Identification of the Last Unknown Genes in the Fermentation Pathway of Lysine

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Although the proteins of the lysine fermentation pathway were biochemically characterized more than thirty years ago, the genes encoding the proteins that catalyze three steps of this pathway are still unknown. We combined gene context, similarity of enzymatic mechanisms, and molecular weight comparisons with known proteins to select candidate genes for these three orphan proteins. We used a wastewater metagenomic collection of sequences to find and characterize the missing genes of the lysine fermentation pathway. After recombinant protein production and purification following cloning in Escherichia coli, we demonstrated that these genes (named kdd, kec, and kal) encode a 1-erythro-3,5-diaminohexanoate dehydrogenase, a 3-keto-5-aminohexanoate cleavage enzyme, and a 3-aminobutyryl-CoA ammonia lyase, respectively. Because all of the genes of the pathway are now identified, we used this breakthrough to detect lysine-fermenting bacteria in sequenced genomes. We identified twelve bacteria that possess these genes and thus are expected to ferment lysine, and their gene organization is discussed.

In the early 1950s, it was demonstrated that lysine was decomposed to acetate, butyrate, and ammonia by Clostridium sticklandii (1) and by two strains of Escherichia coli, which ferment lysine together when both are incubated with the substrate (2). This catabolic pathway comprises ten distinctive reactions (see Fig. 1). The first two steps are catalyzed by lysine-2,3-aminomutase and β-lysine-5,6-aminomutase. Both enzymes have been biochemically characterized (3), and their corresponding genes have been identified (4, 5).

The second intermediate in the fermentation pathway, 1-erythro-3,5-diaminohexanoate, undergoes an oxidative deamination leading to 3-keto-5-aminohexanoate (KAH)4 (see Fig. 2, reaction 1). Baker et al. (6) have purified and characterized a NAD-dependent 1-erythro-3,5-diaminohexanoate dehydrogenase (EC 1.4.1.11) from extracts of Clostridium SB4. Two years later, Baker and van der Drift (7) identified the 3,5-diaminohexanoate dehydrogenase in C. sticklandii. This dehydrogenase has also been characterized from Brevibacterium L5, an obligate aerobe (8, 9), and Fusobacterium nucleatum (10). Although this enzyme has been characterized from several different bacteria, no gene has been assigned to the protein.

The 3-keto-5-aminohexanoate is further converted into 3-aminobutyryl-CoA and acetoacetate in the presence of acetyl-CoA (see Fig. 2, reaction 2). This reaction presents a new type of β-ketoacid cleavage and acetoacetate synthesis by a yet unknown mechanism. This type of β-ketoacid-degrading enzyme, named 3-keto-5-aminohexanoate cleavage enzyme, has been characterized from Clostridium SB4 (11) and F. nucleatum (10), two obligate anaerobes, and from Brevibacterium L5 (9, 12). The three enzymes are quite similar but not identical in terms of substrate specificity, inhibitors, and activation. Again, no gene coding for this protein has been determined.

In the next step, 3-aminobutyryl-CoA is deaminated to crotonyl-CoA (see Fig. 2, reaction 3). The ammonia lyase (EC 4.3.1.14), which catalyzes the reaction, has been purified from Clostridium SB4, and some of its properties have been determined (13). The gene for the ammonia lyase has not been identified so far.

Crotonyl-CoA is then reduced to butyryl-CoA, which reacts with acetoacetate to form butyrate and acetoacetyl-CoA. The latter compound is converted to acetate via acetyl-CoA and acetyl phosphate. All of the genes involved in these steps are known.

In recently sequenced bacterial genomes (Porphyromonas gingivalis, Thermoanaerobacter tengcongensis, Symbiobacterium thermonilum, and F. nucleatum), the two aminomutases characteristic of the lysine fermentation pathway have been identified (accession numbers in UniProt are Q7MVJ1, Q7MV18, Q7MV17, Q8RT6, Q8RT2, Q8RT2, Q67TH3, Q67TH1, Q67TH0, Q8RHX4, Q8RHX7, and Q8RHX8). Of these bacteria, only F. nucleatum has been described to ferment lysine under anaerobic conditions (10, 14). Nevertheless, in all of these genomes but one, S. thermonilum, genes encoding the proteins known to be involved in lysine fermentation (the two aminomutases and the butyrate:acetoacetate CoA-transferase in Fig. 1) appear in a cluster along with a series of
other co-localizing genes. Some are annotated as putative or hypothetical proteins. We therefore examined the possibility that some of these genes could correspond to the missing ones coding for the 3,5-diaminohexanoate dehydrogenase, the 3-keto-5-aminohexanoate cleavage enzyme, and the 3-aminobutyryl-CoA ammonia lyase, named Kdd, Kce, and Kal, respectively. In this paper, we have described the method of identification of these three genes, the expression of the corresponding proteins, and the biochemical characterization of the enzyme activities.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—All chemicals and enzymes were purchased from Sigma-Aldrich. Reagents for molecular biology were from Invitrogen. Oligonucleotides were from Sigma Genosys.

Proteinase inhibitor Pefabloc SC was purchased from Roche Diagnostics. DL-**Erythro**-3,5-diaminohexanoate (DAH) was synthesized by Orga-Link. The purity of the product was checked by 1H-NMR in Me2SO-d6.

KAH was prepared by enzymatic conversion of DAH. KAH was produced using 50 μg of Kdd, 22 mM DAH, and 4 mM NADP+ in 1 ml of activity buffer (50 mM Tris, pH 8.0, 200 mM NaCl, 10% glycerol) according to Fig. 2, reaction 1. Once equilibrium was reached (as indicated by NADP+ reduction kinetics), the reaction was stopped. As KAH concentration could not be determined directly, it was estimated by assaying both NADPH and NH4+ by measuring absorption at 340 nm and pH change, respectively.

**Construction of the Expression Vectors**—The coding sequences of kdd, kce, and kal were amplified by PCR with the following primers: kdd, forward, 5′-CACCATGTGAATTCATCACCACATAG-3′ and reverse, 5′-CTAGGGTACTAGTCTTCTTACTCAG-3′; kce, forward, 5′-ATGCGATCCATCACCACATAG-3′ and reverse, 5′-TTATTTATTTATGCTTCTTCTTACG-3′; and kal, forward, 5′-ATGCGATCCATCACCACATAG-3′ and reverse, 5′-CTAAACCTTCTCTCTCTCTCCTAGTCGTA-3′.

The forward primers introduced a hexahistidine sequence in the proteins after the initial methionine for purification purposes. The amplified sequence of kdd was inserted into the Invitrogen pET101/D-TOPO vector by directional cloning according to the manufacturer's protocol. The amplified sequences of kce and kal were inserted into Invitrogen pCRT7/CT-TOPO vector. The sequences of the resulting plasmids, named pET101-kdd, pCRT7-kce, and pCRT7-kal, respectively, were verified.

**Expression and Purification of the Recombinant Proteins**—The same expression/purification protocol was set up for the three proteins after the initial methionine for purification purposes. The amplified sequence of kdd was inserted into the Invitrogen pET101/D-TOPO vector by directional cloning according to the manufacturer's protocol. The amplified sequences of kce and kal were inserted into Invitrogen pCRT7/CT-TOPO vector. The sequences of the resulting plasmids, named pET101-kdd, pCRT7-kce, and pCRT7-kal, respectively, were verified.

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carbenicillin at 37 °C until reaching an $A_{400}$ of 2. Isopropyl β-D-thiogalactopyranoside was added at a concentration of 500 μM to induce protein production, and the cells were further grown at 20 °C overnight. After centrifugation, the cells were washed and suspended in 50 ml of lysis buffer (50 mM phosphate, pH 8.0, 500 mM NaCl, 10% glycerol, and 10 mM imidazole) containing 1 mM Pefabloc SC and 22 mM of Lysonase™ bioprocessing reagent (Novagen) and sonicated using an Ultrasonic processor. After centrifugation, to clarify the cell extract, the supernatant was loaded onto a 1-ml HisTrap FF column (Amersham Biosciences) using an Äkta Explorer (Amersham Biosciences). After sample loading, the column was washed with lysis buffer and the protein was eluted with the same buffer containing 250 mM imidazole. Removal of imidazole and buffer exchange was carried out using a HiPrep 26/10 desalting column (Amersham Biosciences) and a mobile phase composed of 50 mM Tris, pH 8.0, 50 mM NaCl, 10% glycerol, and 1 mM dithiothreitol. The protein was further purified by ion exchange using a MonoQ 5/50 GL column (Amersham Biosciences). The protein was eluted with a NaCl gradient ranging from 50 mM to 1 M over 100 column volumes. Finally, the protein was submitted to gel filtration on a Superdex 200 column (Amersham Biosciences). The purified protein was stored at −80 °C. The samples were analyzed by SDS-PAGE using the Invitrogen NuPAGE system, according to the manufacturer’s instructions.

Analytical Methods—NADH and NADPH were determined spectrophotometrically at 340 nm using a molar extinction coefficient of 6220 M$^{-1}$ cm$^{-1}$. Acetoacetate was determined according to Williamson et al. (15). Acetyl-CoA was quantified according to Moriymama and Srere (16). NH$_4^+$ was estimated with glutamate dehydrogenase at pH 8.1 in the presence of 10 mM 2-ketoglutarate and 100 μM ADP (17). Enzymatic reactions were stopped by trifluoroacetic acid and neutralized by K$_2$CO$_3$ 5 M.

Enzyme Assays—ni. Erythro-3,5-diaminohexanoate dehydrogenase activity was assayed in the presence of DAH and NAD$^+$ or NADP$^+$ as co-factors by monitoring the formation of the resulting reduced pyridine nucleotide. Reactions were usually performed in 100 μl of activity buffer containing co-factor. The reaction was initiated by the addition of DAH. Experiments assaying the stoichiometry of the Kdd reaction were conducted in 700 μl of activity buffer containing 36 μg of Kdd, 22 mM DAH, and 1 mM NAD$^+$. After 20 min of incubation, the $A_{340}$ was recorded. The enzymatic reaction was stopped and NH$_4^+$ assayed.

3-Keto-5-amino hexanoate cleavage enzyme activity was determined in activity buffer by assaying acetoacetate formation. Various amounts of Kce were incubated in the KAH solution along with 300 μM acetyl-CoA. In each experiment, Kce was allowed to react for 0, 5, and 10 min. Acetoacetate formation rate was linear with time up to 10 min.

3-Aminobutryl-CoA ammonia lyase activity was assayed according to Jeng and Barker (13). Briefly, the activity was monitored for the reverse reaction using crotonyl-CoA as the substrate. The assay was based on the decrease in $A_{260}$ upon crotonyl-CoA amination. Enzyme activity was calculated using a molar extinction coefficient of 6700 M$^{-1}$ cm$^{-1}$.

RESULTS AND DISCUSSION

Searching for Candidate Genes—The 3,5-diaminohexanoate dehydrogenase characterized from different bacteria has been described to possess a molecular weight of ~37 kDa/subunit and catalyzes the reaction using NAD$^+$ as a co-factor (6). Among the 29 dehydrogenases found in the genome of F. nucleatum, six had a molecular weight in the expected range. Interestingly, one (FN1867) is located next to the lysine-2,3-aminomutase. This co-localization is also found in three groups that have a similar chromosomal organization. For gene cluster organization analysis, the F. nucleatum genes were compared with the complete sequenced genomes and those available as a draft (NCBI, WGS section, 263 genomes) using the BLAST2 algorithm (18). Putative orthologous relationships between the two genomes were defined as gene couples satisfying the bidirectional best hit criterion and an alignment threshold of 30% sequence identity on 80% of the length of the smallest protein. Proteins present in E. coli K12 were detected using the same rules. Synteny groups, i.e. conservation of the chromosomal co-localization between pairs of orthologous genes from different genomes, were computed as previously described (19). The data (i.e. syntactic and functional annotations and results of comparative analysis) were stored in a relational data base and explored by using the graphical interface of our microbial genome annotation system, MaGe (19). This data base is publicly available. The phylogenetic profile method was used to find gene candidates. This method, combined with the integration of synteny results, allows one to detect co-evolution of gene groups that have a similar chromosomal organization. For gene cluster organization analysis, the F. nucleatum genes were compared with the complete sequenced genomes and those available as a draft using the Smith and Waterman algorithm (20). Only alignments sharing >40% identity over 80% of the length of the shortest sequence were retained. On some occasions, proteins were retained with a lower percentage of identity when the gene context was conserved.

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enormous enzyme is annotated as a hypothetical protein. As a strategy to find a candidate gene, we searched for proteins present in *F. nucleatum*, *P. gingivalis*, *T. tengcongensis*, and *S. theromophilum* but absent in the non-lysine-fermenting strain *E. coli* K12. Of the resulting 37 candidates, only FN1868 was annotated as a hypothetical protein and had the expected molecular weight. Strikingly, the corresponding gene is located next to the gene of the hypothetical 3,5-diaminohexanoate dehydrogenase in *F. nucleatum*, *P. gingivalis*, and *T. tengcongensis*. These facts support FN1868 as a candidate.

The next step in the lysine fermentation pathway is catalyzed by a 3-aminobutyryl-CoA ammonia lyase. Considering our preliminary results, it seems likely that the genes of lysine fermentation are co-localized in *F. nucleatum*, *P. gingivalis*, and *T. tengcongensis*. Therefore, FN1869, annotated as a hypothetical protein and located upstream from the gene encoding the hypothetical ketoacid cleavage enzyme, might be a candidate for the 3-aminobutyryl-CoA ammonia lyase. We hypothesize that the mechanism of this reaction is similar to that of the β-aminol-CoA ammonia lyase (21). The amino acid sequence alignment of FN1869 and the protein Acl1 encoding the β-aminol-CoA ammonia lyase showed 44.6% identity. Therefore, FN1869 was chosen as the best candidate for encoding enzymatic activity.

The aim of this work was not only to identify and characterize the unknown genes in this pathway but also to check the unknown genes in this pathway but also to check the mechanism of this reaction is similar to that of the β-aminol-CoA ammonia lyase (21). The amino acid sequence alignment of FN1869 and the protein Acl1 encoding the β-aminol-CoA ammonia lyase showed 44.6% identity. Therefore, FN1869 was chosen as the best candidate for encoding enzymatic activity.

**Table 1.** The calculated kinetic parameters were obtained from duplicate experiments by non-linear analysis of initial rates using SigmaPlot version 9.0 (Systat Software, Inc.). Results are presented in Table 1. The calculated $K_m$ values are probably apparent ones, because the DAH used in this study is estimated to be only 75% pure. The remaining 25% are in *threo* conformation, which is known to inhibit L-erythro-3,5-diaminohexanoate dehydrogenase (9, 6). Moreover, D-erythro-3,5-diaminohexanoate has been described to be a non-competitive inhibitor of the reaction in *Clostridium SB4* (6), a competitive inhibitor in *C. sticklandii* (7), and to both decrease the $V_{max}$ and increase the $K_m$ for L-erythro-3,5-diaminohexanoate in *Brevibacterium L5* (9). The DAH concentrations reported in this study were corrected for the *threo* contribution.

Results show that Kdd has DL-erythro-3,5-diaminohexanoate dehydrogenase activity with both NAD$^+$ and NADP$^+$ (Table 1). Although the enzymes from *Clostridium SB4* and *Brevibacterium L5* are rather NAD$^+$-specific (9, 24), Kdd is 223 times more efficient (as indicated by the ratios between $V_{max}/K_m$ of the enzymes for its substrate. In the presence of NADP$^+$, the $K_m$ value for DAH is lowered 16-fold compared with that obtained with NAD$^+$. Experiments performed in the presence of NAD$^+$ were linear with a time up to 20 min, whereas those conducted in the presence of NADP$^+$ were only linear up to 90 s. This lack of linearity could be easily explained by a strong product inhibi-
bition (NADPH). Control experiments indicated that the initial rate of the reaction was 65% inhibited by the addition of 100 μM NADPH (data not shown).

A quantitative estimation of substrate utilization and product formation by purified Kdd was conducted to further confirm the nature of the enzymatic reaction observed. Results in Table 2 (experiment I) show that for 1 mol of NADH formed, 1 mol of NH₄⁺ is liberated, thus confirming that Kdd catalyzes the oxidative deamination of DAH.

Enzymatic Characterization of Kce Activity—Kce was assayed for the cleavage of 3-keto-5-aminohexanoate in 3-aminobutyryl-CoA and acetoacetate in the presence of acetyl-CoA (11). KAHH was first enzymatically produced, and its concentration was estimated to 1.50 mM. As expected, in control experiments devoid of Kce or acetyl-CoA, no acetoacetate formation could be observed (not shown), whereas in the presence of Kce, the rate of acetoacetate formation was proportional to the amount of the enzyme during the course of experiment, 432.7 ± 54.3 pmol/min/μg Kce (estimated from two replicate assays conducted with 0.65, 1.30, and 2.60 μg of Kce). These results are thus consistent with a Kce-dependent cleavage of KAHH in the presence of acetyl-CoA.

Enzymatic Characterization of Kal Activity—In vitro amination of crotonyl-CoA was shown to be Kal-dependent, and the rate of amination was strictly proportional to the amount of the recombinant enzyme (not shown). The kinetic constant of Kal for crotonyl-CoA was estimated at 55 μM (Table 1). This value is very close to the one determined for the Clostridium SB4 enzyme (45 μM, (13)).

Production of Crotonyl-CoA from DAH Using the Tri-coupled Enzymatic Assay—Because each of the putative candidates catalyzes the expected enzymatic reaction, we wanted to test whether crotonyl-CoA could be directly generated from DAH in the presence of acetyl-CoA in a single tri-coupled enzymatic assay containing Kdd, Kce, and Kal. The reactions catalyzed by the combined assay could be monitored by assaying NADPH, acetyl-CoA, acetoacetate, and NH₄⁺.

Results reported in Table 2 (experiment II) are consistent with those expected from the global reaction described by Fig. 2, reactions 1–3 together. For each mol of reduced pyridine nucleotide, 1 mol of acetyl-CoA was consumed, while 1 mol of acetoacetate and 2 mol of NH₄⁺ were formed.

Although not all products could be analyzed, it is unlikely that the observed products result from other uncharacterized and unexpected activities, because the reactions were monitored using pure proteins expressed after cloning in E. coli B, which does not ferment lysine. Furthermore, no homologs of Kdd, Kce, and Kal were identified in this strain.

Gene Cluster Organization in Putative Lysine-fermenting Bacteria—In the sequenced genomes of F. nucleatum, T. tengcongensis, and P. gingivalis, these genes are clustered with all the genes known to be involved in lysine fermentation (Fig. 3, Table 3, and supplemental data).

As mentioned above, the genes of the lysine fermentation pathway are clustered in the genomes of putative lysine-fermenting bacteria. Our initial hypothesis, i.e. the putative and hypothetical genes of these clusters might be the promising

---

**TABLE 2**

Stoichiometry of product formation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Enzymes</th>
<th>Concentration change</th>
<th>NAD(P)H*</th>
<th>Acetyl-CoA</th>
<th>Acetoacetate</th>
<th>NH₄⁺*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Kdd</td>
<td>225 ± 5</td>
<td>209 ± 2</td>
<td>237 ± 6.5</td>
<td>210 ± 7</td>
<td>238 ± 9</td>
</tr>
<tr>
<td>II</td>
<td>Kdd + Kce + Kal</td>
<td>209 ± 2</td>
<td>237 ± 6.5</td>
<td>210 ± 7</td>
<td>411 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

*a* NADH in experiment I, NADPH in experiment II.

---

**FIGURE 3.** Models of the gene cluster organization in the genomes of 12 putative lysine-fermenting bacteria. Completely sequenced bacteria are written in italics, other sequences are almost complete. The symbol “//” means an interruption in the cluster of >5.5 kb. The rectangles indicate the two types of the gene cluster organizations described in the text.
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TABLE 3
Proposed annotation for the genes in the lysine fermentation pathway of F. nucleatum

<table>
<thead>
<tr>
<th>F. nucleatum proteins</th>
<th>Protein names in Fig. 3</th>
<th>Proposed annotation</th>
<th>EC number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN1869</td>
<td>Kal</td>
<td>3-aminobutyryl-CoA ammonia lyase</td>
<td>4.3.1.14</td>
</tr>
<tr>
<td>FN1868</td>
<td>Kce</td>
<td>3-keto-5-aminohexanoate cleavage enzyme</td>
<td>5.4.3.4</td>
</tr>
<tr>
<td>FN1867</td>
<td>Kdd</td>
<td>3,5-diaminohexanoate dehydrogenase</td>
<td>1.4.1.11</td>
</tr>
<tr>
<td>FN1866</td>
<td>KamA</td>
<td>lysine-2,3-aminomutase</td>
<td>5.4.3.2</td>
</tr>
<tr>
<td>FN1865</td>
<td>HP</td>
<td>Hypothetical protein</td>
<td>-</td>
</tr>
<tr>
<td>FN1864</td>
<td>MutS</td>
<td>MutS family protein</td>
<td>-</td>
</tr>
<tr>
<td>FN1863</td>
<td>KamD</td>
<td>β-lysine-5,6-aminomutase (α subunit)</td>
<td>5.4.3.4</td>
</tr>
<tr>
<td>FN1862</td>
<td>KamE</td>
<td>β-lysine-5,6-aminomutase (β subunit)</td>
<td>5.4.3.4</td>
</tr>
<tr>
<td>FN1856</td>
<td>AtoA</td>
<td>Acetoacetate:butyrate CoA-transferase (β subunit)</td>
<td>2.8.3.9</td>
</tr>
<tr>
<td>FN1857</td>
<td>AtoD</td>
<td>Acetoacetate:butyrate CoA-transferase (α subunit)</td>
<td>2.8.3.9</td>
</tr>
</tbody>
</table>

Candidate genes, is now firmly established. Even though three formerly unknown genes of the cluster are now correctly annotated, there is still another hypothetical protein (Fig. 3, in gray color). This protein might be linked to the fermentation of lysine, but its function still remains unknown. In addition, a MutS family protein is co-localized with this hypothetical protein. To our present knowledge, a relationship between lysine fermentation and the MutS protein has never been described.

Considering that eight genes involved in lysine fermentation are now known (kama, kamd, kame, kdd, kce, kkal, atoA, and atoD), we used these sequences from F. nucleatum to detect, in all the sequenced genomes (and those available as a draft), the bacteria potentially able to ferment lysine. This analysis revealed 11 bacterial genomes containing the eight genes and one with six (in S. thermophilum, atoA/atoD were not found). The fact that the acetoacetate:butyrate CoA-transferase (AtotA/AtotD) was not found in S. thermophilum may be explained by the presence of another protein having the same activity. Alternatively, the last steps of lysine fermentation may be catalyzed differently. These 12 bacteria could therefore be considered as potential lysine-fermenting microorganisms. In some bacteria, only KamA and AtoA/AtotD could be identified. However, these bacteria are not supposed to ferment lysine. The existence of KamA might be explained by horizontal gene transfer. AtotA/AtotD belong to the 3-oxoacid CoA-transferase family. Members of this family are involved in several metabolic pathways (25).

The cluster organization of the genes involved in lysine fermentation (observed in F. nucleatum, P. gingivalis, and T. tengcongensis) is conserved to a certain extent in nine other organisms (Fig. 3 and Table 3). In addition, the co-localization of the hypothetical protein and the MutS family protein previously observed in F. nucleatum is retrieved in five genomes. Two main organizational structures of the cluster are observed. In six organisms, the genes encoding Kdd, KamA, the hypothetical protein, the MutS family protein, KamD, and Kame are grouped together. Another configuration is detected in three other bacterial genomes for the genes encoding Kal, Kame, KamD, and Kdd.

CONCLUSION

In this study, we identified the three missing genes of the lysine fermentation pathway. DNA sequence analyses and comparative genomics enabled us to propose a candidate gene for each of the reactions. The purified gene products confirmed their anticipated enzymatic activities. Altogether, these data represent overwhelming evidence that 3,5-diaminohexanoate dehydrogenase, 3-keto-5-aminohexanoate cleavage enzyme, and 3-aminobutyryl-CoA ammonia lyase are encoded by these genes. Of these three reactions, the second one is of special interest, because it presents an unknown mechanism for β-ketoacid cleavage and acetoacetate synthesis.

This work also illustrates the potential of metagenomic resources as reservoirs for discovering genes corresponding to specific enzymatic activities. Such resources can readily bypass the need for dedicated bacterial strains for studying metabolic pathways. Furthermore, it offers the unique opportunity to access genetic material from uncultivable strains or even unknown bacterial species. The assembly of larger metagenomic fragments is in progress. It will give us access to an analysis of the diversity of the lysine-fermenting bacteria. It may also enable us to link these lysine fermentation genes to a group of species that could be anchored on a phylogenetic tree or to discover alternative lysine fermentation pathways.

Nucleotide Sequence Accession Number—Sequences reported in this study have been submitted to the EMBL data base under the accession numbers kal as CU151840, kce as CU151841, and kdd as CU151896.

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