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Complementary Functioning of Two Components from Nitrogen-fixing Bacteria

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Use of alternate substrates (acetylene, azide, cyanide) for estimating N_2 fixation confirmed that recombined fractions from different bacteria are functional but restricted in complementarity.

Detroy et al. (1) described the separation and recombination of the nitrogenase enzyme complex from 4 nitrogen-fixing bacteria and found that 6 of the 12 possible heterologous recombinations of the complementary fractions from different organisms could function together to form ammonia from N_2. The facultative anaerobic bacteria *Klebsiella pneumoniae* and *Bacillus polymyxa* showed the widest range of complementarity, whereas the obligate aerobe *Azotobacter vinelandii* and the obligate anaerobe *Clostridium pasteurianum* were more restricted in heterologous recombination.

Several causes of the apparently negative crosses were suggested, including the possibility that they were so inefficient that activity could not be detected by microdiffusion and nesslerization. This paper reports experiments designed to test this hypothesis by use of azide, cyanide, and acetylene as "alternate" substrates.

The techniques used were described in detail in our previous publication (1). The results of two series of trials are summarized in Tables 1 and 2. In these tables, the homologous cross consists of both protein components from the same organism; in the heterologous cross, component I is from the indicated organism and component II is from the other. Component I corresponds to the protein fraction that Mortenson et al. (3) called molybdoferredoxin and component II corresponds to azoferredoxin. In a third series, all heterologous crosses between *A. vinelandii* and *C. pasteurianum* were negative, independent of the methods of assay. Since these experiments were designed to determine only whether a given cross was active in the reduction of the indicated substrates, the quantitative differences in the results are not significant. Interestingly, however, the control homologous recombination was almost always greater than any of the heterologous, except for an occasional result such as that shown

TABLE 1. *Reduction of substrates by recombined components of extracts of B. polymyxa and K. pneumoniae*

Assay	Organism	Substrate reduction[a]	
		Homologous	Heterologous I[b]
$N_2 \rightarrow NH_3$	*B. polymyxa*	30	4
	K. pneumoniae	17	14
$N_3^- \rightarrow NH_3$	*B. polymyxa*	39	9
	K. pneumoniae	22	30
$CN^- \rightarrow CH_4$	*B. polymyxa*	162	109
	K. pneumoniae	101	65
$C_2H_2 \rightarrow C_2H_4$	*B. polymyxa*	874	1441
	K. pneumoniae	640	872

[a] In both tables expressed as micrograms of NH_3-N, nanomoles of CH_4, and nanomoles of C_2H_4 corrected for controls of individual components (*see* reference 1).

[b] Component I of indicated organism and component II of the other.

TABLE 2. *Reduction of substrates by recombined components of extracts of B. polymyxa and C. pasteurianum*

Assay	Organism	Substrate reduction[a]	
		Homologous	Heterologous I
$N_2 \rightarrow NH_3$	*B. polymyxa*	15	9
	C. pasteurianum	25	0
$N_3^- \rightarrow NH_3$	*B. polymyxa*	14	11
	C. pasteurianum	15	0
$CN^- \rightarrow CH_4$	*B. polymyxa*	99	42
	C. pasteurianum	41	0
$C_2H_2 \rightarrow C_2H_4$	*B. polymyxa*	443	316
	C. pasteurianum	601	0

[a] See footnotes *a* and *b* to table 1.

for the recombination of components from *B. polymyxa* and *K. pneumoniae* with the acetylene reduction assay. The results obtained with these much more sensitive techniques agree with those previously found with the $N_2 \rightarrow NH_3$ assay. Other factors that were tested included alteration of the ratio of the components (usually a 1:1, v/v, is employed) and the time of incubation. Neither caused a negative cross to change to a positive one. Mortenson (2) suggested that a third component found in extracts of cells grown on ammonium might be necessary for N_2 fixation by purified fractions from *C. pasteurianum*. Although much less purification was obtained in this study than in the fractions described by Mortenson, such an extract from ammonium-grown cells of *K. pneumoniae* was tested in recombinations between components from this bacterium and those from *C. pasteurianum*. The extract did stimulate the reduction of acetylene in the homologous recombination of components from *K. pneumoniae* from 724 to 1,293 nm, but it had no effect on the heterologous recombinations.

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