The Utilization of Tween 80 as Carbon Source by *Pseudomonas*

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INTRODUCTION

Members of the genus *Pseudomonas* are unusual among bacteria in their ability to utilize a wide range of exogenous substrates, and some strains harbour plasmids capable of conjugal transfer to several different genera (Clarke & Richmond, 1975). As part of a study of the transfer of genes for catabolic enzymes from *Pseudomonas* to other hosts, we have looked for the capacity to degrade the detergent Tween 80 (polyoxyethylene sorbitan mono-oleate) in a number of strains. Sierra (1957) reported the formation of calcium oleate from Tween 80 in zones surrounding bacterial growth on solid medium, and Tween 80-hydrolysing enzymes have been prepared from pancreatic extracts (Hall, 1961) and from rat adipose tissue (Wallach, Ko & Marshall, 1962).

METHODS

Strains. *Pseudomonas aeruginosa* PAO and PAT (Holloway, 1955) were obtained from Professor B. W. Holloway, Monash University, Australia. *Pseudomonas fluorescens* 950, 8248 and 8249, and *Ps. putida* 9034, were obtained from the laboratory culture collection.

Culture media. Cultures were grown on nutrient medium (Difco heart infusion agar) and minimal medium (Difco Bacto agar) supplemented with Tween 80 (Koch-Light; 1 %, v/v) and CaCl_2 (0.01 %, w/v) where appropriate.

Mutagenesis. *Pseudomonas aeruginosa* PAO and PAT were screened for mutants using the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Koch-Light; Adelberg, Mandel & Chen, 1965), on medium supplemented with Tween 80 + CaCl_2 on which colonies of the parent strain are surrounded by a zone of opacity. Colonies lacking the characteristic zone were selected and purified.

RESULTS AND DISCUSSION

Two mutants of *Ps. aeruginosa* with reduced or absent Tween 80-degrading ability were isolated; one mutant (PAT12275-1) showed a much reduced zone and acquired an additional auxotrophic requirement, and a second (PAO1594-1) showed no detectable zone and also lost the ability to grow on minimal medium with Tween 80 as carbon source. The phenotype of PAO1594-1 was used in direct selection for re-acquisition of the ability to utilize Tween 80 on mating with Tween 80-hydrolysing donor strains. Strain PAO1594-1 recovered the ability to grow on Tween 80-supplemented minimal medium when mated with PAO (FP2⁺) (frequencies: 2.6×10^{-6} to 2.8×10^{-6} /donor cell) and when mated with PAO (RP1⁺) (frequencies: 0.1×10^{-8} to 1.2×10^{-7} /donor cell). Recombinants were scored after 24 h; occasional revertants (about 1 per plate) were seen on control plates, and confirmatory tests showed that these colonies possessed the markers characteristic of the recipient parent. *Escherichia coli* K12 did not show any opacity on Tween 80-supplemented nutrient medium, nor did it grow on Tween 80-supplemented minimal medium; we are currently attempting to transfer the activity from PAO (RP1⁺) to *E. coli*.

In addition to finding this activity in all strains of *Ps. aeruginosa* tested, we have also detected it in three strains of *Ps. fluorescens* (950, 8248 and 8249) and, at a low level, in *Ps. putida* 9034. Strain PAO1594-1 retains the ability to digest the triglyceride tributyrin (al-esterase: Sierra, 1957), an activity absent from *E. coli*, and we therefore tentatively assume that this property is determined by a different enzyme from that which hydrolyses Tween 80. The rat adipose enzyme described by Wallach *et al.* (1962), however, hydrolysed both Tween 80 and tributyrin. Lipases are currently classified among the hydrolases (Desnuelle, 1972) but are considered to act very slowly, if at all, on water-soluble substrates such as Tween 80 (Wills, 1965). According to this definition the activity we have described is not a true lipase but is likely to be an esterase. The further investigation of such enzymes is likely to be important in view of the need for controlling biological degradation of detergents and environmental pollutants.

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