* M : P for strain NRRL Y-2154 = 6.6; strain NRRL Y-7178, 7.5.
* M : P for strain NRRL Y-2154 = 4.3; strain NRRL Y-7178, 5.3.
inability to assimilate D-galactose, L-sorbose, and soluble starch. It also lacks mating competence but so also do many of the haploid isolates of \textit{H. holstii} collected from frass of coniferous trees (12). Many isolates of this species also vary in their ability to assimilate carbon compounds and in the degree to which they produce hyphae and pseudo hyphae. Nevertheless, Y-7178 could not, with any confidence, be assigned to \textit{H. holstii} were it not that its extracellular phosphomannan corresponds to that of this species and is, in fact, indistinguishable from the phosphomannan of Y-2154, a sexually reactive haploid strain (4). The specificity of extracellular polysaccharides has clearly been demonstrated (6, 7), but the procedure has seldom been used for purposes of identification.

Picloxidine digluconate has previously been shown to be useful in retarding various types of postmortem breakdown when incorporated in preserving solutions (1). The strain of \textit{H. holstii} isolated from the cadavers was sensitive to concentrations as low as 1:5,000 but because of other beneficial effects reported (1), the preserving solution was supplemented with picloxidine digluconate at a concentration of 1:100. Since this recommendation was followed, no trouble with microorganisms attacking protected specimens has been encountered for 3 years.

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