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Compatibility of the components of nitrogenase from soybean bacteroids and free-living nitrogen-fixing bacteria

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SUMMARY

The ability of the components of nitrogenase from free-living nitrogen-fixing bacteria to cross-react and form active enzyme complexes with the components of the enzyme isolated from a symbiotic nitrogen-fixing system was tested. Nitrogenase components of *Azotobacter vinelandii, Bacillus polymyxa* cross-reacted with components of nitrogenase from *Rhizobium japonicum* bacteroids. No evidence of a cross reaction was obtained in the case of *Clostridium pasteurianum.*

Nitrogenase from symbiotic and non-symbiotic nitrogen-fixing agents is composed of two components, here designated Fr. 1 and Fr. 2 both of which are necessary for enzyme activity[1,2]. Furthermore, it has been shown that a considerable degree of compatibility exists between the two components of the enzyme prepared from the free-living organisms[3,4]. Results reported here indicate a similarity in some instances, between components of nitrogenase from symbiotic and non-symbiotic nitrogen-fixing bacteria.

Components of nitrogenase were prepared from *Azotobacter vinelandii, Bacillus polymyxa*, and *Clostridium pasteurianum* by procedures already published[2,3,5]. Soybean nodules (obtained from plants –*Glycine max.*– inoculated with a commercial strain of *Rhizobium japonicum*) when collected and chilled slowly to dry ice temperatures contained no Fr. 2 activity after 24 h. It appears that the cold lability of this component parallels that already reported for the same component of the free-living bacteria[2]. Fr. 1 retains activity after this treatment; the cold treatment was used in this study to obtain preparations free of Fr. 2 activity. Active preparations of Fr. 2 were prepared from nodules by the method of Klucas *et al.*[1]. The acetylene reduction reaction[6,7] was used to assay nitrogenase enzyme activity[8].

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TABLE I

CROSS REACTIONS OF COMPONENTS OF NITROGENASE.

Specific activities in nmoles ethylene per min per mg Fr. 2 protein at 25°.

Components		*Spec. act.*	*Enhancement (-fold)*
R. japonicum bacteroid	*A. vinelandii*		
Fr. 1	-	0	
-	Fr. 1	0	
-	Fr. 2	7.8	
-	Fr. 1 + 2	60.0	7.7
Fr. 1	Fr. 2	14.5	1.9
	C. pasteurianum		
-	Fr. 1	0	
-	Fr. 2	21.0	
-	Fr. 1 + 2	71.4	3.4
Fr. 1	Fr. 2	1.0	0
	B. polymyxa		
-	Fr. 1	0	
-	Fr. 2	1.45	
-	Fr. 1 + 2	12.40	8.5
Fr. 1	Fr. 2	6.00	4.1

Table I outlines the results obtained with nitrogenase Fr. 1 of *R. japonicum* bacteroids, prepared by cold treatment, and nitrogenase Fr. 2 of *A. vinelandii*, *C. pasteurianum* and *B. polymyxa*. In all instances Fr. 2 of the free-living nitrogen-fixing bacteria was contaminated with some Fr. 1 and so showed residual activity. The degree of enhancement of enzyme activity obtained with the homologous cross is shown and compared with that obtained for the heterologous cross of Fr. 1 of the bacteroids and Fr. 2 of the free-living forms. Approximately a 2-fold enhancement of enzyme activity was obtained with *A. vinelandii;* this represents 25% of the activity of the homologous cross. *B. polymyxa* Fr. 2 crossed with bacteroid Fr. 1 showed 50% of the activity of the *B. polymyxa* homologous cross, whereas no enhancement of bacteroid Fr. 1 was obtained with Fr. 2 of *C. pasteurianum.* Titration with different concentrations of components would be necessary to establish whether these percentages represent the relative affinities.

TABLE II

CROSS REACTIONS OF COMPONENTS OF NITROGENASE FROM *R. JAPONICUM* BACTEROIDS AND *A. VINELANDII*

Specific activities in nmoles ethylene per min per mg Fr. 2 protein at 25°.

Components		*Spec. act.*	*Enhancement (-fold)*
R. japonicum bacteroid	*A. vinelandii*		
Fr. 1	-	0	
Fr. 2	-	32.8	
Fr. 1 + 2	-	191.5	5.9
-	Fr. 1	0	
-	Fr. 2	4.1	
-	Fr. 1 + 2	57.0	13.9
Fr. 1	Fr. 2	60.0	14.6
Fr. 2	Fr. 1	85.0	2.6

Table II outlines the results obtained with active Fr. 1 and Fr. 2 of *R. japonicum* bacteroids, prepared by ion-exchange chromatography[1], and components of *A. vinelandii* nitrogenase. In this instance the degree of enhancement obtained indicated that a highly active enzyme complex can be formed between Fr. 1 of bacteroids and Fr. 2 of *A. vinelandii.* Results also show that the reverse of this combination is possible and Fr. 2 of bacteroid nitrogenase will combine with Fr. 1 of *A. vinelandii* to give an active enzyme complex.

These results are interesting because they substantiate further the findings that the nitrogenase complexes as they occur in symbiotic and free-living nitrogen-fixing bacteria, are very similar.

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