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.

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1 Shah, V. K., Davis, L. C., Gordon, J., Orme-Johnson, W. H., and Brill, W. J., *Biochim. biophys. Acta,* **292,** 246 (1973).
2 Dixon, R. A., Cannon, F. C., and Kondorosi, A., *Proc. Soc. gen. Microbiol.,* **2,** 43 (1975).
3 Dixon, R. A., Cannon, F. C., and Kondorosi, A., *Nature,* **260,** 268–271 (1976).
4 Newton, J. W., Wilson, P. W., and Burris, R. H., *J. biol. Chem.,* **204,** 445 (1953).
5 Billson, S., Williams, K., and Postgate, J. R., *J. appl. Bact.,* **33,** 270 (1970).
6 Cannon, F. C., Dixon, R. A., and Postgate, J. R., *J. gen. Microbiol.,* **93,** 111 (1976).
7 Gordon, J., and Brill, W. J., *Biochem. biophys. Res. Commun.,* **59,** 967 (1974).
8 Streicher, S., Shanmugam, K., Ausubel, F., Morandi, C., and Goldberg, R., *J. Bact.,* **120,** 815 (1974).
9 Tubb, R. S., *Nature,* **251,** 481 (1974).
10 Postgate, J. R., *The Biology of Nitrogen Fixation* (edit. by Quispel, A.), 663 (North Holland, Amsterdam, 1974).
11 Thorneley, R. N. F., Eady, R. R., and Yates, M. G., *Biochim. biophys. Acta,* **403,** 269 (1975).

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.

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