PROTEIN AS A SPECIFIC CELL SURFACE COMPONENT IN THE MATING REACTION OF HANSENULA WINGEI

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Cells of the haploid mating types of Hansenula wingei (NRRL Y-2340) exhibit a marked attraction for each other when mixed. The result is a marked agglutination which results in a hydrophobic cell mass. This phenomenon has been developed into a quantitative assay for agglutinability which has made it possible to study the mechanism of this attraction (Brock, J. Bacteriol., 75, 607, 1958). Work to be published elsewhere has shown that the two mating types do not differ in electrostatic charge or degree of hydration. Rather, the agglutination seems to be related to the presence of specific cell components on the two strains which are complementary to each other, and the reaction may be analogous to an antibody-antigen reaction. In the work to be reported here, it will be shown that one of the mating types (strain 21) requires a specific protein for agglutination, whereas the other mating type (strain 5) does not require such a protein. This is the first demonstration of a molecular basis for mating type compatibility in a fungus.

The components responsible for agglutination exist on the cell wall. Cell walls isolated by mechanical disintegration with glass beads exhibit strong agglutination, even after extensive washing. However, since this yeast is difficult to disintegrate quantitatively, the work to be reported here is with whole cells prepared as described previously, and killed by heating to 100 C for 5 min. This heating intensifies the agglutination and eliminates autolysis during storage.

When cell suspensions containing about 500 mg wet weight per ml were incubated with 1000 µg per ml of trypsin in 0.02 M tris(hydroxymethyl)aminomethane (Tris) buffer, a rapid removal of protein from the cell surfaces occurred. After 1 hr at 37 C, the cells were removed by centrifugation, washed, and assayed for agglutinability. The supernatant fluids were assayed for protein with the Folin phenol reagent. The results in table 1 demonstrate that only the agglutinability of strain 21 is affected, although almost identical amounts of protein are removed from both strains. Further tests with lower concentrations of trypsin have resulted in essentially the same amount of protein being removed in a longer period of time. Anthrone tests indicate that the trypsin is also removing carbohydrate material. Eddy (J. Inst. Brewing, 64, 19–21, 1958) has shown that trypsin and other proteolytic enzymes are able to remove a protein-mannan complex from the surface of flocculant brewer’s yeasts which is apparently necessary for flocculation (agglutination) to occur. The conclusion seems reasonable that trypsin is removing a carbohydrate-protein complex from the surface of cells of H. wingei. Although almost identical amounts of protein are removed from both cell types, only the agglutination of one of these cell types is affected. Thus it is possible to differentiate between the two mating types of this yeast.

If a protein is not responsible for the be-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Agglutinability</th>
<th>Protein Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 5, buffer...</td>
<td>82</td>
<td>55</td>
</tr>
</tbody>
</table>
| Strain 5 trypsin...
| 88              | 1255            |
| Strain 21, buffer...
| 77              | 69              |
| Strain 21, trypsin...
| 4               | 1105            |
| Control, untreated.
| 87              |                |

1000 µg/ml trypsin in pH 8.0 0.02 M tris(hydroxymethyl)aminomethane (Tris) buffer, 1 hr at 37 C. Protein assay by Folin phenol method, crystalline lysozyme standard, corrected for trypsin present.

* Agglutinability of cells determined against untreated cells of opposite mating type in 1 per cent MgSO4 by the centrifuge method. Values are per cent reduction in turbidity of agglutinated over unagglutinated controls, greater reduction in turbidity meaning stronger agglutination.
behavior of strain 5, it might be hypothesized that it possesses a carbohydrate component which is complementary with the protein of strain 21. Attempts to inhibit the agglutination of normal cells by various sugars, such as inhibit flocculation in brewer’s yeast, have so far been unsuccessful. Also, trypsin extracts of strain 21 cells have not been shown to specifically inhibit strain 5 agglutination. However, since the agglutination reaction is very intense, it may be possible that some inhibitory effect is being overlooked. Further studies to characterize these cell surface components are underway.

BACTERIAL SYNTHESIS OF BUTYRIC ACID IN THE RUMEN OF THE SHEEP

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The mixture of volatile fatty acids produced in the rumen of the sheep during the digestion of wheat or lucerne hay includes all the normal saturated fatty acids from formic to caprylic, and possibly traces of higher acids; and in vitro fermentations of the same substrates in the presence of C¹⁴-labeled acetic and propionic acids have indicated that the higher members of the homologous series are formed, at least in part, from lower acids (Gray, Pilgrim, Rodda, and Weller, J. Exptl. Biol., 29, 57, 1952). It has been suggested that the synthesis might be brought about by the condensation of a 2C-compound with the lower acids, since this process has been demonstrated by Bornstein and Barker (J. Biol. Chem., 172, 659, 1948) in the formation of the higher volatile fatty acids by Clostridium kluyveri. These findings prompted a search for organisms in the rumen fluid capable of carrying out the synthesis; already such powers have been attributed to one rumen organism (Elsden and Lewis, Biochem. J. (London), 56, 183, 1953).

Using media similar to those used by Barker and Taha (J. Bacteriol., 43, 347, 1942) we have isolated an organism which bears some resemblance to C. kluyveri. It is an anaerobic rod, straight or slightly bent, 3 to 6 μ by 1.0 to 1.5 μ, occurring singly or in pairs in young cultures but sometimes as very long chains in older cultures in liquid media. The organism is motile by peritrichous flagella (figure 1), is gram-negative in both young and old cultures and forms a terminal oval spore about 1.5 by 1.8 μ, which causes swelling of the end of the rod. Some differences from C. kluyveri have been noticed, but no strict comparison has been possible without specimens of the type culture being examined under the same conditions of growth. We have grown the organism at 40 C, the temperature of the normal rumen contents, and growth is much more rapid than that reported for C. kluyveri (optimum temperature, 35 C) since surface growth of colonies was visible in less than 18 hr.

The organism produced butyric acid when grown in the medium containing ethyl alcohol.