coagulase-positive. Neither the susceptibility nor the resistance of the coagulase-positive staphylococci was associated with any particular phage type or pattern of antibiotic susceptibility.

The nature and mode of action of such an unusually specific inhibitory agent are of considerable biochemical interest, and identification of the active component is at present being attempted. Meanwhile, in view of the general use of 'Scotch' tape in bacteriology, mycology and virology, the knowledge that its vapour can be biologically active has obvious practical implications.

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Degradation of Yeast Cell Wall by Fractionated Snail Gut Enzyme

Protoplasts are reported as being formed from cells of *Saccharomyces* species by the action of snail crop juice[1], autolysis[2] pounding[3] or enzymes from other micro-organisms[3]. The chemical mechanisms underlying the process have not been fully elucidated.

The cell wall of *Saccharomyces* is composed mainly of glucan, mannan and protein[4–6]. Lipid and possibly hexosamine are also present[5]. It is generally agreed that the mannan and protein components of the cell fabric are closely associated. They can, however, be removed by means of alkali to leave an envelope of glucan which has contours similar to that of the original wall[4].

When cells are treated with snail crop juice, considerable variation in susceptibility is revealed as between one yeast strain and another of the same species, and also between active and stationary-phase cells of the same strain[1]. The work reported here is part of a programme of investigation into yeast cell-wall structure using enzymatic methods of degradation and into the mechanism of protoplast formation.

Crop juice from the snail *Helix pomatia* was dialysed and lyophilized and 20 mg quantities were fractionated on a 'Sephadex *G*–75' column (40 cm × 2 cm²). Elution was effected using 0·85 per cent w/v sodium chloride, at 5° C. Fractions (2 ml.) were collected and the process followed by examining their absorption at 280 mμ. They were also examined for their activity against intact cells and cell walls of strains of *Saccharomyces* from the National Collection of Yeast Cultures (N.C.Y.C.). The cell walls were prepared by disintegration of whole cells by agitating with glass beads and subsequent thorough washing[4]. Cell walls or whole cells (about 1 mg dry weight) were suspended in 1·0 ml. 0·01 M citric acid/disodium hydrogen phosphate buffer at *p*H 5·8 and incubated at 37° for 30 min with 1·5 ml. of the enzyme fractions. After removal of solid material by centrifugation, the supernatant fluid was deproteinized by adding 0·5 ml. 15 per cent trichloroacetic acid, de-salted using 'Dowex 1' and 'Amberlite *IR* 120' and freeze dried. The lyophilized material was taken up in a small quantity of water and chromatographed on Whatman No. 4 paper using as solvent methylethyl ketone, 9: acetic acid, 1 : water, 1 : the whole being saturated with boric acid[7]. Development was effected by spraying with aniline phthalate reagent[8].

When the individual fractions were incubated with cell-wall preparations of strains N.C.Y.C. 74*S* and 366, which formed protoplasts readily with the crude enzyme, the results showed that the enzyme activity could be fractionated into at least three components, one of which released both mannose and glucose, the others releasing glucose only. All three components gave rise in addition to what was assumed to be short-chain polysaccharide; this was particularly prominent when strain 366 had been treated. Confirmation that the spots on the chromatograms were glucose and mannose was obtained by running parallel chromatograms in a solvent system (butanol 45, ethanol 5, water 49, ammonia 1), dissimilar from that above. Glucose oxidase[9] was also used to establish the authenticity of the glucose in the hydrolysates. No other hexoses were detected nor was there any evidence of hexosamine.

Cell-wall preparations of two strains of yeast which did not form protoplasts with the crude enzyme (N.C.Y.C. 239, 1056) behaved differently on being tested with enzyme fractions. No mannose was released, glucose only appearing on the chromatograms. In these cases all the eluent fractions from the column behaved in the same way and there was no apparent division into components.

However, when living or heat-killed (65° for 10 min) intact cells of the resistant strains 239 and 1056 were incubated with enzyme fractions, no release of hexose could be demonstrated. There thus seems to be important differences between the inner and outer wall surfaces in their susceptibility to attack. When the cell-wall materials from the four strains were hydrolysed for 3 h at 105° with 2 N sulphuric acid, glucose and mannose were found in all the hydrolysates.

The ability of the eluent fractions from the 'Sephadex' column to form protoplasts was tested by incubating them at 30° with a suspension of log phase intact cells of the susceptible strains 74*S* and 366 in 0·01 M citrate + phosphate buffer *p*H 5·8, using 0·6 M mannitol as osmotic stabilizer. Microscopic observations were carried out during 48 h incubation. The regular ellipsoidal appearance of the cells was modified suggesting attack on the walls in each case, but protoplasts were only observed with the fractions which gave rise to both glucose and mannose when incubated with cell-wall preparations.

These results suggest that to achieve protoplast formation in the two susceptible strains investigated the mannan moiety of the cell wall must be degraded in some manner. Important differences in the accessibility or structure of the mannan in the cell walls of different yeast strains are also indicated. It might be interesting to see the extent of wall attack that the cell will tolerate without impairment of its capacity to grow and divide.

Two distinct membranes were reported in yeast cell walls by Northcote and Horne[4]; furthermore, Eddy[5] has suggested that the mannan–protein complex overlays the glucan. Our observations support this view as glucan is seemingly attacked in resistant strains only when the 'inside' of the wall is accessible.

Nečas[2] has suggested that since he could not detect extracellular enzymes in the protoplast suspension which he prepared autolytically, lysis must take place from within. The mechanism which we have been studying appears to be different, however, resembling that described by Eddy and Williamson[1]. No doubt several different treatments result in protoplast formation from whole cells by mechanisms which in some respects are dissimilar. Our findings suggest that using certain active components of snail crop juice protoplasts are only formed when both the mannan and the glucan of the cell wall are at least partially degraded. Attack on the glucan alone does not of itself lead to protoplast formation.

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