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Erythritol Metabolism in a Dome Mutant of Schizophyllum commune.

by

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(5)

Submitted to the faculty of the Graduate School in
partial fulfillment of the requirements for
the degree of Master of Science in the
Department of Microbiology
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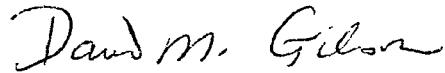
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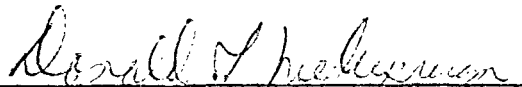
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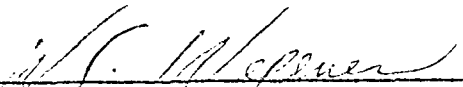
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SUMMARY

This study was undertaken to determine the nature of the lesion responsible for dome's inability to utilize erythritol. The results reported in this study indicate that the mutant, dome, is unable to utilize erythritol due to a lack of the enzyme, erythritol DH, the activity of which was never demonstrated. Dome did have the capacity to transport erythritol into its hyphae in a manner similar to wild-type.

Characteristics of the pathway and metabolism of erythritol as uncovered in S. commune during the process of demonstrating dome's lesion are summarized as follows:

1. The utilization of various polyols as sole carbon sources by strains of S. commune and the inability of dome to utilize erythritol were demonstrated.
2. Dome's inability to utilize erythritol was independent of the commercial source of erythritol and the media supplements, but the presence of erythritol did not affect dome's utilization of glucose.
3. Dome failed to utilize erythrose, but had the same growth capacity on glucose as wild-type, on a dry weight basis, with the production of an acidic culture broth.
4. The ultrastructure of dome was similar to other strains of S. commune.
5. The dome morphology was genetically separable from the ability to utilize erythritol.
6. The soluble carbohydrate pools of dome and wild-type were determined and compared.

7. NADPH₂-linked reductase activity was found with the substrates erythrose, galactose, xylose, and ribose, but not sorbose, glucose or mannose in wild-type and dome.
8. Erythritol DH had a pH optima of 8.8 in tris buffer (0.05 M) and K_m of 0.04 M; erythrose reductase had a pH optima of 7.0 in phosphate buffer (0.08 M) and K_m of 0.005 M.
9. The enzyme activities for polyol oxidation and aldose reduction were separate enzymes found only in the 10,000 x g supernatant solution. The basis for these activities being separate enzymes was evidenced by ammonium sulfate fractionation, utilization of NAD analogs, heat inactivation, differential change with culture age, and substrate competition experiments.
10. Enzyme activity of erythritol DH and erythrose reductase was sulfhydryl-dependent as evidenced by inhibitor studies.
11. NAD analog studies revealed the importance of the amide configuration, (the amide ammonia group), in the binding of the enzyme to coenzyme.
12. Activity of the various polyol dehydrogenases, aldose reductases, and reference carbohydrate enzymes were found in both wild-type and dome when grown on glucose.
13. Erythritol DH was not found in dome under any of the conditions which led to induction in wild-type.
14. Erythritol and erythrulose were demonstrated as the enzymatic products of erythrose reduction and erythritol oxidation, respectively.
15. Erythritol DH activity was reversible because enzyme activity was demonstrated with erythrulose as substrate and NADH₂ as coenzyme.
16. The properties of the uptake of erythritol and other carbohydrates by dome were similar to those seen in wild-type.