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Identification of Novel Genes Involved in Long-Chain *n*-Alkane Degradation by *Acinetobacter* sp. Strain DSM 17874[▽]

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***Acinetobacter* sp. strain DSM 17874 is capable of utilizing *n*-alkanes with chain lengths ranging from that of decane ($C_{10}H_{22}$) to that of tetracontane ($C_{40}H_{82}$) as a sole carbon source. Two genes encoding AlkB-type alkane hydroxylase homologues, designated *alkMa* and *alkMb*, have been shown to be involved in the degradation of *n*-alkanes with chain lengths of from 10 to 20 C atoms in this strain. Here, we describe a novel high-throughput screening method and the screening of a transposon mutant library to identify genes involved in the degradation of *n*-alkanes with C chain lengths longer than 20, which are solid at 30°C, the optimal growth temperature for *Acinetobacter* sp. strain DSM 17874. A library consisting of approximately 6,800 *Acinetobacter* sp. strain DSM 17874 transposon mutants was constructed and screened for mutants unable to grow on dotriacontane ($C_{32}H_{66}$) while simultaneously showing wild-type growth characteristics on shorter-chain *n*-alkanes. For 23 such mutants isolated, the genes inactivated by transposon insertion were identified. Targeted inactivation and complementation studies of one of these genes, designated *almA* and encoding a putative flavin-binding monooxygenase, confirmed its involvement in the strain's metabolism of long-chain *n*-alkanes. To our knowledge, *almA* represents the first cloned gene shown to be involved in the bacterial degradation of long-chain *n*-alkanes of 32 C's and longer. Genes encoding AlmA homologues were also identified in other long-chain *n*-alkane-degrading *Acinetobacter* strains.**

Long-chain (LC) alkanes, with chain lengths of >20 C atoms, are environmental pollutants and may also cause problems in recovery, transportation, and processing of crude oil by e.g., clogging pipes. The possibilities of using processes based on the microbial biodegradation of hydrocarbons for removal of pollutants from the environment and upgrading oil refinery products have been suggested (27). Several bacterial enzymes for aerobic degradation of alkanes have been identified, e.g., cytochrome P450 (11), monooxygenase (6), and dioxygenase (10). The best-characterized system for alkane degradation is the Alk system of *Pseudomonas putida* GPo1 (26), sequentially converting alkanes to the corresponding alcohols, aldehydes, carboxylic acids, and acyl-coenzyme A's (CoAs), which then enter the β-oxidation pathway. Most of these systems catalyze the degradation of relatively short-chain alkanes, and very little is known about enzymes involved in the degradation of LC alkanes.

Strains of the genus *Acinetobacter*, capable of utilizing alkanes with C chain lengths ranging from 10 to 44 have been described (1, 12, 18, 21, 24). *Acinetobacter* sp. strain DSM 17874 (initially described as *A. venetianus* 6A2 [24]) is capable of utilizing C_{10} to C_{40} *n*-alkanes as a sole carbon source. We have recently identified two *alkB* paralogs, *alkMa* and *alkMb*, which were shown to be involved in the utilization of *n*-alkanes with C chain lengths up to 20 in this strain (24). Furthermore, we postulated the existence of at least one other enzyme system in *Acinetobacter* sp. strain DSM 17874 involved in degradation of LC alkanes.

Here we describe the construction and high-throughput screening (HTS) of a library of transposon mutants of *Acinetobacter* sp. strain DSM 17874 (24), which have led to identification of several genes possibly involved in LC alkane degradation. Detailed analysis of one of these genes, designated *almA*, confirmed its involvement in the degradation of LC alkanes with C chain lengths of 32 and longer. Interestingly, the *almA*-deficient mutant, MAV1, could still grow with C_{24} and shorter alkanes as a sole carbon source, indicating the occurrence of yet another enzyme system for the degradation of C_{20} and longer alkanes in *Acinetobacter* sp. strain DSM 17874.

MATERIALS AND METHODS

Bacterial strains, plasmids, and primers. The bacterial strains, plasmids, and oligonucleotide primers used in this study are presented in Table 1.

Media and growth conditions. Bacterial strains were grown in Luria broth (LB; 10 g tryptone [Oxoid], 5 g yeast extract [Oxoid], and 5 g NaCl per liter of deionized water) or Czapek broth (CB; 3 g $NaNO_3$, 1 g K_2PO_4, 0.5 g $MgSO_4$, 0.5 g KCl, and 0.01 g $FeSO_4$ per liter of deionized water, pH 7.5, supplemented with various carbon sources). The same media were solidified with 15 g agar per liter medium to make plates. Antibiotics at the following concentrations were used when appropriate: ampicillin, 100 mg/liter; apramycin, 50 mg/liter; chloramphenicol, 30 mg/liter; and kanamycin, 25 mg/liter. *n*-Alkanes of defined chain lengths were purchased from Sigma-Aldrich. They will be referred to by the number of carbon atoms they contain, e.g., decane will be referred to as C_{10}, throughout the paper. Growth of *Acinetobacter* strains in liquid and on solid media supplemented with *n*-alkanes was carried out as described before (24).

Construction of an *Acinetobacter* sp. strain DSM 17874 transposon mutant library. Plasmid pLOFKm (7), carrying a mini-Tn*10* delivery system, was used to construct an *Acinetobacter* sp. strain DSM 17874 transposon mutant library. The pLOFKm plasmid contains a mini-Tn*10* transposon harboring a kanamycin re-

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TABLE 1. Bacterial strains, plasmids, and oligonucleotide primers used in this study

Strain, plasmid, or primer	Source and/or reference; description or sequence (5′-); restriction site[a]
Bacterial strains	
Acinetobacter sp. strain DSM 17874	24; Cam^r, deposited in the DSMZ strain collection under accession no. DSM 17874
Escherichia coli S17-1 (λ*pir*)	4
Acinetobacter baylyi ADP1	A. Steinbüchel, 8
Acinetobacter sp. strain M-1	Y. Sakai, 21
Acinetobacter sp. strain RAG-1	Purchased from ATCC, accession no. ATCC 31012
Acinetobacter sp. strain MAV1	This study; *almA* knockout mutant, Cam^r Apr^r
Acinetobacter sp. strain BKO2	This study; *almB* knockout mutant, Cam^r Apr^r
Plasmids	
pLOFKm	7; mini-Tn*10* delivery vector, Kan^r
pSOK201	28; Apr^r
pSOK804	3; Apr^r
pDLM02.1	13; *A. baylyi* ADP1 and ColE1 replication origin, Tdk/Kan^r selection/counter-selection cassette, Amp^r
pLAL50	This study; part of pSOK804 containing a 607-bp *almA* fragment, Apr^r
pBKO2	This study; derivative of pSOK201 containing a 300-bp *orf1* fragment, Apr^r
pALMA1	This study; derivative of pDLM02.1 carrying the *almA* gene from *Acinetobacter* sp. strain 6A2, Amp^r Kan^r
pACIAD1	This study; derivative of pDLM02.1 carrying the ACIAD3192 gene from *A. baylyi* ADP1, Amp^r Kan^r
Primers	
Tn10:1	GGATCATATGACAAGATGTG
Kan1	GCTCTAGACCGTCAAGTCAGCGTAATGC
almA1	GACATGTGTATTGTCAAATTTGTGC
almA2	CCAATGAGATCATGGAAGAAC
almA3	GCTCTAGACTATCCTGGTATTCGTTCAG; XbaI
almA4	CGGGATCCTAAATACCACGTTGCATACC; BamHI
almA5	GCGCGCGCATGCGACATGTGTATTGTCAAATTTGTGC; SphI
almA6	GCGCGCGGCGCCCCCTATGCCATGCATAGGGTTTC; NarI
orf1:1	GCGCGCGCATGCGACAGCAATTGCTGAGAAGTTGG; SphI
orf1:2	GCGCGCGAATTCGTGAAATGAGCAACCTCTCCTCC; EcoRI
ACIAD1	GCGCGCGCATGCCGCGAAGTAAACACTTCAGCAG; SphI
ACIAD2	GCGCGCGGCGCCCCTTTTTTTGTTTAGCTCAAGTTAGGATAC; NarI

[a] Antibiotic resistance markers: Amp^r, ampicillin resistance; Cam^r, chloramphenicol resistance; Kan^r, kanamycin resistance; Apr^r, apramycin resistance. Restriction sites are underlined.

sistance marker, the conjugal transfer origin *oriT*, and the Tn*10* transposase under the control of an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible promoter. This plasmid cannot replicate in *Acinetobacter* sp. strain DSM 17874. pLOFKm was introduced into *Acinetobacter* sp. strain DSM 17874 via conjugation. *Escherichia coli* S17-1 (λ*pir*)/pLOFKm and *Acinetobacter* sp. strain DSM 17874 were grown in 50 ml LB containing the appropriate antibiotics in 500-ml baffled Erlenmeyer flasks at 30°C on a rotary shaker at 200 rpm. When the cultures had reached an optical density at 600 nm of about 0.4, several aliquots of 1 ml of each culture were mixed and immediately centrifuged at 8,000 rpm for 5 min. The pellets were resuspended in 100 μl LB and placed as drops on LB plates supplemented with 500 μM IPTG to induce the expression of the transposase. The plates were incubated at 30°C overnight. Bacteria were harvested from the plates and resuspended in LB medium. The resulting cell suspension was diluted and spread onto LB plates supplemented with kanamycin and chloramphenicol to select against the *E. coli* donor cells (24). The plates were incubated at 30°C for about 48 h, and colonies were picked using a QPix robot (Genetix) and transferred into 96-well microtiter plates (Nunc) containing 120 μl LB supplemented with chloramphenicol and kanamycin in each well. All liquid handling using 96-well microtiter plates was carried out using a Genesis RSP200 robot (Tecan). The plates were incubated at 30°C and 900 rpm in a Multitron shaking incubator (Infors) for 24 h. A library containing approximately 6,800 *Acinetobacter* sp. strain DSM 17874 transposon mutants was created.

HTS of the *Acinetobacter* sp. strain DSM 17874 transposon mutant library for mutants deficient in LC alkane degradation. Transposon mutants from the 96-well library plates were replicated onto Omnitray plates (Nunc) containing solid CB medium without a carbon source. After transfer of the mutants, C_{32} alkane was added as a powder to the Omnitray plates as described before (24). The plates were incubated at 30°C for 48 h, and growth was detected by overlaying the plates with a top agar containing 0.05% (wt/vol) iodonitrotetrazolium chloride (INT). Reduction of INT by the active respiratory chain of growing cells led to purple staining of colonies (5). Mutants not showing growth on the C32 alkane were rearrayed using the QPix robot from the original library into new 96-well microtiter plates containing 120 μl LB, supplemented with chloramphenicol and kanamycin in each well. The plates were incubated overnight at 30°C and 900 rpm, resulting in a library enriched for mutants no longer capable of utilizing C_{32} as a carbon source. To confirm the initial screening results and further analyze these mutants, they were replicated onto Omnitray plates containing CB supplemented with 0.5% (wt/vol) sodium acetate, C_{16}, or C_{32} alkanes as a sole carbon source. Mutants showing no growth on C_{32} and coincidentally growing at wild-type level on plates supplemented with acetate or C_{16} were chosen for further analysis.

Molecular biology methods. Total chromosomal DNA was isolated from *Acinetobacter* strains using the QIAGEN DNeasy tissue kit. PCR amplification and sequencing of chromosomal regions flanking the transposons of selected *Acinetobacter* sp. strain DSM 17874 mutants were carried out by inverse PCR (15) followed by ABI sequencing using primers Tn10:1 and Kan1 (Table 1), specific for the transposon sequence. PCR and subsequent sequencing of the *almA* region from *Acinetobacter* sp. strain DSM 17874 and homologous regions from *Acinetobacter* sp. strain M-1 and *Acinetobacter* sp. strain RAG-1 were carried out using primers almA1 and almA2 (Table 1), followed by primer walking. The sequence of the *Acinetobacter baylyi* ADP1 ACIAD3192 gene was obtained from GenBank, accession no. NC_005966 (2).

Construction of the *Acinetobacter* sp. strain DSM 17874 *almA* and *orf1* disruption mutants, MAV1 and BKO2. Suicide vectors pLAL50 and pBKO2, for disruption of *almA* and *orf1*, respectively, in *Acinetobacter* sp. strain DSM 17874 were constructed. A 607-bp XbaI-BamHI *almA* fragment and a 300-bp SphI-EcoRI *orf1* fragment were PCR amplified using *Acinetobacter* sp. strain DSM 17874 chromosomal DNA as the template and the primer pairs almA3 and almA4 for *almA* amplification and orf1:1 and orf1:2 for *orf1* amplification (Table 1). The *almA* PCR fragment was digested with XbaI and BamHI and ligated into

TABLE 2. Genes identified in HTS of the *Acinetobacter* sp. strain DSM 17874 transposon mutant library

Gene[a]	Organism	Function or functional category	No. of mutants[b]	% Identity/ length (bp)
ACIAD3192	*A. baylyi* ADP1	Putative monooxygenase	1	81–91/35–447
gacS/barA	*A. baylyi* ADP1	GacS-like sensor kinase protein	5	79–96/71–157
xcpS	*A. baylyi* ADP1	General secretion pathway protein F	1	82–84/107–152
xcpR	*A. baylyi* ADP1	General secretion pathway protein E, putative ATPase	2	87–88/61–115
ACIAD0294	*A. baylyi* ADP1	General secretion pathway protein	2	80/121–209
32_384	*A. baumannii* AYE	Putative major facilitator superfamily drug transporter	1	80/257
filA	*Acinetobacter* sp. strain BD413	Pilus assembly system *fil* gene cluster	1	96/29
ACIAD0505	*A. baylyi* ADP1	Formyltetrahydrofolate deformylase	1	86/365
ACIAD2911, *panD*	*A. baylyi* ADP1	Aspartate 1-decarboxylase precursor CoA	1	83/179
ugd	*Acinetobacter* sp. strain RAG-1	UDP-glucose DH (close to the *wee* gene cluster for emulsan production)	1	100/461
himD	*A. baylyi* ADP1	Integration host factor, β-SU	1	83/218
ACIAD0049	*A. baylyi* ADP1	Putative lineoyl-CoA desaturase	1	80–91/43–187
ACIAD0473	*A. baylyi* ADP1	Putative trancriptional regulator (AraC family)	2	83–96/26–136
truA	*A. baylyi* ADP1	tRNA-pseudouridine synthase	1	84/150
acnA	*A. baylyi* ADP1	Aconitate hydratase	1	81–84/242–294
ACIAD3547	*A. baylyi* ADP1	Hypothetical protein, putative enzyme	1	84/140

[a] Gene with closest homology in BLASTn homology search.
[b] For six mutants, there was no reliable match; five mutants could not be analyzed.

the 3-kb XbaI-BamHI fragment of pSOK804 (which is identical to that of pSOK201 [28]) containing the ColE1 ori, *oriT*, and an apramycin resistance gene, resulting in plasmid pLAL50. The *orf1* PCR product was digested with SphI and EcoRI and cloned into the 3-kb SphI-EcoRI fragment of pSOK201 (28) to give plasmid pBKO2. Each plasmid was first transferred into *E. coli* S17-1 (λ*pir*) via heat shock transformation and subsequently transferred into *Acinetobacter* sp. strain DSM 17874 by conjugation. Transconjugants were selected on LB plates containing apramycin and chloramphenicol. Integration of the plasmids into the *Acinetobacter* sp. strain DSM 17874 chromosome resulted in the *almA* and *orf1* disruption mutants, MAV1 and BKO2, respectively. The insertion of the respective vectors into the *Acinetobacter* sp. strain DSM 17874 chromosome was confirmed by Southern blot analysis using the DIG nonradioactive nucleic acid labeling and detection system (Roche) and probes for detection of *almA* or *orf1*.

Construction of plasmids pALMA1 and pACIAD1 for complementation studies. The *almA* gene of *Acinetobacter* sp. strain DSM 17874 was amplified by PCR using the primers almA5 and almA6 (Table 1) and chromosomal DNA from *Acinetobacter* sp. strain DSM 17874 as a template. The PCR product was digested with SphI and NarI and ligated into similarly digested vector pDLM02.1, giving plasmid pALMA1.

The ACIAD3192 gene from *A. baylyi* ADP1 was amplified by PCR using the primers ACIAD1 and ACIAD2 (Table 1) and chromosomal DNA from *A. baylyi* ADP1 as a template. The PCR product was digested with SphI and NarI and ligated into similarly digested vector pDLM02.1, giving plasmid pACIAD1.

Nucleotide sequence accession numbers. The GenBank accession numbers for the sequences reported in this paper are EF212873 for the *Acinetobacter* sp. strain DSM 17874 *almA* region, EF212874 for the *Acinetobacter* sp. strain RAG-1 *almA* region, and EF212875 for the *Acinetobacter* sp. strain M-1 *almA* region.

RESULTS

A novel high-throughput screening method allows identification of genes involved in *n*-alkane degradation in *Acinetobacter* sp. strain DSM 17874. The presence of at least one metabolic pathway for degradation of *n*-alkanes with chain lengths of over 20 C atoms in *Acinetobacter* sp. strain DSM 17874 has been postulated (24). To identify genes involved in the metabolism of LC alkanes in *Acinetobacter* sp. strain DSM 17874, we have designed and used a novel HTS method for screening an *Acinetobacter* sp. strain DSM 17874 transposon mutant library for mutants which can no longer utilize solid LC alkanes as a sole carbon source. This screening yielded 34 mutants showing no significant growth with C_{32} as a sole carbon source but wild-type-like growth with acetate or C_{16} as a sole carbon source. The mutants were analyzed for the site of transposon insertion, and 16 different putative genes were identified (Table 2). All of these genes showed highest homology to genes from other *Acinetobacter* strains, predominantly *A. baylyi* ADP1, for which the entire genome has been sequenced (2). One mutant harbored the transposon insertion within a gene encoding a homologue of a putative flavin-binding monooxygenase, ACIAD3192 from *A. baylyi* ADP1. This gene, designated *almA* (*n*-*al*kane *m*etabolism A), was chosen for further analysis.

The *almA* locus is present in other *Acinetobacter* spp. capable of degrading LC alkanes. The *almA* gene and a part of the surrounding chromosomal region in *Acinetobacter* sp. strain DSM 17874 were sequenced, and the sequence of the surrounding region was found to be very similar to that of the region surrounding *A. baylyi* ADP1 ACIAD3192 (Fig. 1), with the exception of the presence of an additional open reading frame 127 bp downstream of *almA* in *Acinetobacter* sp. strain DSM 17874. The latter putative gene, designated *orf1*, presumably encodes a 166-amino-acid polypeptide, but no significant homology to any known gene or protein could be found in the GenBank database.

A. baylyi ADP1 and *Acinetobacter* sp. strains RAG-1 and M-1 were also found to grow with C_{32} and C_{36}, respectively, as a sole carbon source (our unpublished data; 21). Genes homologous to *almA* and *orf1* were also identified in *Acinetobacter* sp. strain RAG-1 and *Acinetobacter* sp. strain M-1 (Fig. 1). Several AlmA homologues identified in the GenBank database were phylogenetically analyzed using MEGA version 3.1 (9) and the neighbor-joining algorithm (20). The AlmA homologues from *Acinetobacter* sp. strain DSM 17874, *A. baylyi* ADP1, and *Acinetobacter* sp. strains RAG-1 and M-1 clustered together with homologues from, e.g., *Marinobacter aquaeolei* VT8, *Alcanivorax borkumensis* SK2 (two proteins), *Oceanobacter* sp. strain RED65, *Ralstonia* spp., *Mycobacterium* spp., a

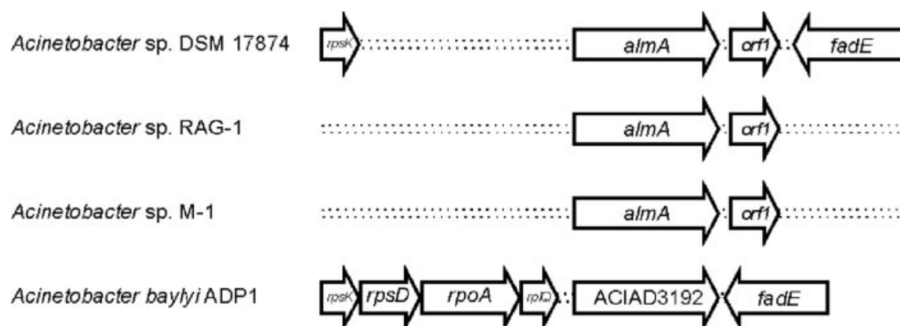

FIG. 1. Schematic representation of the *almA* regions in different *Acinetobacter* strains. Arrows indicate the relative orientations of the genes. Gaps indicate estimated distances. The *Acinetobacter* sp. strain DSM 17874 *almA* coding region is 1,491 bp and shows 74.8% sequence identity to ACIAD3192 from *A. baylyi* ADP1. The identity between the deduced AlmA and ACIAD3192 peptide sequences is 76.7%. The *almA* and *orf1* genes from *Acinetobacter* sp. strain RAG-1 showed 100% nucleotide sequence identity to *Acinetobacter* sp. strain DSM 17874. The nucleotide sequence identity between *Acinetobacter* sp. strain DSM 17874 and *Acinetobacter* sp. strain M-1 was found to be 85.7% for *almA* and 75.5% for *orf1*, with predicted peptide sequence identities of 95.4% for AlmA and 80.1% for Orf1.

Photorhabdus sp., *Psychrobacter* spp., and *Nocardia farcinica* IFM10152 as close neighbors (data not shown). All of these are annotated solely according to sequence homology, and functions have not yet been experimentally confirmed.

AlmA is involved in the degradation of LC alkanes in *Acinetobacter* sp. strain DSM 17874. To analyze the function of the *almA* gene in LC alkane utilization, the *Acinetobacter* sp. strain DSM 17874 *almA* mutant MAV1 was constructed and used in growth experiments with various *n*-alkanes as a sole carbon source. MAV1 did not grow with C_{32} and C_{36} alkanes as a sole carbon source, while showing wild-type growth with C_{20} and C_{24} alkanes (Fig. 2), indicating that AlmA functions specifi-

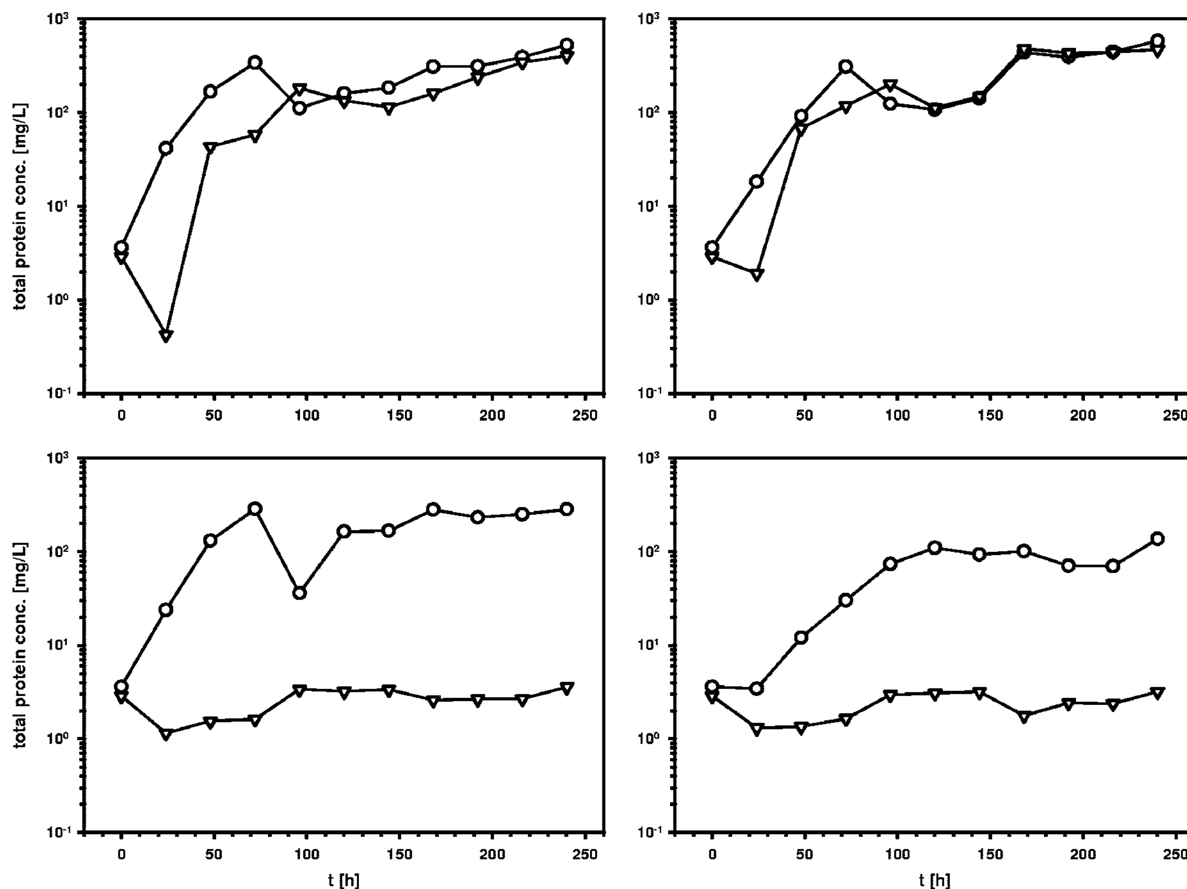

FIG. 2. Growth of *Acinetobacter* sp. strain DSM 17874 (wild-type; ○) and *Acinetobacter* sp. strain MAV1 (*almA*-deficient mutant; ▽) in 100 ml CB medium supplemented with seven 50-μl solid droplets of C_{20} (top left), C_{24} (top right), C_{32} (bottom left), or C_{36} (bottom right). Growth was measured as an increase in protein in the cultures over time. The values presented are the averages of two independent experiments.

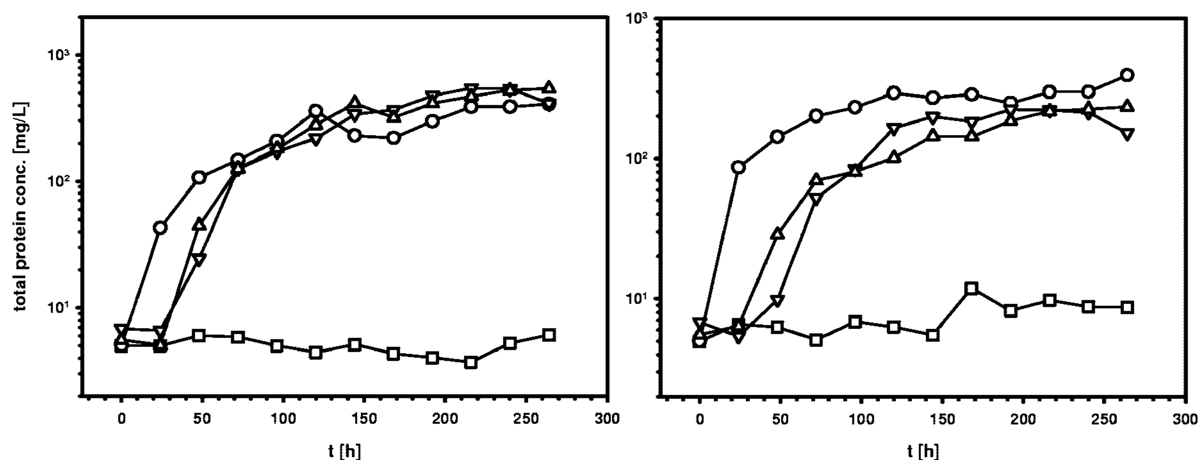

FIG. 3. Complementation of *almA* activity in the *almA*-deficient mutant *Acinetobacter* sp. strain MAV1 with the *almA* gene on a plasmid (see Table 1). Shown are data on the growth of *Acinetobacter* sp. strain DSM 17874/pDLM02.1 (○), *Acinetobacter* sp. strain MAV1/pDLM02.1 (□), *Acinetobacter* sp. strain MAV1/pALMA1 (▽), and *Acinetobacter* sp. strain MAV1/pACIAD1 (△) in 100 ml CB medium supplemented with seven 50-μl solid droplets of C_{32} (left) or C_{36} (right). Growth was measured as an increase in protein in the cultures over time. The values presented are the averages of two independent experiments.

cally in LC alkane utilization in *Acinetobacter* sp. strain DSM 17874.

Introduction of the *almA* gene on a plasmid in the *almA*-deficient strain MAV1 restored the strain's ability to grow with C_{32} and C_{36} alkanes as a sole carbon source to almost wild-type level (Fig. 3). This result confirmed that the LC alkane degradation deficiency of MAV1 is not caused by a polar effect on the genes downstream of *almA* and that the *almA* deficiency is responsible for the inability of the MAV1 mutant to utilize LC alkanes.

The ACIAD3192 gene from *A. baylyi* ADP1 represents the closest homologue to the *almA* gene found in the databases. Therefore, this gene was chosen for heterologous functional complementation of the *almA*-deficient mutant MAV1. *Acinetobacter* sp. strain MAV1 carrying the ACIAD3192 gene on a plasmid showed growth with C_{32} and C_{36} alkanes (Fig. 3), clearly showing that the product of this gene is a functional homologue of AlmA in the LC alkane utilization pathway.

To investigate whether *orf1* is involved in *n*-alkane metabolism by *Acinetobacter* sp. strain DSM 17874, an *orf1* disruption mutant strain, BKO2, was constructed. Growth of BKO2 was found to be indistinguishable from that of the wild-type strain with C_{20}, C_{24}, C_{32}, and C_{36} alkanes as sole carbon sources (data not shown), suggesting that *orf1* most likely is not involved in the strain's alkane metabolism, at least not under the conditions tested.

DISCUSSION

We here describe the identification of novel genes involved in LC alkane degradation by *Acinetobacter* sp. strain DSM 17874 using HTS of a transposon mutant library consisting of ca. 6,800 mutants. Although this number of mutants most likely does not cover insertions into all of the strain's nonessential genes, it allowed identification of several genes presumably involved in LC alkane degradation (Table 2). Some of the genes identified here have been found to be involved in (short-chain) alkane utilization previously, e.g., the general secretion pathway gene *xcpR* (16), the regulator of the AraC family (17), and the *ugd* gene (14). Further investigation is necessary to confirm and analyze the involvement of these genes specifically in alkane metabolism in strain DSM 17874. We analyzed the involvement of the newly identified *almA* gene in LC alkane degradation in more detail. This gene is homologous to the ACIAD3192 gene from *A. baylyi* ADP1 and encodes a putative flavin-binding monooxygenase. The majority of the enzymes reported to be involved in the initial step of aerobic alkane metabolism are represented by monooxygenases/hydroxylases (6, 17, 23, 25), and for *Alcanivorax borkumensis* SK2, a number of putative monooxygenases and oxidoreductases have been implicated in alkane degradation (19, 22).

The involvement of AlmA in the utilization of C_{32} and C_{36} alkanes by *Acinetobacter* sp. strain DSM 17874 was confirmed by mutational analysis and complementation studies. Interestingly, the *almA*-deficient mutant could still grow on alkanes with a C chain length up to 24, indicating the presence of at least one more enzyme system involved in the degradation of C_{20} to C_{24} alkanes in the strain.

The LC alkane utilization deficiency of the *almA* disruption mutant could also be complemented by the ACIAD3192 gene from *A. baylyi* ADP1, suggesting a similar function for the latter gene in ADP1. The present study demonstrates the utility of the novel HTS system and paves the way for comprehensive analysis of genes and enzymes involved in bacterial alkane utilization.

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