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NIC, a Conjugative Nicotine-Nicotinate Degradative Plasmid in *Pseudomonas convexa*

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The plasmid nature of genes specifying degradation of nicotine and nicotinate in *Pseudomas convexa* strain 1 (Pc1) is indicated by mitomycin curing and conjugational transfer to other strains. The NIC plasmid appears to be compatible with other metabolic plasmids in *Pseudomonas putida*.

The ability of certain strains of *Pseudomonas putida* to grow on octane, camphor, toluene and methyl benzoates, salicylate, naphthalene, and xylenes can be lost spontaneously, and the frequency of loss can be increased by mitomycin C. These observations along with high conjugational transfer (except xylene) have been correlated to the extrachromosomal nature of genes responsible for the expression of these pathways (3). *Pseudomonas convexa* strain 1 (10) degrades nicotine via pseudooxynicotine, 3-succinoyl pyridine, and 6-hydroxy 3-succinoyl pyridine to 2,5-dihydroxy pyridine, where it converges with the biodegradation of nicotinate (Fig. 1). Pathways are the same as those utilized by other *Pseudomonas* strains (1, 2, 4–7, 11). On the basis of transductional analysis, it has been shown that the degradation of nicotinate is coded for by chromosomal genes in *P. putida* (8). In this report we present data which suggest that the nicotine-nicotinate degradation pathways are borne on a transmissible plasmid in *P. convexa* strain 1 (Pc1).

When Pc1 was grown in the presence of growth-limiting concentrations of mitomycin C, about 3% of the cells lost their ability to grow on nicotine and nicotinate. This is similar to the frequency of mitomycin C curing of *Pseudomonas* plasmids such as SAL and TOL (3) and strongly suggests the extrachromosomal nature of the genes involved.

The conclusion that these cells are cured of all the genes coding for nicotine-nicotinate degradation enzymes rather than just having lost the common enzyme in the two pathways, 2,5-dihydroxy pyridine oxygenase, by mutation or curing is based on the following evidence: (i) they do not revert even when plated at a cell density of 10^{10} on selective media; (ii) they are not able to utilize nicotine as a carbon source, whereas mutants lacking 2,5-dihydroxy pyridine oxygenase are able to do so; (iii) they lack the inducible enzymes nicotinate and 3-succinoyl pyridine hydroxylases, 6-OH nicotinate and 6-OH 3-succinoyl pyridine monooxygenases, and 2,5-dihydroxy pyridine oxygenase, which are present before mitomycin curing.

To determine whether there are two separate plasmids coding for these degradation pathways, or only one, and whether the plasmid is transmissible, we mated the wild-type strain of *P. convexa* with a cured strain of *P. convexa* or with *P. putida* (PpG) strains (see Table 1). The results in Table 2 show that both NIC and NCT are highly transmissible (compared with chromosomal transfer) not only to cured strains of Pc, but also to PpG strains, attesting to the plasmid nature of the genes in question. Also, in all cases NIC and NCT are transferred together, suggesting that both nicotine and nicotinate degradations are coded by one and the same plasmid (designated NIC plasmid).

Since the NIC plasmid was transmissible to PpG strains at a rather high frequency and PpG

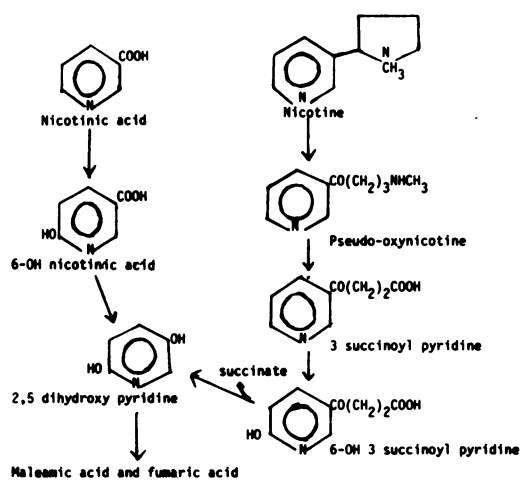

FIG. 1. *Degradation pathways of nicotine and nicotinate.*

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TABLE 1. *List of strains*[a]

Strain derived from:	Parent strain	Phenotype	Derivation	Plasmid
P. convexa (Pc1, Nic$^+$ Nct$^+$)				
Pc101	Pc1	Cys$^-$ Nic$^+$ Nct$^+$	NG	NIC
Pc204	Pc1	Met$^-$ Nic$^+$ Nct$^+$	NG	NIC
Pc3	Pc1	Nic$^-$ Nct$^-$	M	
Pc309	Pc3	Trp$^-$ Nic$^-$ Nct$^-$	NG	
P. putida (PpG1, Nic$^-$ Nct$^+$ Cam$^+$, Pf16^s)				
PpG273	PpG1	Trp$^-$	S, PC	CAM (9)
PpG277	PpG273	Cam$^-$ Trp$^-$	M	
PpG972	PpG277	Oct$^+$ Trp$^-$	C	OCT
PpG1273	PpG273	Cam$^+$ Trp$^-$	UV	CAM
PpG1274	PpG1273	Cam$^-$ Trp$^-$	M	
PpG u116	PpG1274	Tol$^+$ Trp$^-$	C	TOL
PpG u119	PpG1274	Sal$^+$ Trp$^-$	C	SAL
PpG u120	PpG1274	Nah$^+$ Trp$^-$	C	NAH

[a] Nic, Nct, Cam, Tol, Sal, Nah, and Oct designate degradations of nicotine, nicotinate, camphor, toluene, salicylate, naphthalene, and octane, respectively. NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; M, mitomycin C curing (9); S, PC, spontaneous penicillin-cycloserine selection; C, conjugation; Pf16^s, phage Pf16 sensitivity.

TABLE 2. *Conjugational transfer of NIC plasmid to other strains*[a]

Donor × recipient	Selected[b]	Frequency of transfer
Pc101 × Pc309	*nic*$^+$ *trp*	2.5×10^{-6}
Pc101 × Pc309	*trp*$^+$	$<10^{-8}$
Pc204 × Pc309	*nic*$^+$ *trp*	10^{-5}
Pc204 × Pc309	*trp*$^+$	$<10^{-8}$
Pc101 × PgG273	*nic*$^+$ *trp* Pf16^s	2×10^{-7}
Pc101 × PpG277	*nic*$^+$ *trp* Pf16^s	3×10^{-7}
Pc204 × PpG273	*nic*$^+$ *trp* Pf16^s	4×10^{-7}
Pc204 × PpG277	*nic*$^+$ *trp* Pf16^s	10^{-6}

[a] Pf16^s, Sensitivity to phage Pf16; *nic*, degradation of nicotine. Exconjugants from crosses 1 and 3 were all found to be positive for nicotinate degradation (*nct*$^+$).

[b] Exconjugants were selected on nicotine plus amino acid plates. After growth, the exconjugant colonies were tested for their auxotrophy by replicating on nicotine plates with and without the required amino acid.

strains containing different metabolic plasmids were readily available, we studied the compatibility of the NIC plasmid with the other metabolic plasmids (see Table 1). Results show that the NIC plasmid is compatible with CAM, OCT, NAH, SAL, and TOL metabolic plasmids.

In *P. putida* nicotinate degradation genes are located on the chromosome linked with those for other peripheral catabolic functions, such as degradation of benzoate, *p*-hydroxybenzoate, skimimate, quinate, mandelate, phenylacetate, histidine, tyrosine, and phenylalanine. These genes are closely clustered on about 10 to 15% of the chromosome. Our report shows that in *P. convexa* nicotinate genes are located on a plasmid along with nicotine degradation genes. Of interest in this context is the observation that *p*-hydroxybenzoate degradation genes are not curable in Pc1 and are most probably chromosome bound. The different location of nicotinate genes, chromosomal in *P. putida* and plasmid bound in *P. convexa*, may reflect different evolutionary patterns. The possibility of transposition also must be considered.

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