Aldehydes and Ketones Produced During Fermentation of Glucose by *Escherichia coli*

DONALD W. THAYER³ AND JAMES E. OGG

*Department of Microbiology, Colorado State University, Fort Collins, Colorado 80521*

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Although considerable attention has been given to the identification of keto acids produced during the fermentation of glucose by *Escherichia coli*, relatively little information has been obtained on the production of aldehydes and ketones. During an investigation of the keto acids produced during glucose fermentation by *E. coli* 82/r and P6 (D. W. Thayer and J. E. Ogg, Bacteriol. Proc., p. 96, 1964), the study was extended to include aldehydes and ketones.

The keto acids, aldehydes, and ketones produced during glucose fermentation were converted to their 2,4-dinitrophenylhydrazone derivatives. Samples were taken from 24-hr cultures of *E. coli* P6 or 82/r grown anaerobically in 0.3 M glucose basal salts medium (J. E. Ogg and R. D. Humphrey, J. Bacteriol. 85:801, 1963). The medium was supplemented with 5 μg of adenine sulfate per ml. Cell-free growth liquor was mixed with 2,4-dinitrophenyl hydrazine (0.2%) in 2 N HCl and incubated at 37 C. The hydrazone derivatives were extracted from the reaction mixture with ethyl acetate, and the acidic products were extracted from the organic phase with 2 N NH₄OH. The ethyl acetate phase was retained for the analyses of aldehydes and ketones. Details of technique used have been described elsewhere (D. W. Thayer, Ph.D. Thesis, Colorado State Univ., Fort Collins, 1965). Additional samples were prepared by a selective extraction technique. Cell-free growth liquor was acidified and subjected to repeated extraction with cold HOH saturated diethyl ether. The combined ether extract was then washed with saturated aqueous NaHSO₃ followed by Na₂CO₃ (10%, w/v). Relatively pure classes of compounds were obtained: the aldehydes and methyl ketones in the NaHSO₃ solution and the ketones in the ether. The solutions were then treated to form the 2,4-dinitrophenylhydrazone derivatives of the solutes.

Neutral carbonyls obtained above were sub-

³ Present address: Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md. 20014.
not be obtained with this chromatographic system, the 2,4-dinitrophenylhydrazone of 2-butane was used as a standard of comparison of relative rates of migration. Nine chromatographically different neutral carbonyls were found. On the basis of solubility, $R_{MEK}$ values, $R_M$ values, and reactions with alkali, these were identified as methanal ($R_{MEK}$: 0.26), ethanal ($R_{MEK}$: 0.43), 2-propanone ($R_{MEK}$: 0.75), 2-butane ($R_{MEK}$: 1.00), and 3-pentane ($R_{MEK}$: 1.31). The hydrazone with an $R_{MEK}$ of 0.35 may be that of 3-hydroxy-2-butane, but it did not turn pink in alkali as did an authentic sample. The violet color reaction produced in alkali by the hydrazone(s) at the origin is characteristic of bis-2,4-dinitrophenylhydrazones of dicarbonyls. The average $R_{MEK}$ value of 0.92 obtained for one of the carbonyls may indicate heptanal, but a standard was not available for comparison. The identification of these carbonyls must be considered as tentative, since only one chromatographic system was employed.

The variety of neutral carbonyls tentatively identified in this study is surprising. For example, on the basis of the intensity of its chromatographic zone, 2-butane was present in fairly large amounts in extracts from cultures of both strains of *E. coli*. No significant differences were observed between the neutral carbonyls produced by the two strains. The variety of products found in this study, some of which to the knowledge of the authors have not been described as fermentation products of *E. coli*, indicates a need for an investigation of their source and significance in *E. coli* fermentations.

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