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Bacterial Distribution of the Use of Succinyl and Acetyl Blocking Groups in Diaminopimelic Acid Biosynthesis

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The use of acetate, rather than succinate, as a blocking group in diaminopimelate biosynthesis has been found only in species of the genus *Bacillus*.

Since the discovery of α,ϵ-diaminopimelic acid (DAP) by Elizabeth Work in 1949 (6), the pathway for its biosynthesis has been elucidated in the organisms *Escherichia coli* and *Bacillus megaterium*. The pathways in these two bacteria, although generally similar, differ in the identity of the acyl blocking group which is first added to tetrahydrodipicolinic acid and later cleaved from the acylated DAP derivative. *E. coli* has been found to utilize a succinyl blocking group, whereas *B. megaterium* uses an acetyl group (3, 5). The variation of the DAP pathway in these bacteria led to this study of the distribution of the use of succinate and acetate in other bacterial species.

The two pathways were differentiated on the basis of the presence of the particular deacylase able to cleave the acyl-DAP derivative, producing DAP and the free acyl group. That is, an organism using succinate should have a succinyl-DAP deacylase, whereas one using acetate should have an acetyl-DAP deacylase. The assay for the deacylases has been described (5). The assay depends on the greater chromogenicity of DAP, as compared to the acyl derivatives in an acidic ninhydrin assay.

All bacteria were harvested during the logarithmic phase of growth, after which a cell-free extract was prepared for use in the assay. *B. subtilis* and *E. coli* were grown on medium A as described by Davis and Mingioli (1). *B. cereus* was grown on a medium containing: $FeSO_4 \cdot 7H_2O$, 0.00005%; $CuSO_4 \cdot 5H_2O$, 0.005%; $ZnSO_4 \cdot 7H_2O$, 0.0005%; $MnSO_4 \cdot H_2O$, 0.005%; $MgSO_4$, 0.02%; $(NH_4)_2SO_4$, 0.2%; $CaCl_2 \cdot 2H_2O$, 0.008%; K_2HPO_4, 0.05%; yeast extract, 0.2%; and glucose, 0.5%. *Azotobacter vinelandii* was grown by the method of Still and Wang (4). *Micrococcus lysodeikticus* was grown in a medium containing 1.25% Brain Heart Infusion broth, 0.54% nutrient broth, and 0.25% yeast extract. *Rhodospirillum rubrum* was grown by the method of Evans (2). *Arthrobacter globiformis*, *Alcaligenes viscolactis*, *Bacillus mycoides*, *Bacillus licheniformis*, and *Bacillus pumilus* were grown in 0.8% nutrient broth. *B. megaterium* was grown by the method of Sundharadas and Gilvarg (5).

The results, summarized in Table 1, show that the utilization of an acetyl blocking group was limited to species classified within the genus

TABLE 1. *Distribution of acetyl- and succinyl-DAP deacylases*

Species	Specific activity[a]	
	Acetyl	Succinyl
Alcaligenes viscolactis (local strain)	0	0.052
Arthrobacter globiformis (local strain)	0	0.003
Azobacter vinelandii (ATCC 12518)	0	0.066
Escherichia coli (ATCC 9637)	0	0.087
Micrococcus lysodeikticus (ATCC 4698)	0	0.033
Rhodospirillum rubrum (local strain)	0	0.018
Bacillus cereus[b] (ATCC 10876a)	0.005	0
B. licheniformis (ATCC 8187)	0.002	0
B. megaterium (local strain)	0.019	0
B. mycoides (local strain)	0.003	0
B. pumilus (ATCC 18)	0.002	0.013
B. subtilis (Marburg strain W-168)	0.007	0

[a] Expressed as micromoles per minute per milligram of protein.

[b] It was not possible to confirm a previous report of the occurrence of *N*-succinyl-DAP deacylase activity in this organism (3).

Bacillus. The presence of the succinyl-DAP deacylase, on the other hand, was found in all the non-*Bacillus* species tested. The demonstration of both acetyl- and succinyl-DAP deacylase activities in *B. pumilus* could have indicated that both pathways existed for this organism. This explanation was eliminated when it was shown that crude extracts of *B. pumilus* have the acetyl transferase responsible for the reaction of acetyl coenzyme A with tetrahydrodipicolinic acid, although they do not exhibit the corresponding succinyl transferase. Therefore, the succinyl blocking group is not utilized in *B. pumilus*, and the succinyl deacylase activity is due either to a nonspecific enzyme, unrelated to the pathway, or to a less rigid specificity on the part of the *B. pumilus* deacylase than is exhibited by all the other deacylases tested.

According to *Bergey's Manual of Determinative Bacteriology*, 7th edition, all the *Bacillus* species mentioned in Table 1 belong to the first of three subgroups within the genus. Further work will have to be done to determine the blocking group in group II and group III *Bacillus* species. However, the fact that acetyl and succinyl blocking groups were limited to *Bacillus* and non-*Bacillus* species, respectively, already indicates that the type of blocking group employed in DAP biosynthesis should be a useful taxonomic marker and may also help to trace out evolutionary relationships.

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