Breakdown of Cellulose by Yeast Species

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Yeasts as defined by Lodder (1970) are collectively capable of utilizing many different substrates, including mono-, di-, and trisaccharides, polyols, ethanol and organic acids. Some yeasts are capable of utilizing polysaccharides, but the attack is usually limited to soluble starch, inulin, and more rarely pectin (Luh & Phaff, 1951; Bell & Etchells, 1956; Wieringa, 1956; Ingram, 1958), and as far as the author is aware there are no previous reports of yeasts attacking cellulose (cf. Ingram, 1958; Do Carmo-Sousa, 1969). This communication reports cellulolytic activity by *Trichosporon* species.

During an investigation of the microbial flora of forced rhubarb (*Rheum rhaponticum* var. Timperly Early and var. Early Victoria) *Trichosporon cutaneum* was isolated from freshly harvested petioles and leaf blades in numbers up to 2.5×10^3 and 3.5×10^4 per g respectively. This yeast was also frequently isolated from the leading edge of rotted areas of petioles, sometimes in association with pectolytic bacteria. Inoculation of surface-sterilized cut petioles of rhubarb with *T. cutaneum* isolates confirmed that this yeast could act as a primary invader. Pectin and cellulose are the main constituents of plant cell walls, hence the ability of these isolates to degrade these substances was examined.

Pectin breakdown was tested by growing the yeasts on yeast extract-glucose-peptone-malt extract agar (Wickerham, 1951), containing 0.5 % calcium chloride, overlaid with 2 % sodium polypectate. All the isolates of *Trichosporon cutaneum* degraded pectate as shown by the formation of craters around the colonies. Isolates of *T. pullulans*, *Cryptococcus macerans* and *C. albidus* var. *albidus* also were observed to degrade pectate. This is contrary to the findings of Di Menna (1959).

Cellulose breakdown was tested by the method used by Bravery (1968) to demonstrate the cellulolytic activity by soft-rotting fungi of wood. The mineral salts-cellulose media (media I and VII - see Bravery, 1968) were used except that the concentration of cellulose (ball-milled Whatman no. 1 filter paper) was reduced to 0.5%. Incubation was at 20 °C for 3 weeks. Cellulolytic activity was indicated by the appearance of clear zones around the yeast colonies in the opaque cellulose media.

Nine out of 12 strains of *Trichosporon cutaneum* and 12 out of 13 strains of *T. pullulans* showed cellulolytic activity. The most active strains showed this activity after 6 to 7 days of incubation at 20 °C. The presence of small amounts of yeast extract (0.5 mg/ml) and asparagine (0.5 mg/ml) stimulated cellulolytic activity in some of the isolates of *T. cutaneum* and *T. pullulans* but reduced and/or delayed activity in others.

Levinson, Mandels & Reese (1951) reported cellobiose to be the main end-product of cellulose hydrolysis by the mould *Trichoderma viride*. Yeasts, other than *Trichosporon* species, and the yeast-like organism *Aureobasidium pullulans*, all of which can utilize cellobiose as sole carbon source, were therefore assayed for cellulolytic activity. With the same test no cellulolytic activity was observed with ten strains of *A. pullulans*, three strains of *Torulopsis fragaria*, two strains of *Kloeckera apiculata* and single strains of *Cryptococcus*

laurentii, *C. laurentii* var. *flavescens*, *C. diffluens*, *C. albidus*, *Torulopsis candida*, *Hansenula anomala*, *H. saturnus*, *H. silvicola*, *Pichia pinus*, *P. etchellsii*, *Endomyces capsularis*, *Debaryomyces hansenii* and *Saccharomycodes ludwigii*. The inability of *A. pullulans* to degrade cellulose is in agreement with previous work (White, Darby, Stechert & Sanderson, 1948).

The cellulase activity of two strongly cellulolytic strains (on the basis of plate clearing) of each of *Trichosporon cutaneum* and *T. pullulans* was studied further. The ability to attack a less degraded form of cellulose was tested with Whatman no. 1 filter-paper discs, 5.5 cm in diameter. The strains were grown in shaken liquid culture on media I and VII (without agar) at 20 °C on a gyratory shaker (200 rev/min). Degradation of the filter-paper cellulose was visible after 7 days of shaking at 20 °C. The filter-paper discs were completely macerated after 14 days of incubation, when up to a 10 % loss in dry weight was determined by filtering off the residual cellulose.

Table 1. *Release of reducing sugars from cellulose by a culture filtrate of Trichosporon cutaneum*

Reaction mixture consisted of 2 ml culture filtrate plus 8 ml of 1 % cellulose substrate in NaOH/succinic acid buffer pH 5.0 (see Reese, Siu & Levinson, 1950). Culture filtrate from *T. cutaneum* (strain Y 1) grown on medium I for 14 days at 20 °C.

Total reducing sugar was estimated on 1 ml. samples by the Somogyi-Nelson method (Whistler & Wolfrom, 1962). Samples taken at time 0 were used as blanks. The Boehringer Blood Sugar GOD-Perid-Method was used for estimating glucose. The protein content of the filtrate (128 µg/ml) was estimated by the Folin-phenol method of Lowry, Rosebrough, Farr & Randall (1951), after dialysis against distilled water at 2 °C for 24 h. Bovine plasma albumen was used as a standard.

Carboxymethylcellulose (sodium salt, low viscosity)			Ball-milled Whatman no. 1 filter-paper cellulose		
Time (h)	Total reducing sugar* (µg/ml)	Glucose (µg/ml)	Time (h)	Total reducing sugar* (µg/ml)	Glucose (µg/ml)
2	4.0	3.0	6	10.0	9.0
4	13.0	8.0	24	32.0	21.0
6	44.0	14.0	30	82.0	29.0
24	94.0	13.0	48	132	34.0

* Expressed as equivalent glucose.

The presence of cellulase in culture filtrates was shown by filling a well, cut in an agar plate containing 0.25 % ball-milled filter-paper cellulose, with filtrate. After 48 h at 20 °C the clearing zones around the well were recorded.

When culture filtrates were incubated with ball-milled filter-paper cellulose on carboxymethyl cellulose, reducing sugars were released (Table 1). In both cases most of the reducing sugar in the early stages could be accounted for as glucose. Thereafter, there was little increase in release of glucose, although total reducing sugar continued to increase. Examination of the hydrolysate by paper chromatography, as described by Levinson *et al.* (1951), showed that cellobiose was the only other reducing sugar detected.

The glucose released was less than 1 % of the theoretical maximum. This yield is relatively low, but the conditions used were not necessarily optimal for production or activity of cellulase. Katz & Reese (1968) suggest a 5 % yield as more usual with other fungal enzymes, though a value as high as 30 % has been observed with *Trichoderma viride* cellulase.

Cellulase production appeared to be restricted to *Trichosporon* species and therefore may be of use in yeast identification and classification. However, most of the yeasts used here, other than *Trichosporon cutaneum* had been maintained in culture for at least a year on media lacking cellulose, and it is possible that cellulolytic activity may have been lost under

these circumstances. Similarly, strain differences may account for the discrepancy between the present observations and those of Di Menna with regard to pectolytic activity.

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