of both *Rhizobium* and *Agrobacterium*, suggesting that at least one *nif* gene from *K. pneumoniae* can be transcribed and translated in these bacteria.

We thank John Barzdo and Pauline Harvey for technical assistance and Dr V. Krishnapillai for carrying out the tests with *Pseudomonas aeruginosa*. We also thank Professor J. R. Postgate for constructive criticism of the manuscript. This work was supported by a grant from the Agricultural Research Council and by the Hungarian Academy of Sciences.

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Received December 18, 1975; accepted February 2, 1976.

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Expression of *Klebsiella* nitrogen fixation genes *(nif)* in *Azotobacter*

NITROGEN fixation mutants have been isolated and biochemically characterised in *Azotobacter vinelandii*[1], but the absence of chromosomal gene transfer systems such as conjugation in this genus has prevented a genetic analysis of such mutants. We now report the intergeneric transfer of *Klebsiella nif* genes carried on the P-type plasmid, RP41 (refs 2 and 3), to *Azotobacter*, and their expression in this host.

The *A. vinelandii nif* mutants lacked nitrogenase molybdoprotein (AvI) activity, iron protein (AvII) activity or were pleiotropically negative for both activities. Spontanenous derivatives resistant to 25 $\mu g\ ml^{-1}$ nalidixic acid were isolated before mating with an *Escherichia coli* K12 strain carrying the *nif* plasmid RP41. Matings were done on agar plates: cultures of donor *E. coli* grown at 37 °C in Oxoid nutrient broth and *A. vinelandii* recipients grown at 28 °C in Burk's sucrose medium[4] supplemented with 2 mg ml^{-1} ammonium acetate were grown to mid logarithmic phase and mixed in a 1 : 1 ratio; 0.1-ml portions of mixtures were spread on Oxoid nutrient agar plates and incubated overnight at 34 °C. Mating mixtures and parental azotobacter cultures were resuspended and diluted in sucrose-free Burk's medium[5] and spread on selective agar media: Burk's sucrose medium containing 25 $\mu g\ ml^{-1}$ nalidixic acid to select Nif^+ exconjugants and a similar medium with 2 mg ammonium acetate and 10 $\mu g\ ml^{-1}$ tetracycline when tetracycline-resistant exconjugants were sought. Selective plates were incubated at 28 °C; all incubations were in air.

Presumptive nitrogen-fixing exconjugants were tested for acetylene-reducing ability by transfer to 5 ml liquid Burk's sucrose medium in 25-ml conical flasks. After growth at 30 °C, the cotton wool plugs were replaced by Suba-seal rubber closures. 2 ml C_2H_2 was injected and gas samples were removed at intervals for gas chromatography on a Porapak N column with a flame ionisation detector. Activities were compared with that of wild-type *A. vinelandii* grown in similar conditions.

Among the markers carried by RP41 are *nif, his,* resistance to tetracycline (Tc), kanamycin (Km) and carbenicillin (Carb). The transfer frequencies of *nif* and Tc to *Azotobacter* mutants are shown in Table 1. In contrast to the situation in *Klebsiella* and *E. coli,* Nif^+ exconjugants could be selected directly on plates in air. Although the transfer frequencies were low, they were significantly higher than reversion frequencies. The low observed frequencies arose at least partially from the poor growth of *A. vinelandii* on the mating plates: $\sim 10^7$ *A. vinelandii* compared with $\sim 10^9$ *E. coli* ml^{-1} when resuspended at the end of the mating period.

Fifteen of the 83 Nif^+ exconjugants listed in Table 1, including representatives of all positive recipients, were tested for nitrogenase by the acetylene test and all showed activity between 40% and 50% of that of the wild type.

Table 1 shows that nitrogenase activity was restored to three mutants which lacked AvI and one which lacked AvII activity but not to the one strain examined (UW1) which

Table 1 Intergeneric transfer of plasmid RP41 from *E. coli* K12 to *A. vinelandii*

Recipient strains	Nitrogenase activity*	Frequency of reversion to Nif^+†	Selection§	Transfer frequency‡	Exconjugant phenotype§		
UW1	I⁻ II⁻	$<4\times10^{-8}$	Tc^R	3.8×10^{-7}	28/28Km^R,	27/28$Carb^R$,	0/28Nif^+
			Nif^+	$<10^{-7}$	—		
UW10	I⁻ II⁺	9×10^{-8}	Tc^R	2.0×10^{-6}	27/28Km^R,	24/26$Carb^R$,	25/26Nif^+
			Nif^+	5.4×10^{-7}	17/17Tc^R,	17/17$Carb^R$,	13/17Km^R
UW100	I⁻ II⁺	$<5\times10^{-8}$	Tc^R	4.0×10^{-7}		0/9 $Carb^R$,	9/9Nif^+
			Nif^+	1.3×10^{-6}	18/18Tc^R,	18/18$Carb^R$	
UW38	I⁻ II⁺	$<2\times10^{-8}$	Tc^R	1.4×10^{-7}		27/28$Carb^R$,	27/28Nif^+
			Nif^+	3.8×10^{-6}	21/28Tc^R,	18/28$Carb^R$	
UW91	I⁺ II⁻	$<3\times10^{-8}$	Tc^R	9.0×10^{-7}			22/28Nif^+
			Nif^+	4.1×10^{-7}	19/19Tc^R		

*W. J. Brill, personal communication (I and II are the molybdoprotein and iron protein, respectively, of nitrogenase).
†Reversion frequencies determined as for transfer frequencies but without donor cells. Minima signify no revertants detected with the population tested.
‡Transfer frequencies determined after plate matings (carried out as described in text) and expressed relative to the numbers of donor bacteria *E. coli* JC5466(RP41), at the end of the mating period.
§Symbols: Tc^R, resistance to 10 μg tetracycline; Km^R, resistance to 20 μg kanamycin; $Carb^R$, resistance to 200 μg carbenicillin ml^{-1}.

Table 2 Transfer of RP41 from *A. vinelandii* to *E. coli* K12

A. vinelandii donor strains	*E. coli* recipients	Selection	Transfer frequency	Exconjugant phenotype
UW100(RP41)	JC5466	His^+	3.2×10^{-7}	7/8 Km^R Tc^R Nif^+
		Tc^R	2.0×10^{-7}	1/7 His^+ Nif^+, 7/7 Km^R
		Km^R	1.4×10^{-7}	0/9 His^+ Nif^+, 9/9 Tc^R
UW100(RP41)	SB1801	His^+	3.0×10^{-4}	28/28 Nif^+ Tc^R
		Km^R	3.0×10^{-2}	28/28 Tc^R, 0/28 His^+ Nif^+
UW91(RP41)	SB1801	His^+	5.0×10^{-6}	8/8 Km^R Nif^+
		Km^R	1.0×10^{-5}	8/8 Tc^R, 0/8 Nif^+ His^+

Plate matings as described in text. Genotype of JC5466 is *trp his* recA56 *spc;* Genotype of SB1801 is *his*750 (deletion extending through *his gnd* RHA-2A) *str* λ^R *ara gal mal*A *xyl mtl* (λ^-). Symbols as in legend to Table 1.

was pleiotropically negative for both component activities. The presence of RP41 *nif* genes in one of each class of Nif^+ exconjugants, UW91(RP41) and UW100(RP41), was confirmed by using these strains as donors and observing transfer of *his, nif* and drug resistance markers back to *E. coli* (Table 2). Some segregation of markers occurred in these matings: exconjugants selected directly for drug resistance were mainly His^- and Nif^- while all but one of the His^+ exconjugants were Nif^+ and drug resistant. Such segregation has been observed in other transfers involving *nif* plasmids[3,6].

Loss of RP41 *nif* genes ought to restore the Nif^- phenotype in these exconjugants. One *Azotobacter* strain UW91 (RP41) was cultured in non-selective conditions and spontaneous loss of *nif* genes occurred: After 10 subcultures in Burk's medium supplemented with 2 mg ml^{-1} ammonium acetate, all clones among 52 tested were Nif^- and 28, screened for drug resistance, were resistant to Tc, Km and Carb. These drug-resistance markers, which originated on RP4, the parent plasmid of RP41 (ref. 2), were stable in *A. vinelandii.*

One *Azotobacter* strain, UW100(RP41) was examined for immunological evidence for the expression of *Klebsiella nif* structural genes. Crude extracts of this strain, grown in Burk's medium, contained material which cross reacted antigenetically with antiserum prepared against purified *K. pneumoniae* nitrogenase molybdoprotein (KpI). This antigen was absent from extracts of the parental strain, UW100, grown in ammonium-limited conditions (100 μg N (as ammonium acetate) ml^{-1} Burk's medium). Material cross reacting with antiserum to purified *Azotobacter* nitrogenase molybdoprotein (AvI) was, however, present in both extracts. When these strains were grown with excess NH_4^+ ion, no such cross-reacting materials were detected in extracts prepared similarly.

We conclude from these experiments that the Nif^+ *Azotobacter* exconjugants carried RP41 and expressed its *nif* genes, which originated in *Klebsiella pneumoniae* strain M5a1 (ref. 2). The ability of *Azotobacter* to transcribe and translate *Klebsiella nif* genes and, in contrast to *Agrobacterium*[3], actually to make use of their products has several important consequences:

(1) The regulatory apparatus, whereby ammonia repressed nitrogenase synthesis in parents and exconjugants, is common to *Klebsiella* and *Azotobacter,* even at a molecular level. For example, if glutamine synthetase (GS) is accepted as a positive activator of *nif* genes[7-9], then *Azotobacter* GS can activate *Klebsiella nif* genes.

(2) *Klebsiella nif* genes normally function only in an anaerobic environment, yet transfer to the obligate aerobe *Azotobacter* permitted their expression in air. Clearly they must have shared the oxygen exclusion processes[10] of *Azotobacter.*

(3) The possibility arises that hybrid nitrogenases, such as the active mixtures of AcI+KpII or KpI+AcII which can be studied *in vitro*[11], are functioning *in vivo,* but comparable experiments with characterised mutants of RP41 would be necessary to establish this point conclusively.

RP41 is thus potentially useful for a genetic complementation analysis of *nif* mutants in *Azotobacter.* It could also be used for the isolation of selected mutants in *Azotobacter* by introducing RP41 with characterised *nif* mutations into *Azotobacter* and preparing homogenates.

We thank J. Bardzo and J. Simkins for assistance, Dr R. A. Dixon for criticism of the manuscript and Professor W. J. Brill for the *A. vinelandii nif* mutants.

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Received December 24, 1975; accepted January 22, 1976.

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Errata

In the article "Cell differentiation by 3′,5′-cyclic AMP in a lower plant" by A. K. Handa and M. M. Johri (*Nature,* **259**, 480; 1976) there is an error in Table 3. The entries under the heading 'Medium' should read . . . MM, MM+1% glucose, MM+1 μM IAA, MM+1% glucose . . . and not as printed.

In the paper "Dopamine-like renal and mesenteric vasodilation caused by apomorphine, 6-propylnorapomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene", by H. J. Crumley, R. M. Pinder, W. B. Hinshaw and L. I. Goldberg (*Nature,* **259**, 584; 1976) Dr Pinder's address should be : . . . Birkenhead, Auckland, New Zealand.