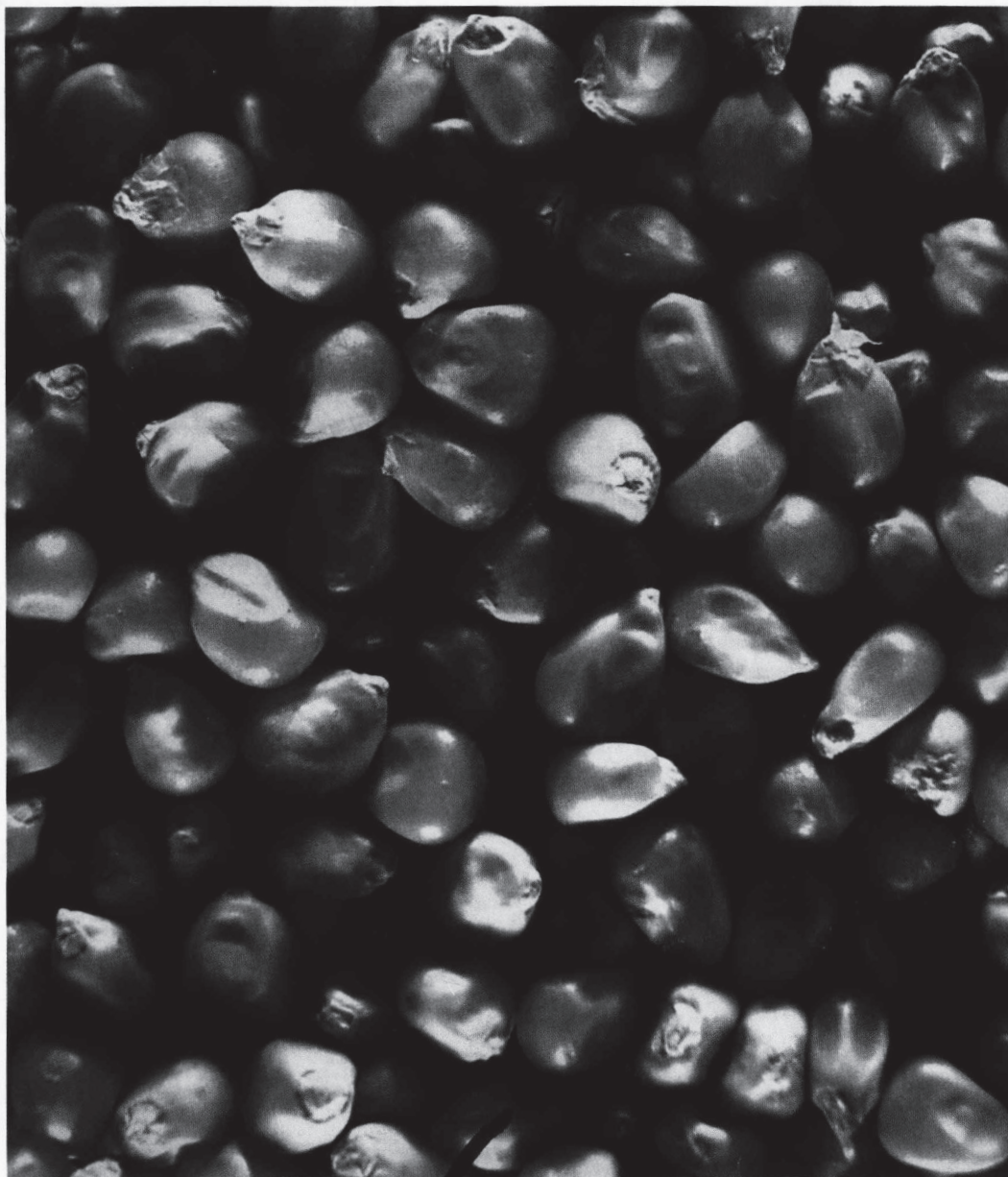




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Cover photograph (Copyright © 2000, American Society for Microbiology. All Rights Reserved.): Corn and the soil bacterium *Pseudomonas putida* KT2440 have been used as a model system to study the genetic basis of bacterial attachment to plant seeds. Isolation and characterization of *P. putida* mutants defective in adhesion to corn seeds have uncovered novel functions in this strain and suggest the existence of different, partially overlapping pathways for bacterial attachment to either biotic or abiotic surfaces. (See related article on page 2363.)

TABLE 2. Effect of different carbon sources on UAS_{ALK}-mediated transcriptional activation

Carbon source	β-Galactosidase activity (pmol min ⁻¹ mg ⁻¹) ^a		Activation (fold)
	Control	+UAS _{ALK}	
Glucose	<1.0	<1.0	
Glycerol	18.2	15.8	0.87
<i>n</i> -Alkane	3.5	71.7	20.5
Acetate	11.1	10.9	0.98
Propionate	24.7	127	5.15
Butyrate	381	1,260	3.30

^a Control indicates pUTA230-transformed cells, and +UAS_{ALK} indicates pUTA05F-transformed cells. The initial cell density (optical density at 570 nm) for each carbon source was as follows: 0.05 for glucose, 0.1 for glycerol, 0.2 for *n*-alkane and acetate, and 0.4 for propionate and butyrate. The cultivation time for each condition was as follows: 24 h for glucose- and glycerol-grown cells, 36 h for *n*-alkane- and acetate-grown cells, and 88 h for propionate- and butyrate-grown cells.

ity of 22.4 and 26.0 pmol/min/mg, respectively) compared with the activity in those harboring pUTA230 (without UAS_{ALK}) (2.51 pmol/min/mg), indicating that UAS_{ALK} is also active in the oleic acid-grown cells. However, the cells harboring pUTA550 (with total *UPR-T34*) had twice the β-galactosidase activity (45.0 pmol/min/mg) as that of the pUTA05F-harboring cells, demonstrating the possible existence of another UAS(s) in addition to the UAS_{ALK} in response to oleic acid on *UPR-T34*.

DISCUSSION

We have identified the UAS sequence that responds to *n*-alkane (UAS_{ALK}) in the *n*-alkane-assimilating yeast *C. tropicalis*. Deletion analysis delimited the sequence of UAS_{ALK} within 29 bp (from positions -289 to -261 of *UPR-T34*). Further mutation analysis showed that the nucleotides that were changed in the M4 mutant (positions -268 and -269) were the most critical for the UAS_{ALK} activity. The 12-bp

sequence including these positions was selected, and similar motifs were searched for promoters of genes encoding several *C. tropicalis* peroxisomal enzymes (Fig. 3). In this 12-bp sequence, the marginal positions were not crucial for the UAS_{ALK} activity, because, as for *CT-T3B*, the marginal positions of the corresponding 12-bp sequence were different from those of *CT-T3A* but the region still had the UAS_{ALK} activity (Fig. 2). In *POX18* and *KAT*, regions were found in which internal 10 bp of UAS_{ALK} (5'-TCCTGCACAC-3') was completely conserved. *KAT* encodes catalase, a marker enzyme of peroxisome, which is highly induced by *n*-alkane (28, 34, 40, 41). Therefore, it is reasonable to consider that this region functions as a UAS_{ALK}. *POX18* of *C. tropicalis* (*POX18*) encodes a nonspecific lipid transfer protein which is induced by oleic acid (35, 36). Although it is not clear at present whether the expression of *C. tropicalis* *POX18* is induced by *n*-alkane, the expression of *Candida maltosa* *POX18* is inducible by *n*-alkane (7). The UAS_{ALK} can also induce transcription by oleic acid; therefore, it seems probable that the expression of *C. tropicalis* *POX18* is induced by *n*-alkane by the common mechanism through UAS_{ALK} as in the oleic acid-grown cells. However, whether the sequences shown in Fig. 3 actually have the UAS_{ALK} activity should be determined by experiments.

In *C. maltosa*, NADPH-cytochrome P-450 reductase which is localized in the endoplasmic reticulum, is highly induced by *n*-alkane. By a reporter gene assay, the 0.47-kbp 5' noncoding region of the gene was shown to be sufficient for the induction on *n*-tetradecane (25). We compared this region with UAS_{ALK}. Although no region closely homologous to UAS_{ALK} was detected, there were two CACAT motifs, the pentanucleotide often found in the 5'-noncoding regions of P-450alk genes, encoding cytochrome P-450, of *C. maltosa* (25). UAS_{ALK} of *C. tropicalis* contains a CACACA sequence at its 3' end. Physiological and genetic evidence suggests that *C. tropicalis* and *C. maltosa* are closely related strains. Therefore, it is likely that the similar activation mechanisms are present in these yeasts, in which the CACA motif sequence might play an important role.

Besides *n*-alkane and oleic acid, UAS_{ALK}-dependent transcription also occurred with butyrate and propionate. These carbon sources can also induce the proliferation of peroxisomes in *C. tropicalis* (17, 39). *n*-Alkane or long-chain fatty acids incorporated in *C. tropicalis* are degraded through the fatty acid β-oxidation system localized in peroxisomes and are ultimately converted into butyryl- or propionyl-CoA. Therefore, the results of this study show that these short-chain fatty acids and/or their derivatives might be a true inducer(s) that causes UAS_{ALK}-dependent transcriptional activation.

In *S. cerevisiae*, the consensus sequence of ORE is suggested as inverted repeats of the CGG triplet with a spacing of 15 to 18 nucleotides (CGG_N₁₅₋₁₈CCG) (13, 30). The CGG triplet repeat is the common consensus sequence for the C₆ zinc cluster family of fungal transcriptional regulators, such as Gal4p (32, 38). In fact, a heterodimeric protein complex consisting of Oaf1p and Oaf2p/Pip2p, both of which have a C₆ zinc cluster motif, was identified as the factor that binds to ORE (12, 22, 30, 31). On the other hand, UAS_{ALK} does not contain the CGG triplet repeat. This fact strongly suggests that the ORE-like regulation mechanism does not exist in *C. tropicalis*. Sloots et al. (33) investigated the regulation mechanism of the gene (*HDE*) encoding the peroxisomal bifunctional enzyme of *C. tropicalis* by introducing its upstream region into *S. cerevisiae*. By deletion analysis, they identified an oleic acid-responsive region located between positions -393 and -341. When this region was compared with UAS_{ALK}, no homologous sequence was observed, which supports our notion that these two

		M3	M4	M5	M6	Score
CT-T3A	[-274]	ATCCTGCACACA				[-263] (12/12)
POX18	[-297]	CTCCTGCACACA				[-308] (11/12)
CT-T3B	[-268]	CTCCTGCACACG				[-257] (10/12)
KAT	[-998]	CTCCTGCACACCT				[-1009] (10/12)
POX4	[-264]	TTTCTGCACACA				[-253] (09/12)
BFE	[-226]	ACACTGCACACA				[-237] (09/12)
CAT	[-272]	CTGCTGCACACA				[-261] (09/12)
POX2	[-432]	CTCCTGCCTCTCC				[-443] (08/12)
BFE	[-253]	CACCTGCACACT				[-242] (08/12)
KAT	[-378]	GCCAGCACAAC				[-367] (07/12)
POX2	[-251]	GGCATGCACGCT				[-240] (07/12)

FIG. 3. Nucleotide sequences similar to UAS_{ALK} found on promoters of *C. tropicalis* peroxisomal enzyme genes. *POX2* and *POX4* encode acyl-CoA oxidase (accession numbers for *POX2* and *POX4* are M18259 and M12160, respectively); *BFE* encodes the bifunctional enzyme (X57854); *CAT* encodes carnitine acetyltransferase (D84549) (unpublished data); *KAT* encodes catalase (X13978, E01922) (unpublished data); *POX18* encodes nonspecific lipid transfer protein (X53633 and M24440). The score indicates the number of the nucleotides that are the same as those of *CT-T3A*. The positions of changed nucleotides in the UAS_{ALK} mutants (M3, M4, M5, and M6) are indicated above the sequences. Numbers on both sides of the sequences indicate the distance relative to the translational start codon.