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Camphor biodegradation by Pseudomonas putida plays a key role in carbon cycling in the biosphere

The key role of microorganisms in promoting carbon cycling in the biosphere is well illustrated by the biodegradation of the natural plant terpenoid camphor by *Pseudomonas putida* ATCC 17453.

Research has established that several different monooxygenases (cytochromeP450 monooxygenase [step A), 2,5-diketocamphane 1,2-monooxygenase [Step C], 3,6-diketocamphane 1,6-monooxygenase [Step D] , 2-oxo-Δ^3-4,5,5-trimethylcyclo- pentenylacetyl-CoA monooxygenase [Step F]) play key sequential roles in the progressive conversion of both enantiomers of the chiral bicyclic monoterpene, firstly to the same monocyclic pathway intermediate 2- oxo-Δ^3-4,5,5-trimethylcyclopentenylacetic acid (OTE), and then subsequently via 5-hydroxy-3,4,4-trimethyl-Δ^2-pimelyl-CoA-δ-lactone (HTP-CoA) to $\Delta^{2,5}$-3,4,4-trimethylpimelyl-CoA, the first aliphatic catabolic intermediate (Fig. 1). Significantly, the genes coding for all these key oxygen-dependent enzymes are located on the large CAM plasmid present in this bacterium. The pathway is then completed by a number of oxygen-independent enzymes that serve to hydrolyse the C10 CoA ester to short-chain aliphatic intermediates (1 x C4 succinyl-CoA plus 3 x C2 acetyl-CoA) able to gain direct entry into the TCA cycle, and hence complete the degradation of both enantiomers of alicyclic camphor to the oxidation level of CO_2, a key molecule in the global Carbon Cycle.

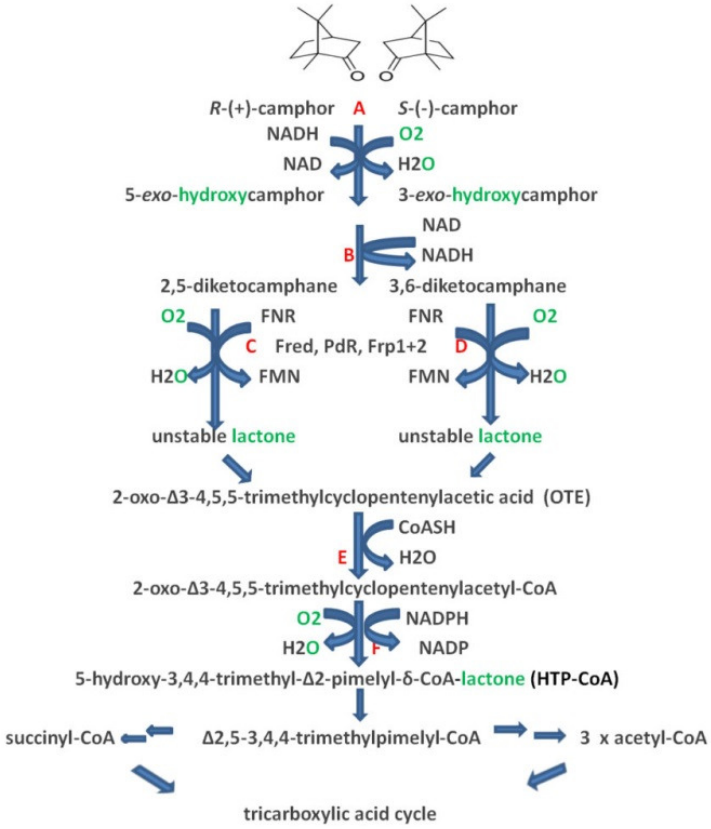

Fig. 1. Pathway of (+)- and (−)-camphor degradation in *P. putida* ATCC 17453. A = cytochrome P450 monooxygenase (*camCAB*): B = exo-hydroxycamphor dehydrogenase (*camD*): C = 2,5-diketocamphane 1,2-monooxygenase (*camE25-1* + *camE25-2*): D = 3,6-diketocamphane 1,6-monooxygenase (*camE36*): E = 2-oxo-Δ^3-4,5,5-trimethylcyclopentenylacetyl-CoA synthetase (*camF1* + *F2*): F = 2-oxo-Δ^3-4,5,5-trimethylcyclopentenylacetyl-CoA monooxygenase (*camG*): FNR = reduced flavin mononucleotide; Fred = 36 kDa chromosome-coded flavin reductase: PdR = putidaredoxin reductase subunit of cytochrome P45O monooxygenase (*camA*): Frp 1 + 2 = chromosome-coded ferric reductases: diatomic oxygen molecules participating in the four monooxygenase-catalysed steps is shown in green, as in each case are the fates of each component oxygen atom.

The importance of transcriptional control in up-regulating the pathway for the degradation of alicyclic camphor to aliphatic $\Delta^{2,5}$-3,4,4-trimethylpimelyl-CoA has been confirmed by using the relevant inhibitors rifampicin and actinomycin D. This comprehensive study monitored the differential rates of synthesis of a number of CAM plasmid-coded activities including the two diketocamphane monooxygenases (DKCMOs, Steps C and D, Fig. 1) in response to both camphor and key degradation pathway intermediates to establish the various induction and repression circuits that regulate relevant activities in *P. putida* ATCC 17453 (Fig. 2). The results confirmed that the genes that code for the enantioselective DKCMOs are subject to induction by the corresponding camphor enantiomer, along with the *camRDCAB* polycistronic operon that codes for cytochromeP450 monooxygenase (Step A, Fig. 1) and 5-*exo*- hydroxycamphor dehydrogenase (Step B, Fig. 1). This coordinate transcriptional control of the first three successive steps of the catabolic pathway by the initial substrate represents 'from the top' coordinate pathway regulation, and has been reported in a number of other catabolic pathways in other *Pseudomonas* spp. Further, the relevant differential rates of synthesis demonstrated that each enantioselective ketolactonase as well as being induced by its own corresponding diketone substrate, was cross-induced by the complementary chiral diketocamphane from the opposite enantiomeric series, and additionally back-induced by OTE. These two interesting forms of induction confirm cross-inducibility and product induction respectively as two additional important elements of transcriptional regulatory control of the ketolactonases in camphor-grown *P. putida* ATCC 17453.

Product induction or so-called 'from the bottom' regulation is not unique to the DKCMOs of camphor-grown *P. putida* ATCC 17453. It has been characterised in a number of other bacterial catabolic pathways, and has been speculated to reflect the evolution of catabolic pathways 'from the bottom to the top' by the sequential acquisition of additional units of physiological function.

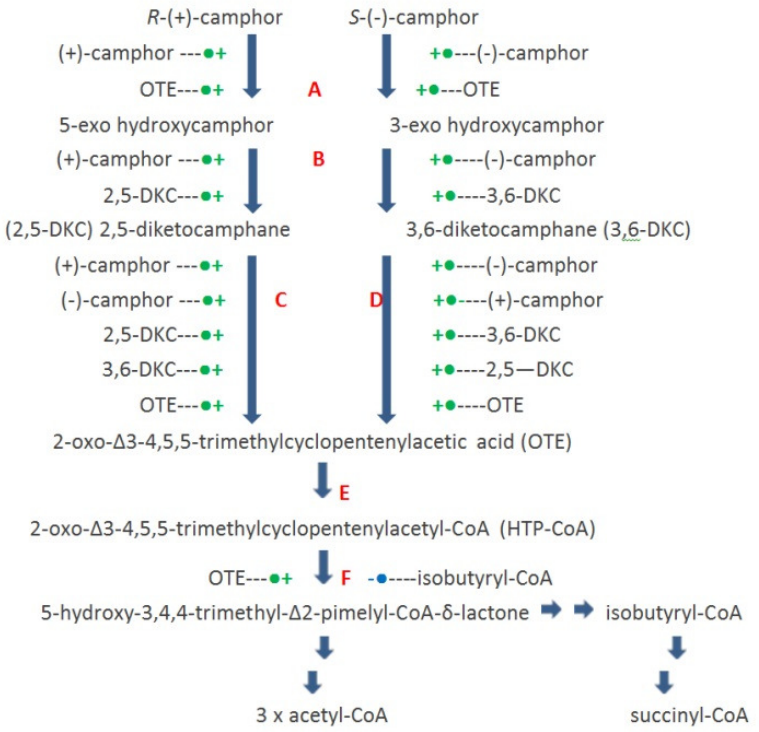

Fig. 2. Transcriptional controls of the pathway of (+)- and (-)-camphor degradation in P. putida ATCC 17453. —-●+ = induction: -●—- = repression: A = cytochromeP450 monooxygenase (camCAB): B = exo-hydroxycamphor dehydrogenase (camD): C = 2,5-diketocamphane 1,2-monooxygenase (cam25-1 + cam25-2): D = 3,6-diketocamphane 1,6-monooxygenase (camE36): E = 2-oxo-Δ3-4,5,5-trimethylcyclopentenylacetyl-CoA synthetase (camF1 + F2): F = 2-oxo-Δ3-4,5,5-trimethylcyclopentenylacetyl-CoA monooxygenase (camG}.

The confirmation of cross-inducibility of the DKCMOs by their complementary chiral diketocamphane pathway intermediates supports earlier studies of the camphor degradation pathway which have consistently reported an equivalent significant element of cross-inducibility of both ketolactonases by each enantiomer of camphor. The broad specificity of the relevant repressor proteins implicated by these various transcriptional controls, allied to the established patterns of coordinate induction has been suggested as illustrative of a more general phenomenon characteristic of a number of different catabolic pathways of pseudomonads, thereby vesting many species of this genus of bacteria with their acknowledged impressive metabolic versatility.

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Publication

Regulation of Camphor Metabolism: Induction and Repression of Relevant Monooxygenases in Pseudomonas putida NCIMB 10007.
Willetts A, Masters P, Steadman C
Microorganisms. 2018 May 7