

Sequence and organization of genes encoding enzymes involved in pyruvate metabolism in *Mycoplasma capricolum*

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Abstract

The region of the genome of *Mycoplasma capricolum* upstream of the portion encompassing the genes for Enzymes I and II^{A_{glc}} of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) was cloned and sequenced. Examination of the sequence revealed open reading frames corresponding to numerous genes involved with the oxidation of pyruvate. The deduced gene organization is *naox* (encoding NADH oxidase)-*lplA* (encoding lipoate-protein ligase)-*odpA* (encoding pyruvate dehydrogenase EI α)-*odpB* (encoding pyruvate dehydrogenase EI β)-*odp2* (encoding pyruvate dehydrogenase EII)-*dldH* (encoding dihydrolipoamide dehydrogenase)-*pta* (encoding phosphotransacetylase)-*ack* (encoding acetate kinase)-*orfA* (an unknown open reading frame)-*kdtB*-*ptsI*-*crr*. Analysis of the DNA sequence suggests that the *naox* and *lplA* genes are part of a single operon, *odpA* and *odpB* constitute an additional operon, *odp2* and *dldH* a third operon, and *pta* and *ack* an additional transcription unit. Phylogenetic analyses of the protein products of the *odpA* and *odpB* genes indicate that they are most similar to the corresponding proteins from *Mycoplasma genitalium*, *Acholeplasma laidlawii*, and Gram-positive organisms. The product of the *odp2* gene contains a single lipoyl domain, as is the case with the corresponding proteins from *M. genitalium* and numerous other organisms. An evolutionary tree places the *M. capricolum* *odp2* gene product in close relationship to the corresponding proteins from *A. laidlawii* and *M. genitalium*. The *dldH* gene encodes an unusual form of dihydrolipoamide dehydrogenase that contains an aminoterminal extension corresponding to a lipoyl domain, a property shared by the corresponding proteins from *Alcaligenes eutrophus* and *Clostridium magnum*. Aside from that feature, the protein is related phylogenetically to the corresponding proteins from *A. laidlawii* and *M. genitalium*. The phosphotransacetylase from *M. capricolum* is related most closely to the corresponding protein from *M. genitalium* and is distinguished easily from the enzymes from *Escherichia coli* and *Haemophilus influenzae* by the absence of the characteristic amino-terminal extension. The acetate kinase from *M. capricolum* is related evolutionarily to the homologous enzyme from *M. genitalium*. Map position comparisons of genes encoding proteins involved with pyruvate metabolism show that, whereas all the genes are clustered in *M. capricolum*, they are scattered in *M. genitalium*.

Keywords: acetate kinase; dihydrolipoamide dehydrogenase; lipoate-protein ligase; NADH oxidase; PTS; phosphotransacetylase; pyruvate dehydrogenase

Mycoplasmas, the smallest free-living organisms, contain the least complex genomes (Razin, 1985, 1992). They appear to have evolved from Gram-positive bacteria by selective elimination of nonessential genes (Maniloff, 1983). Growth of these organisms requires complex media, reflecting the loss of many genes encoding anabolic enzymes, but retention of genes encoding cat-

abolic pathways (Miles, 1992). *Mycoplasma capricolum* has a genome of 1,155 kb, sufficient to encode approximately 350 genes (Miyata et al., 1991).

It was shown previously (Cirillo, 1979) that *M. capricolum* can metabolize carbohydrates by the ubiquitous phosphoenolpyruvate:sugar phosphotransferase system (PTS) (for review, see Cirillo, 1979). This system promotes phosphotransfer from phosphoenolpyruvate to the heat-stable phosphocarrier protein HPr in a reaction proposed to be catalyzed by the homodimeric form of Enzyme I. Phosphorylated HPr then transfers a phosphoryl group to the sugar-specific acceptor proteins, referred to as Enzymes II. Each Enzyme II complex consists of one or two membrane embedded proteins or domains (IIC and IID), as well

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¹ Nucleotide sequence(s) reported in this paper has (have) been submitted to the GenBank™/EMBL Data Bank with accession number (U62057).

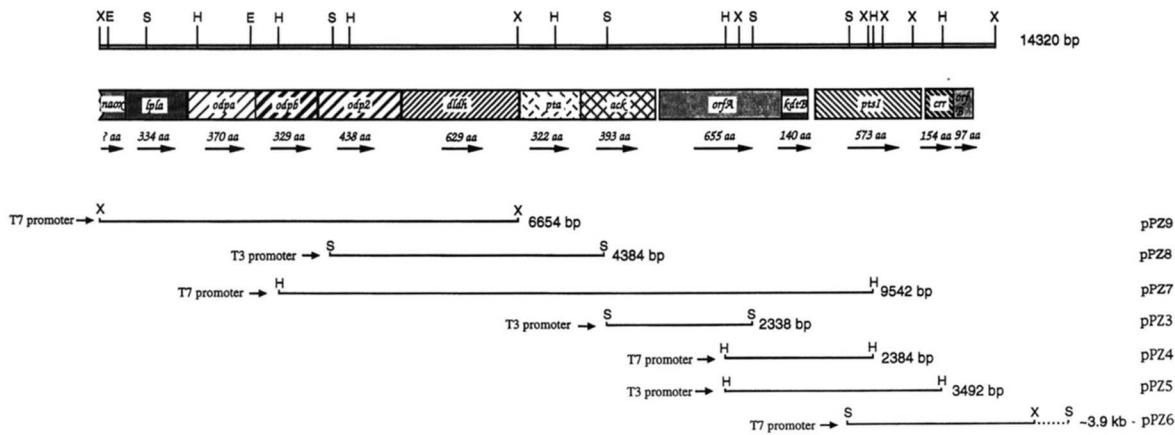


Fig. 1. Restriction and genetic maps of the region of the *M. capricolum* genome upstream of the *ptsI-crr* operon. The three clones that were isolated and sequenced are (A) pPZ7, the 9,542-bp clone derived from a partial *Hind* III digest, (B) pPZ8, the 4,384-bp *Spe* I clone, (C) pPZ9, the 6,654-bp *Xba* I clone. The internal *Xba* I site is located at base 10209 (see Fig. 2). The internal *Eco* I sites are located at bases 141 and 2396; the internal *Spe* I sites are located at bases 720, 3710, and 8094; the internal *Hind* III sites are at bases 1551, 2834, 3966, 7266, and 9992. Horizontal arrows indicate direction of transcription of the indicated genes. --- aa, the number of amino acid residues of the deduced open reading frame. *naox*, the gene encoding NADH oxidase; *lplA*, the gene encoding lipoate-protein ligase; *odpA*, the gene encoding the EI α subunit of pyruvate dehydrogenase; *odpB*, the gene encoding the EI β subunit of pyruvate dehydrogenase; *odp2*, the gene encoding the EII subunit of pyruvate dehydrogenase; *dldh*, the gene encoding dihydrolipoamide dehydrogenase; *pta*, the gene encoding phosphotransacetylase; *ack*, the gene encoding acetate kinase; *kdtB*, an open reading frame homologous to the *E. coli* KDTB protein product; *ptsI*, the gene encoding Enzyme I of the PTS; *crr*, the gene encoding Enzyme II A^{glc} of the PTS.

as two cytoplasmic proteins or domains (IIA and IIB) that pass the phosphoryl group from HPr to a specific sugar substrate during its translocation across the membrane (Postma et al., 1993).

In previous studies from this laboratory (Zhu et al., 1993, 1994), we reported the sequences of cloned *pts* genes from *M. capricolum*. It was shown that the *ptsH* gene, encoding HPr, constituted a unique monocistronic operon (the *ptsH* operon) in contrast to the typical occurrence in all *pts* operons sequenced to date of the *ptsH* gene immediately upstream of the *ptsI* gene, encoding Enzyme I. It was also established that *ptsI* is located in a dicistronic operon containing the gene encoding the glucose-specific Enzyme IIA (*crr*). In the present study, the region of the *M. capricolum* genome upstream of the *ptsI-crr* operon is characterized. It is shown that this region includes essentially all the genes necessary for the use of pyruvate.

Results

Cloning of the genes required for pyruvate metabolism

Previously we sequenced the region of the *M. capricolum* genome encoding the *ptsI-crr* operon (Zhu et al., 1994). Clones that were isolated in the course of that sequencing project were designated pPZ3, pPZ4, pPZ5, and pPZ6 (see Fig. 1). With the intention of investigating the organization of genes located upstream of the *ptsI-crr* operon, further clones were isolated. A partial digest with *Hind* III provided DNA for the isolation of a 9,542-bp clone, designated pPZ7, which was screened using an oligonucleotide based on the pPZ4 sequence (from bases 3466 to 3422 of the sequence published previously) (Zhu et al., 1994) (see Materials and methods). Other DNA clones were isolated by a similar approach. pPZ8 was isolated from an *Spe* I digest

and identified using an oligonucleotide sequence from pPZ7 corresponding to base numbers 8018–8062. The clone pPZ9 (from an *Xba* I digest) was also detected with a pPZ7 probe corresponding to bases 2882–2926 (see Fig. 1).

Sequence analysis of the genes required for pyruvate metabolism

Clones pPZ7, pPZ8, and pPZ9 were sequenced on both strands and then analyzed for open reading frames to reveal the gene organization illustrated in Figure 1. It was surprising to observe that a block of eight genes in this upstream region was in some way involved with the metabolism of pyruvate. The deduced gene organization, starting from the 5'-end of the sequence, is *naox* (encoding NADH oxidase), *lplA* (encoding lipoate-protein ligase), *odpA* (encoding the EI α subunit of pyruvate dehydrogenase), *odpB* (encoding the EI β subunit of pyruvate dehydrogenase), *odp2* (encoding the EII subunit of pyruvate dehydrogenase), *dldh* (encoding dihydrolipoamide dehydrogenase), *pta* (encoding phosphotransacetylase), *ack* (encoding acetate kinase). The region downstream of *ack* (*orfA*, *kdtB*, *ptsI*, *crr*, and *orfB*) has been described previously (Zhu et al., 1994).

The combination of the three sequenced clones (pPZ7, pPZ8, and pPZ9) allowed us to deduce a total of 10,214 bp of DNA sequence (see Fig. 2). Bases 1–280 coded for an open reading frame that is homologous to the carboxyl-terminal region of the amino acid sequence of NADH oxidase from *Enterobacter faecalis* (Genbank accession no. P37061) and *M. genitalium* (MG275 from the TIGR database) (Fig. 3). Comparison (by BESTFIT analysis) of the *M. capricolum* and *E. faecalis* sequences indicated 64% similarity and 39% identity of the compared sequences over a length of 88 residues. When the *M. capricolum* sequence was compared with the *M. genitalium* se-

TCTAGATAGACCAAGATTATGTCACAGCAAATGAAGGTTTATCAGAACTGTGTTGAGATAAAAACAGAAAATCATGGTCTCAAGTAGCTACTGAAAAAAACCATACATGAAAGT
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 M Y M L A L G I Q K D L T I D E L F L V D T F F L P H F N K P F N F I S L A G L 240
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 E V L Y F K K E K * M I N I V Y K H P A M P A N L A I E E Y L T 360
 TATCATTATAAGCAAAGAACCTATTGTTACCAAAATGCTAATCTATAGTTGAGAAGAACTCAAACGCTTGTCTGAAATTAACTTAGAAGCTGCTAAAAGATAAC
 Y H Y K A K E P I V F F W Q N A N T I V V G R N Q N A F A E I N L E A A Q K D N 480
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 V K I V K R N T G G G T V Y Q D L G N V C Y S L I V D N S T D D V D Y Q K A L Q 600
 CCTATTATCACTTAAATCAAATTAATGCAATTCTGGAGAAAATGACATGTTGATTGATGGTTAAGGTTTCAGGAAATGCTCAATTAAAACATAAGAAAACA
 P I T Y L N Q K N I N A M F S G R N D M V I D G Y K V S G N A Q L K T N E K T 720
 CTAGTTCATGGTACATTGCTATTGATGTTGCTAAATGCTTAATTTAGTTGATGTTGATCCAGAAAATTAACATCAACAAATCAGATCACCCTGCCAGAGTTGAAAT
 L V H G T L L F D V D L S K M P K Y L V V D P E K L K H Q Q I R S K P A R V R N 840
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 I K E F F K D I N I D I D L S T F I N D V V S S Y V K N E K I K W I E L T D Q E 960
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 L N L D V D N G V I T N I K I Y G D F L G T Q G E T K L E A K L G I V G U K D D K K 1200
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 D V E K V L N Q F D L E A I F A K N F T S S D D I T N L L F K D * * 1320
 odpa
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 M T Y L G K F D P L K N E K V C V L D K D G K V I N P K L M P K I S D Q E 1440
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 E G Y F Q V N P P K K V L V K M Q E L L D K F P * 3480
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 R G D F A N N K I O M H N I N I G A D V T P N G L M V P V I K G A D H L S V 4440

Fig. 2A. (*Figure continues on next two pages.*)

B	ATTTGAAATTGCAATTAAAATTAGTAGCAACTAGCAAATAAGGCCAAGATGGTAAATTACAAGAGCTGAATGACTGAAGCAACATTACTGTTCAACCTTGGTCAGTAGGTTAGA F E I A I K I S E L A N K A K D G K L T R A E M T E A T F T V S N F G S V G L D	4560
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	TAAAGAAAAATTGCTGACATAGGTGAAGGTTAACAGAAGGAACAGTCGCTGAAGTTTGTAAAGTGGTGTGTTAAAGAAGGACAAACCTTAACTTTGTTGAAACTGATAA K V K F A D I G E G L T E G T V A E V L V K V G D V V K E G Q P L Y F V E T D K	4920
	AGTAAATAGTGAATTCCCTCCCGTTGCCGAAAATTGCAATAATCAATCTACTGGTCAAGAAATTAAAGTGGTGTGTTATTGAAATTGATGATGGAAGTCAACTC V N S E I P S P V A G K I A I I N I S T G Q E I K V G D V V I E I D D G S S T S	5040
	TACAGCTTCAACTTCAAAAGTGAAGTAGTGTGAAGAAAAGTCTAGTGTAGTTGGTGCACTCCAGTTCGAATGATGTTTACCAAGTAGGACCAAAAGCTGAAGCTAAAGT T A S T S K V E V V E E N A S V V G A T P V S N D V L P S R A P K P K A E A K V	5160
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	AACAGCTGATACAATTAGACAGCTTACAATTGGAACAAACCCAGGATATAATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG T A D T I R P A L Q I I G T K P G Y N I A S S I F V U M S K G N E N Y I F T D C A	7200
	TTTAAATTTAAACCAACAGTGAACAAATTAGGCTATTGCAAAATGCAATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG L N I K P T E Q L V E I T Q M A V D F A K T V N K V E A A L S Y S T N G	7320
	TAGTGGTAAAGGTGAAGATGTTGATAGAGTACATCAAGCAGTTGAATTAAAGGAAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG S G K G E D V D R V H Q A V E I L K S E K D Y V C E G E I Q F D A A F D K K T	7440
	TAGAGATAAGAAATTAAAATGTCATTAAAACACCCAGATATTGTTGTTTCCAGATATAATTGCTGAATATCGGTTATAAAATCGCTAAAGAATGGTGGTTTGA R D K K F K N C S L L K Q T P D I F V F P D I N A G N I G Y K I A Q R M G G F E	7560
	AGCAATTGGACCTTTGTTAGGTTAAACCAAGCTTAATGACCTAAGTAGGGTCAACATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG A I G P F V L G L N Q P V N D L S R G A T F V D V L N T A I M T L Y L S Y *	7680
ack	AGTAAATGTTGTTAGTAAATTCAAGGAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG M I L V I N S G S S S I K F K L F D T S K A I E P I L D G L A E R I G I D G	7800
	GATTTTAAAGTTGAACATAATAAATATAATTGAGATCCACTCCAGATCATGAACTGTTCAATTAAATTAAATTGCTGAATTAAATTAAATTGCTGAATTAAATTATTC F L K F E H N N Q K Y K F E D P L P D H E H A I Q L I L N K L L E L K I I S N I	7920
	TTGATGAAATAAAGGTGTAGGTTTAGGTTCATGGTGGAAATTTCACATTCACTAAATTAAATGAGAAGTATTCAAGAAAGTGTAAATTGCTCCTTAC D E I K G V G F R V V H G G E I S H S I I N E E V L Q K I Q E S V K L A P L H	8040
	ATAATCCTGTCGAATTATTGCAATAAAAGCAGTAAACAAACTATGCAATTGCTGAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG N P A A I I I A I K A V K K L M P N T S M I A C F D T A F H Q T M P Q V N Y L Y S	8160
	CTGTTCTTATAATGATGAGAAGATTGGCTGAAGAAAATGGTTTCAAGGAAATTGTTGAAATTGATGAAATTGTTGAAATTGAGAAGATTTAAAGGAAACACTTGA V P Y K W Y E E F G V R K Y G F H G I S Y E Y I V N K C E E I L N K K K E H L N	8280
	ATTAATAGTTGTCATTAGGAAATGGCGCAAGTATTGCAATTGAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG L I V C H L G N G A S I S C I K D G K S Y D T S M G L T P L A G L M M G T R S G	8400
	GAGATATTGTTGATGTTGAAATTGAGTGGCAAAACAAACAAATTGAGATGTTGCAATTGAGTATTGCAAGTATTGCAAGTATTGCAAGTATTGCAAGTATTG D I D V S I C E Y V A K Q T N S D I F A I T Q I L N K Q S G L G L S Q T S A D	8520
	ATATGAGAGATGTTGAGAACATATGAGAACATGAGAAAAAGCTTATTGAGTGGTGAAGGAAAGTGTGTTAGGTTATGTTAGGTTACAAATTGCAAGA M R D V L E Q Y D R N D K K A I I A V E K Y V Q V V A D F I V K Y A N Y L D S I	8640
	TTGATGCACTAGTCTTACAGCAGGAATTGGTGAAGAACATGAGATGTCATTAGAGATTATTGAGTAAAGAGTTAACCTTGTGTTAGGTTACAAATTG D A V V F T A G I G E N A D V I R D L I C K R V K L L G L Q I D Q E K N E S K Y	8760
	ATTCAAGACTATAATTAAATTCTAGTAGGAAATCAAAATTCCAGTTATGCTATTAGAACAAATGAGAACAAATGATGTTAGGTTACAAATTGCAAGA S D Y K L I S S E K S K I P V Y A I R T N E E K M I C L D T L N L I K *	8880

Fig. 2B.

C	orfA	9000
AGTTAAAAAAACTTAGCTTAAATGCTAAGTTTAATGTTAATAAAGAAAGCATAAAAGAAAACAATAAAGTATTAAAGTTTGTAGGTTAATTCATTTATCAATAACAACT		
M K R T I K Y L S F L G L I P F L S I T T		
ATAAGTTGTGTTAACAGCTAAAGAAAATAATAAAATCAATTAAATTAGTCATTAAACAACCTTATTTATCTTAAACAGCTTGTAGTTAGATAATAAAACTAGAATCAA		9120
I S C V K Q A K E N N N K N Q L I S Q F K Q L I F I L N S F D L D N K K L E S K		
ATCATAAAAGCTATTGAAAAAGTATTGTTAATAAAATTAGTAACTATAAATTAGAATTAACTATAAAGTTTAACAGATAATAAAAGTGGAAACTAAACAAATTAGTCAGTA		9240
I T K A I E K S D F N K I S N I N L E L T I K F L T R I K N E L E T K T I S Q L		
AATAAAAATGATAAGCTAGATATTAACTAAATAAAAGTCATTAGGTCTATTAAATTAGAATTAACTATAAAGTTTAACAGATAATAAAAGTGGAAACTAAACAAATTAGTCAGTA		9360
N K N D K L D I L T K I K V H L G S L N L I E L V N I V D E L V N K L N Q K E E		
ATTAAAAATACTCATAAAGATAAAAATGAAAAAAATAAAAGATAATATAGAAGATATTGATGATTCAAAACTTGAGATTAGATCAAATATACCTAATCAGCACAACTACCCAGAT		9480
I K N T H K D K I E K N K D N I E D I D D S K L E I L E S K Y I P N Q H N Y P D		
TATGTTAAAACCTTTAACAGCTTACAGAGAAATTATAAGACCTTATGATAGAATTTCATAAGTTTGTAAATTAAAGATGGTGGTTATTAGTAATGGAAC		9600
V K K F T V S A E E I Y K E L D Y R T F S I K K F L V K L K D G G L L S N G T		
GGAACCTGGTGTGATTAGATTATGATCATAAATATAGAATACTAAATAAAATAAAATGTTATTGCAACAACTTACATGTTGGCAGATTAGCAATTCAACTGATGAACAAAT		9720
G T G W L L D Y H K Y S N T N K Y K M P I A T N L H V L A D F S N S L T D E Q N		
AAAGAATTAACTACTATGATCCATCAGGAATAAAGTGTAGGTCTGGTTAGGAAAGCTGATAATGTTACTGATTTAGAGAAAAATAATAATCAGAAAATAATA		9840
K E F N Y Y D P S G N K V I G L G L G K A D N V T D F S R K N N N S K S E N N I		
GCTAATTATTATTTAAATAATCAGATTGAAAGATTATCTTAAAGTATTGAAAGTGTAAATTATCTAAAGGTTATTGCAACACCCAAATTGTTGGCAGATTAGCAATTCAACTGATGAACAAAT		9960
A N Y Y L N N Q D F E N Y L K W F S V N K F S G K I S E P K I V F G A V D F		
ATGAAAGATCGTGTATTAAATCATTAGAAGCTTACAAAAAGACCAATTAAATTATACTATAAAATAATAAGTAAATTATGATGACAATAAGATAGCTGAATAAT		10080
M K D R A I K N H Y E A L Q K E A I N Y Y N Y K K N N N E I N D D N K I A W N N		
TTTTAAATAATAAAAGATATTCTATAATGATAGATTGCTAGATTGATGTTGCAATTGATGTTGACCTAGATTGGTGTATAATTAAATCATGAATTCTAATGCTATTAGTGGTTA		10200
F L L N N K D I P I M I D F A V F E F D V D L D L V D Y N L K S W I S N A I S G L		
GATAATTATCTAGA 10214		
D N Y L		

Fig. 2. Nucleotide sequence of the region of the *Mycoplasma* genome encoding genes involved with pyruvate metabolism.¹ A partial sequence of *naox* and the entire nucleotide sequences of *lplA*, *odpA*, *odpB*, *odp2*, *dldH*, *pta*, *ack*, and an ORF designated *orfA* (Zhu et al., 1994), as well as the deduced amino acid sequences, are shown. The putative promoter regions [i.e., 35 regions (underlined) and 10 regions (boxed)] are shown for the *lplA*, *odpA*, *odpB*, *odp2*, *pta*, and *orfA* genes. A sequence corresponding to a likely transcription termination site downstream of *ack* is marked with diverging arrows. Shine-Dalgarno ribosome binding sites for *lplA*, *odpA*, *odpB*, *odp2*, *dldH*, *pta*, *ack*, and *orfA* are highlighted with shaded boxes. Asterisks denote translation stop codons.

quence, it showed 77% similarity and 52% identity over a length of 92 residues.

After a gap of 11 bases, an open reading frame (from bases 292 to 1312) was identified. The end of this sequence was characterized by the presence of four in-frame stop codons (all TAA). Use of FASTA analysis of the deduced protein product led to its identification as the gene encoding lipoate-protein ligase. Alignment of the sequence with the lipoate-protein ligases from *M. genitalium* (MG270 from the TIGR database²) and *Escherichia coli* (Genbank accession no. P32099) is shown in

² The *M. genitalium* sequence MG279 is listed in the TIGR database as unknown in function. The FASTA analysis reported here identifies the sequence as the gene encoding lipoate-protein ligase.

Figure 4. BESTFIT analysis of the *Mycoplasma* sequence (334 amino acid residues) with the lipoate-protein ligase from *E. coli* (337 amino acid residues) showed 55% similarity and 32% identity over a length of 335 residues (data not shown). A similar analysis with the enzyme from *M. genitalium* (336 residues) showed 62% similarity and 36% identity over a length of 335 residues.

After a gap of 19 bases, an open reading frame (from bases 1331 to 2443) was observed. FASTA analysis showed the protein product to be the α -subunit of pyruvate dehydrogenase Enzyme I. Figure 5 shows an alignment of the sequence from *M. capricolum* with those from other sources recovered from the Genbank and TIGR databases. It is clear that the regions of total amino acid conservation (shown in reverse shading) are

1		110
<i>Mycge</i>	MKKVIVIGIN HAGTSFIRTL LSKSKDFKVN AYDRNTNISF LGCGIALAVS GVVKNDDLF YSNPEELKQM GANIFMSHDV TNIDLIKKQV TVRDLTSNKE FTQFQDQLVI	
<i>Entfa</i>	.MKVIVLGSS HGYYEAVEEL LNLLHPDAEQ WYKEKGDFISL LSCGMQLYLE GKVKDVNSVR YMGEKMSR GVNVFSNTEI TAIQPKEHQV TVKDLVSGEE RVENYDKLII	
111		220
<i>Mycge</i>	ASGAWPICMN VENKVTHKPL EFNYTDKYCG NVKNLISCKL YQHALTLIDS FRKDFTIKSV AIVGSGYIGL ELAEAAWLCK KVTVTVIDLLD KPAGNNFDHE FTDELEKVMQ	
<i>Entfa</i>	SPGAVPFELD IPGK..... LDLDNIYLMRG RQWAIKLKQK .TVDPEVNNSV VVIGSGYIGI EAAEAFAKAG KKVTVIDILD RPLGVYLDK FTDVLTEEME	
221		330
<i>Mycge</i>	KDGLKLMGMC SVKGFVUDST NNVVKGVETD KGIVNADLN QSIGFRPSTK FPKDQNPEF IHNGSIKUNE FQALNHHKD VYIGGCCAIY NAASEQYENI DLATNAVKG	
<i>Entfa</i>	ANNITIATGE TVERYEGRDR ..VQKVTD KNAYDADLVV VAVGVRPNIA WL .KGTTEL HPNLKIKTDE YMFT .SEPDVF FAVGDATLIK YNPADTEVNI ALATNARKKG	
331		440
<i>Mycge</i>	
<i>Mycge</i>	LVAAMHIIGS NQVKLQSIVG TNALHIFGLN LAACGLTEQR AKKLGFDPVGI SVVDDNDRPE FMOSYDKVRF KLVYDKKLR ILGAOLLENN TNHSEIIFYI ALAIQKMLL	
<i>Entfa</i>	RFAVKNLEEP VK.PFFGVQG SSGLAVFVDYK FASTGNEVM AQKLGKETKA VTVVEDYLMF ENPDQKQAWF KLVYDPEQTO ILGAOLMS.K ADLTANAINAI SLAIQAKMTI	
441	478	
<i>Mycge</i>	DEPLPLVDDIFF LPHFNKPFPNF ISLAGEVVLG LNYFKKEK	
<i>Mycge</i>	TEPLGLVDVYF LPHYNKPFPNF VLATVHQALG FSYYIPKK	
<i>Entfa</i>	EDAYADFFF QPAFDPENNI INTAAAEAV.KQER	

Fig. 3. Alignment of sequences of characterized NADH oxidase proteins from various bacteria. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed NADH oxidase proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (*Mcapr*) (this work); *M. genitalium* (*Mgeni*) (MG275 from the TIGR database); *E. faecalis* (*Entfa*) (Ross & Claiborne, 1992).

1	.MINLLI...SK YHEDAMMII EYLTYHYKA KE...PIVFFF CHANLIVR NQAFAPLIL EAQAKNKKI VKNTTNTWY YQHLSWYS LIVDNSTDV D...QKALQP Mycca .MQTTIAP VPNTYFPAI EWLTLERPK NELVKVYFE CHANLIVR MDTYAVRL KELESKKNL PRPSFSTAA PHDLSLFS IILPRTGKVM ENAEGTRNN Mycce Ecoli STRLRLISD SYDWFNFAV EECIFIRQMPA TQ...RVLFLW RRADEWVIG AOPWPKCET RRMEEBMLR ARISSEGRAY PHDLSLFS FTMAGKPE... ...DKTIST	110
111	.IITYLNQK NINAMFSGRN DMVI....DG YVNSCNOLQK TNEKTLVHG TDFDVLLKRM PKLVVPPEN LKHQQIREKP ARVRIKHF KDNIDIDL3 TFINDVVS Mycca .VVPFLPLSL NNPVAFPHGRD ELEI....NN KEPNLQAYI AKDRLLVHG MFDATPLKRL AVKNDVTKN IASKGVDFVA KRWVWKRYL PNWT... A KFLEEMINPF Mycce Ecoli SIVLNALN GVSABASGRN DLVVKTVEGD RPSGSVCEA TKDRGFGHGT DLVNLNLRLR ANVLPNPKR LAKGITSV SVWVTELL PGITHEQVACE AITEFAAFFH	220
221	VNKNEKIKWAL LTDXQEKYIQ SRKET...KFD QDVKLIEKNTN ESKVPLVKKQYL ESKGOFITNL DWDNIVITNI KIYGDFLGTQ GTEKLEAKHII GVKF... K DVDEKVNLNF Mycca VTIKECAETIV LTDXDALKAWE KRKE...HFKQ SIEUHBLKTY EYNFKNKKRF NNAGLFCENV QMEKFTVVDI KFYGDFLSV DITPVTKKII QKYD... Y KTFKLNLNF Mycce Ecoli ..GERVEAEI ISPKNTPDPLW NFAETFARQS SIEUHBLQAP AFSHLLDERF TWGG VELHF DUEKCHITRA QVFTPSLNPW PLEALAGRQ CLYRADMLQ QCEALLVDP	330
331	353	
Mycca	DLEIAFIKNTN TSDDITNLLF KD.	
Mycce	DHFSDYFDSL KPEQLGVIP DNK	
Ecoli	PQEKEKRELRL SAWMAGAVR. .	

Fig. 4. Alignment of sequences of characterized lipoate–protein ligase proteins from various bacteria. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed lipoate–protein ligase proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (*Mcapr*) (this work); *M. genitalium* (*Mgeni*) (MG270 from the TIGR database); *E. coli* (*Ecoli*) (Morris et al., 1994).

Fig. 5 (on next page). Alignment of sequences of characterized Enzyme I- α proteins of the pyruvate and α -ketoadic dehydrogenases and acetoain catabolism complexes from various bacteria. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in all listed Enzyme I- α proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (odpa-mycca) (this work); *M. genitalium* (odpa-myce) (MG274 from the TIGR database); *A. laidlawii* (odpa-achla) (Wallbrandt et al., 1992); *B. subtilis* (odpa-bacsu) (Hemila et al., 1990) and (odba-bacsu) (Wang et al., 1993); *B. stearothermophilus* (odpa-bacst) (Borges et al., 1990); *A. thaliana* (odpa-arath) (Luethy et al., 1995); yeast (odpa-yeast) (Behal et al., 1989); human (odba-human) (McKean et al., 1992), (odpa-human) (Ho et al., 1989), and (odpt-human) (Dahl et al., 1987); pig (odpa-pig) (Sermon et al., 1990); mouse (odpa-mouse) (Fitzgerald et al., 1992) and (odpt-mouse) (Fitzgerald et al., 1992); rat (odba-rat) (Zhang et al., 1987), (odpt-rat) (Cullingford et al., 1993), and (odpa-rat) (Cullingford et al., 1994); *Ascaris suum* (odpt-ascu) (Johnson et al., 1992) and (odpa-ascu) (Johnson et al., 1992); *C. magnum* (acoa-cloma) (Kruger et al., 1994); *A. eutrophus* (acoa-aleuc) (Priefer et al., 1991); *Pelobacter carbinolicus* (acoa-pelca) (Oppermann & Steinbuchel, 1994); *K. pneumoniae* (acoa-klepN) (Deng et al., 1994); bovine (odba-bovin) (Hu et al., 1988); *Pseudomonas putida* (odba-psepu) (Burns et al., 1988). odpa and odpt designations correspond to pyruvate dehydrogenase, whereas odba designations correspond to α -ketoadic dehydrogenase complex gene products, respectively, and the acoa designation corresponds to acetoain catabolism complexes.

also shared by the sequence from *M. capricolum*. The signature sequence (shown as residues 250–280) G(D/E)(G/A)(X26)NN, characteristic of thiamine diphosphate-dependent enzymes, is also found in the *M. capricolum* sequence. A phylogenetic tree (Fig. 6) of the sequences of the α -subunit family shows that the *M. capricolum* protein is related closely to the corresponding proteins from *M. genitalium*, *Acholeplasma laidlawii*, and those from Gram-positive organisms (*Bacillus subtilis* and *B. stearothermophilus*).

The last base of the termination codon (TAA) of the sequence encoding the α -subunit of pyruvate dehydrogenase Enzyme I is also the first base of an open reading frame (from bases 2443 to 3432) that codes for the β -subunit of pyruvate dehydrogenase Enzyme I. Figure 7 shows an alignment of the sequence from *M. capricolum* with those from other sources recovered from the Genbank and TIGR databases. The alignment shows that residues that are totally conserved (shown as reverse shading) are also identical in the sequence from *M. capricolum*. It is noteworthy that all the bacterial sequences are from 40 to 70 residues shorter at the amino-terminal end than those sequences from eukaryotes. A phylogenetic tree (Fig. 8) shows a similar pattern to that observed for ODPA; the protein from *M. capricolum* is most closely related to those from *M. genitalium* (MG273, TIGR, database) and *A. laidlawii*.

After a gap of 28 bases, a new open reading frame (from bases 3461 to 4877), corresponding to the Enzyme II subunit of py-

ruvate dehydrogenase, was identified. An alignment of the *M. capricolum* sequence with other Enzyme II sequences (Fig. 9) shows clearly that the conserved residues (reverse shading) are found in the *M. capricolum* sequence. Boxes shaded in grey cor-

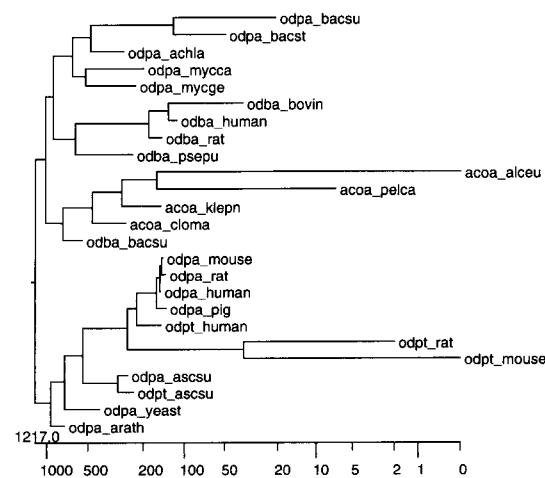


Fig. 6. Phylogenetic tree of sequenced proteins of the Enzyme I- α sub-unit family. Relative evolutionary distances are shown on the horizontal scale. Abbreviations are as in the legend to Figure 5.

1									
odpb-mycca									
odpb-myge									
odpb-achla									
odpb-bacsu									
odpb-bact									
odbb-human	MAVAAAAGW	LRLRRAAGAE	GHWRRLPGAG	LARGFLHPAA	TVEDAAQRQ	VAHFTFOPDP	EPRREVGOTOK	MMLFQSUTSA	MAI INNIKAVTDA
odbb-bovin	MAAVAAPAGW	LRLRRAAGAD	GFWRKLGAG	LSRGFLQAS	AYGAAGAQRQ	VAHFTFOPDP	EPEVYQGTQK	MNLFQAVTS	MSKIQ VNNIEALNNA
odbb-rat	CNSAD WP.	AA	LVOGLQPA	.VDDASQKRR	VAHFTFOPDP	ESLYQGOTQR	MNLFQSUTSA	LENSLADNPT	MAI ITLLEAINQA
odbb-psepu									
odpb-ascsu									
odpb-human	MAAVS G.	LVRPPI	REVSGLLKKR	FHWTAPAAQV	VITVRAINQG	MTVREALNSA	MAEELDRDPP	VIMLGEDIVG	MAI ITLLEAINQA
odpb-yeast									
odpb-arath									
acob-alceu									
acob-peleca									
acob-klepn									
acob-cloma									
11									
odpb-mycca	CTEGVVFR	ATOGIAVAKFG	NDFCFNABIS	BAMFACVGLC	MANNGKPKVL	EFOEGGLGLA	SQNIFNTIS	RMRNRTRGKY	TAPMVIRMP
odpb-myge	GPEGVVFR	ATAGLJQKQYQ	SERWDQDIA	BNSMACJIGVC	AAICLKPIV	IQPSGSFSP	AMQQTIVHAA	KLRNRSRGVII	TAPLVIRMP
odpb-achla	GPEGVVFR	ATAGLJQKQYQ	ETRWDPLBIA	BSAIVGSAVE	MAINGLPKIV	IQFDGFIPF	GTYDLVTHAA	RMRNRSRGQF	MGCGIKALEH
odpb-bacsu	GVNGGVFR	ATEGLJQKQFG	EDRVFPLBLA	PSGIGGLAEL	LGLGFRPV	IQFFGPVYE	VMDVSQSGQNA	RMRYRSRGRW	SEALEAVLFG
odpb-bact	GVNGGVFR	ATEGLJQKQFG	EDRVFPLBLA	ESGGIGGLAEL	LALOGLFRPV	IQFFGPVYE	WMDSICQCMQA	RIRYRTGGRY	SETLEAIYAH
odbb-human	AF.GOVFR	CTVGLRDYKG	KERFVN1BLC	EOGIVGFGIC	IVATICATAIA	IQFQADYIFF	AEDQIVNEAA	KYRYRSDFL	IDEEMSTDP
odbb-bovin	AF.GOVFR	CTVGLRDYKG	KERFVN1BLC	EOGIVGFGIC	IVATICATAIA	IQFQADYIFF	AEDQIVNEAA	KYRYRSDFL	MTDEELERDEK
odbb-rat	AF.GOVFR	CTVGLRDYKG	KERFVN1BLC	EOGIVGFGIC	IVATICATAIA	IQFQADYIFF	AEDQIVNEAA	KYRYRSDFL	MTDEELRDP
acob-alceu									
acob-peleca									
acob-klepn									
acob-cloma									
221									
odpb-mycca	HIPFVQIVCP	STPYDTKG	LAAIDSPDPV	IVVPTKLY	RAFKQ	EVPDEHYIVP	IGEGYKQEQ	NDLTIVTYGA
odpb-myge	QIAGLKTVMP	SNPYDTG	LAAIESPDPV	IFPFPKLY	RAFRQ	EIPSDYYTVP	IGEANLISEP	QTIVDCQKAIA
odpb-achla	SIFGLKVTMP	SNPYDTG	LAAINDPDV	VFLPKRKY	RAGKQ	EVPAEYIEIP	IGKAGVVKQOS	SELITIVSYGP
odpb-bacsu	OOPGLKVVIP	SNPYDTG	LAAIRDNDPV	VFLPKRKY	RSRFO	EPEEEYITP	LGKADVKRE	TDMDTIVAWGS
odpb-bact	OOPGLKVVIP	SNPYDTG	LAAIRDNDPV	VFLPKRKY	RSRFO	EPEGEYITP	IGKADIKREG	TDLSITYIYG
odbb-human	HCPGLKVVIP	SNPYDTG	LSCIEDKNPC	IPFEPKILY	RAAEF	EVPIEYPNIP	LSQAEVQIOP	VMHESLKAAD
odbb-bovin	HCPGLKVVIP	SNPYDTG	LSCIEDKNPC	IPFEPKILY	RAAEF	EVPVPEYNP	LSQAEVQIOP	ELEKOGIS
odbb-rat	HCPGLKVVIP	SNPYDTG	LSCIEDKNPC	IPFEPKILY	RAAEF	EVPVPEYNP	LSQAEVQIOP	ESQDFTAWFM
odbb-bacsu	NOPEGLKVVIP	SNPYDTG	LAARIVEDDPV	LEFEPKRKY	RAAEF	EVPVPEYNP	LSQAEVQIOP	ESQCFAWAYG
odbb-psepu	QCGLKVVIP	SNPYDTG	LIAISCDPDPV	IPFLBKRLW	GPFDGHDRP	TPWSKPHIS	AVPDCYTYVE	LDKAAITRPG	ESQDFSPWYG
odbb-ascsu	HCPGLKVVIP	YDCEDEDARGL	KAARDDNDPV	IGCLMELING	MKEFVSPF	AQSPDFEPV	FQCAOKIOPG	ESQCYAAYA
odpb-human	HCPGLKVVIP	YDCEDEDARGL	KAARDDNDPV	IGCLMELING	MKEFVSPF	AQSPDFEPV	FQCAOKIOPG	ESQDFPLMFT
odpb-yeast									
odpb-arath									
acob-alceu	HIPFVQIVCP	STPYDTKG	QIAIRDNNDPV	IFCENHKNLYG	LEGEVPE	RLIKG	EPPADDVY	IGKADVKRE
acob-peleca	HIPFVQIVCP	SNPYDTG	QIAIRDNNDPV	IFCENHKNLYG	LEGEVPE	RLIKG	EPPADDVY	DDITIVTYGL
acob-klepn	HIPFVQIVCP	SNPYDTG	QIASTADDPPC	VFFEPHQKMLY	MKEVPE	RLIKG	EPPADDVY	CVDHFALQAAE
acob-cloma	HIPFVQIVCP	SNPYDTG	QISIRADDPPC	VFFEPHQKMLY	MKEVPE	RLIKG	EPPADDVY	ESLEKOGIS
331									
odpb-mycca	IDIDLRSIK	PCKKMKVIES	VKIKTGRLVV	HEAVKSFSVS	AEIIATVNE	ECEFYIKAPL	SRCTGYDVIT	EFDRG	EGY FQVNPKKVLV
odpb-myge	IIELDLRTIS	PDKOTVFNS	VKIKTGRLVV	TEAVKSPFTS	AEIITSVTE	ELEFTYKLKAP	QRVTFIDIVV	ELARG	KMQEELLDFKF
odpb-achla	VELIIDLRTIS	PDKOTVFNS	VKIKTGRLVV	TEAVKSPFTS	AEIITSVTE	ELEFTYKLKAP	QRVTFIDIVV	ELARG	EKY QFENIARVID
odpb-bacsu	AEVUDLRTVS	PDKOTVFNS	VKIKTGRLVV	TEAVKSPFTS	AEIITMVE	KAFPHLEAP	VRFTGFDITV	ELARG	AVNOLLK..
odpb-bact	AEVUDLRTVS	PDKOTVFNS	VKIKTGRLVV	TEAVKSPFTS	AEIITMVE	RAILSLEAPV	LRVAAPD.T	PEFSSQA	EKY QFENIARVID
odbb-human	CEVIDLRTII	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	RAILSLEAPV	LRVAAPD.T	PEFSSQA	AVNOLLK..
odbb-bovin	CEVIDLRTII	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECFLNLEAPI	SRVCGYDT	PEFSSQA	EKY QFENIARVID
odbb-rat	CEVIDLRTII	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECFLNLEAPI	SRVCGYDT	PEFSSQA	AVNOLLK..
odbb-bacsu	CEVIDLRTII	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECFLNLEAPI	SRVCGYDT	PEFSSQA	EKY QFENIARVID
odbb-psepu	CEVIDLRTII	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECLFLDLPAT	KRLGDPIDA	PEFSSQA	AVNOLLK..
odbb-ascsu	CEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECLFLDLPAT	KRLGDPIDA	PEFSSQA	EKY QFENIARVID
odpb-human	CEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	ECLFLDLPAT	KRLGDPIDA	PEFSSQA	AVNOLLK..
odpb-yeast									
odpb-arath									
acob-alceu	AEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	DAFGYLIGP1	LRVTGFDVPM	PEAQALIET	AVNOLLK..
acob-peleca	AEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	DAFGYLIGP1	LRVTGFDVPM	PEAQALIET	AVNOLLK..
acob-klepn	AEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	DAFGYLIGP1	LRVTGFDVPM	PEAQALIET	AVNOLLK..
acob-cloma	AEVINLRSIK	PWDVDTICKS	VKIKTGRLVV	HEALPITCGFA	SEIISSTVQE	DAFGYLIGP1	LRVTGFDVPM	PEAQALIET	AVNOLLK..
431									
odpb-mycca									
odpb-myge									
odpb-achla									
odpb-bacsu									
odpb-bact									
odbb-human									
odbb-bovin									
odbb-rat									
odbb-bacsu									
odbb-psepu									
odpb-ascsu									
odpb-human									
odpb-yeast									
odpb-arath									
acob-alceu									
acob-peleca									
acob-klepn									
acob-cloma									

Fig. 7. Alignment of sequences of members of the Enzyme I-β subunit family. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed Enzyme I-β proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (odpb-mycca) (this work); *M. genitalium* (odpb-myge) (MG273 from the TIGR database); *A. laidlawii* (odpb-achla) (Wallbrandt et al., 1992); *B. subtilis* (odpb-bacsu) (Hemila et al., 1990) and (odbb-bacsu) (Wang et al., 1993); *B. stearothermophilus* (odpb-bact) (Borges et al., 1990), human (odbb-human) (Nobukuni et al., 1990a) and (odpb-human) (Ho & Patel, 1990); bovine (odbb-bovin) (Nobukuni et al., 1990b); rat (odbb-rat) (Zhao et al., 1992); *P. putida* (odbb-psepu) (Burns et al., 1988); *A. suum* (odpb-ascu) (Wheelock et al., 1991); yeast (odpb-yeast) (Miran et al., 1993); *A. thaliana* (odpb-arath) (Luethy et al., 1994); *A. eutrophus* (acob-alceu) (Priefer et al., 1991); *P. carbinolicus* (acob-peleca) (Oppermann & Steinbuchel, 1994); *K. pneumoniae* (acob-klepn) (Deng et al., 1994); *C. magnum* (acob-cloma) (Kruger et al., 1994). odpb, odbb, acob refer to the genes encoding the β-subunits of Enzyme I of the pyruvate dehydrogenase, 2-oxoisovalerate dehydrogenase, and acetooin catabolism complexes.

respond to the lipoyl domains (approximately 70 residues). The proteins from *E. coli* and *Azotobacter vinelandii* contain three such domains; those from *E. faecalis*, *A. laidlawii*, *Alcaligenes eutrophus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Dictyostelium discoideum*, *Arabidopsis thaliana*, and human contain two; and all the others shown, including the *Mycoplasma*

sequences, contain one lipoyl domain. Each one of the lipoyl domains contains a conserved lysine residue (the site of lipoylation), generally preceded by an aspartyl residue. In the case of *A. vinelandii*, the aspartate of the first lipoyl domain is replaced by alanine. For *Klebsiella pneumoniae*, the conserved aspartate is replaced by serine. All the lipoyl domains are also character-

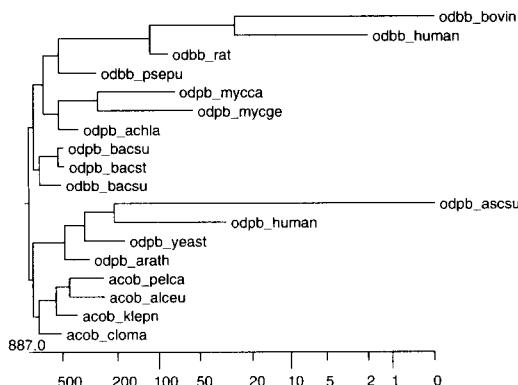


Fig. 8. Phylogenetic tree of sequenced proteins of the Enzyme I- β subunit family. Relative evolutionary distances are shown on the numerical scale. Abbreviations are as in the legend to Figure 7.

ized by the presence of a conserved GD pair. In the case of odp2 from *B. subtilis*, the G is replaced by N. For odo2 of *A. vinelandii*, odp2 of rat, and odp2 of human, the D is replaced by E. In approximately half of the proteins (odp2-psepu, odo2-azovi, odp2-rat, odo2-rat, odo2-human, odp2-entfa, odp2-achla, odp2-alceu, odp2-haein, odp2-pseae, odp2-dicdi, odp2-arath, odp2-human, odp2-ecoli, and odp2-azovi), the lipoyl domain is separated from the remainder of the protein by an A,P-rich linker. It is worth noting that the linker in odp2-dicdi is enriched in serine. The central portions of the proteins contain an E3 binding domain of approximately 50 residues and the carboxyl-terminal 250 residues corresponds to the catalytic domain, which effects the acyl transfer reaction. The conserved histidine (at residue 753) acts as the general base catalyst (Mattevi et al., 1992). A phylogenetic tree (Fig. 10) shows the preservation of the evolutionary relationship of the proteins from *M. capricolum*, *M. genitalium*, and *A. laidlawii*.

Following a gap of 18 bases, an open reading frame (from bases 4796 to 6686) was found; it corresponds to the gene encoding dihydrolipoamide dehydrogenase. The alignment shown in Figure 11 emphasizes a unique feature of the dihydrolipoamide dehydrogenase from *M. capricolum*. This protein contains an aminoterminal lipoyl domain (shown as a boxed grey area), a feature observed previously only in the DLDH proteins from *A. eutrophus* (Hein & Steinbüchel, 1994) and *Clostridium magnum* (Kruger et al., 1994). These lipoyl domains contains the characteristic conserved lysine residue (the site of lipoylation) (Mattevi et al., 1992), preceded by an aspartyl residue (residues 42 and 43). There are a number of totally conserved residues (shown in reverse shading) characteristic of dihydrolipoamide dehydrogenase sequences. The region from residues 171 to 180 corresponds to the motif GXGXXGYXXA, which is a possible nucleotide binding site. The conserved region from residues 206 to 218 corresponds to the signature GGXCLNXGCXP(S/T)K. In this region, the combination of the flavin ring with an adjacent disulphide bridge forms the redox center, which is involved with the transfer of the reducing equivalents from dihydrolipoamide to NAD⁺. The vicinal cysteines in this region may bind lipoic acid and undergo reversible oxidation-reduction. The active species (dimeric) of E3 has four domains: FAD binding,

NAD binding, central, and interface (Mattevi et al., 1992). A phylogenetic tree (Fig. 12) documents the consistency of the relationship of the *M. capricolum*, *M. genitalium*, and *A. laidlawii* sequences.

A space of 21 bases separates the coding sequence for dihydrolipoamide dehydrogenase from the next open reading frame. The region from bases 6707 to 7675 corresponds to the gene encoding phosphotransacetylase. The seven known sequences for phosphotransacetylase are aligned in Figure 13. The proteins from *E. coli* and *H. influenzae* are unique in that they have amino-terminal extensions of approximately 400 residues. All of the sequences show characteristic regions of total conservation, highlighted by reverse shading. A phylogenetic tree (Fig. 14) shows clearly a distinct branch for the proteins from *H. influenzae* and *E. coli* and a separate branch for the proteins from *M. genitalium* and *M. capricolum*.

A 13-base spacer separates the *pta* gene from the next open reading frame, encoding acetate kinase (from bases 7689 to 8866). The alignment shown in Figure 15 compares the six known acetate kinase sequences. The amino-terminal regions are characterized by a highly conserved sequence (residues 12–17, shown in reverse shading), GSSS(I/L)K, that may be involved in nucleotide binding. The phylogenetic tree shown in Figure 16 demonstrates the sequence relatedness of the *M. capricolum* and *M. genitalium* proteins, as well as the *E. coli*, *H. influenzae* similarities. Seventy-one bases separate the end of the sequence encoding acetate kinase from the one encoding *orfA*.

The sequence shown in Figure 2 was examined for regulatory features. Upstream of the *lplA* gene, within the coding sequence of *naox*, there is a possible –35 (TTGACA, bases 180–185, underlined) and –10 (TTTAAT, bases 204–209, boxed) promoter sequence. Upstream of the *odpA* gene, within the *lplA* coding sequence, there is a possible –35 (TTGACT, bases 1226–1231, underlined) and –10 (AAGAAT, bases 1249–1254, boxed) sequence. Upstream of *odpB*, within the *odpA* coding sequence, is found the potential –35 (TTGACA, bases 2328–2333, underlined) and –10 (CAAAAT, bases 2353–2358, boxed) sequence. Within the *odpB* coding sequence, upstream of *odp2*, potential –35 (TTGATA, bases 3350–3355, underlined) and –10 (TAAA AA, bases 3384–3389, boxed) sequences are located. Within the *dldH* coding region, upstream of *pta*, there are two potential –35 sequences (TTGGAA, bases 6523–6528 and TTGCTA, bases 6594–6599, underlined) and two potential –10 (AGAAAT, bases 6550–6555 and AACAAAT, bases 6619–6624, boxed) sequences. Near the end of the *ack* coding sequence is located a potential –35 (TTGTTT, bases 8841–8846, underlined) and –10 (TTAAAT, bases 8864–8869, boxed) sequence. The spacer between the end of the *ack* gene and the beginning of *orfA* (71 bases) is AT-rich (marked with diverging arrows) and may form a stem-loop structure that plays a role in transcription termination of the *ack*-containing message.

Probable ribosome-binding sites are recognizable at appropriate positions before coding sequences. AGGAAAA (bases 270–276, shaded) is found before *lplA*; AGAAAGG (bases 1315–1321, shaded) is found before *odpA*; AGGAGGA (bases 2425–2431, shaded) is localized preceding *odpB*; AGAAAGG (bases 3447–3453, shaded) precedes *odp2*; AGTGAAG (bases 4781–4787, shaded) is localized in front of *dldH*; AGAAAGA (bases 6696–6702, shaded) precedes *pta*; AGGAGAA (bases 7675–7681, shaded) is found in front of *ack*; AGAAAGC (bases 8927–8933, shaded) is found in front of *orfA*.

A

Fig. 9A. (*Figure continues on facing page.*)

B	551	
odp2-mycca	VRKATVKAMT KSHTEIAAFT GMKNTDITET HKMRTELEK, HAASAS.GIKL TYLAFLIIKAV AKSLRDMPNI	IVYRGDFANNK IQFMHNINIG IADTPNGCIV VPIVKGADHL
odp2-mycge	MRAKIAEAMT KSHAIIPPTV LTFYVYNATKL QYRESVNGY ALSKY. SMK SYFAFPVKAV VNALKKFVVF	IVYGDPMQNE IVLNNDINVG IVDTTEEGCI VPIVKQAQTK
odp2-bacst	IRRAIAKAMV HSXHTAPHRV LMDEADVTKL VAHRRKKFKA IAEEK. GIKL TFLPYVVKAL VSALREYPVLF	IVSISDTEETE IIQKHYNIG IADTDRCGII VPIVKHADR
odp2-bacsu	IRKAIAKAMV NSXHTAPHRV LMDEVDTVNLS VAHRRKKFQK, VAADQ. GIKL TFLPYVVKAL TSALKKFVVL	IVSISDCTDE VIQKHYFNIG IADTDTEGKLU VPIVKXNADR
odc2-bacsu	RQQTIAKMLR EVQOTSAMLT TFEVENDMTAV MNLRKRRKDQ FFE. QNEVKL GFMSFPFTKAV	VAALKKYPPL NAEIQQ.. DE LIVVKFYDID IVNAAVEGCV VPIVVRADRL
odb2-bacsu	VRKAIALSNMK RSKTEI PHAM TMMEVDVTNM VAYRNNSIKDQ FFKTE. GFNL TFFAFPVKAQ AQLKEFPOI	NSMM.. AGDK IIQKQDINIS IVNATEDSF VPIVKNADEK
acoc-cloma	IRKIIASRHM ESWITSEPTVY YDIKVDMTSL RKRFDALDKDV	NSIN.. GNE LITRNYNVNG VVVAIDGGMV VPIVVKYANEK
acoc-klepn	MRAIASRLQ TSKQSPHPF LSVLDLQLERL LALRQDINIE VPG .. . VKI SVNDLVLKA	VIQFDEAAOS INRFTDADIS VVVALPAGI TPIVSAERK
acoc-pelca	IGAAISNTMV NSK TIPOPF VTMGEEA KEPFRAGLKAK GKA.. . . V SMNDMVIRAL	GKAIEQYPMV NATLGKEYG L.. . NADVNI VVAGTDDALM VPIVVKGCQAL
odb2-psepu	DLRKLIQRMQ DAKRRVAFHS YVEEIDVTAL SALRQQLNS. KHCDGS.RGKL TLLPFLVRLA	VATYDDEAQI ITRHGAHVRS ITQGDNCN VPIVLRHAEAG
odc2-azovi	EAQSSMAMLS TFEVNNMKPV MELRAKYKDL FETHINGVRL GFMSFPVKAQ	VEALKRQPVG PASIDG.. ND IVVHYGQDQG VVSSSDRGCV VPIVLRNAEFL
odc2-ecoli	LRLRKVARBL EAKNSTAMLS TFEVNNMKPI MDLRKQVGEA FERKHR. GIRL GFMSFPVVKAV	PASIDG.. DD VVHHNYFDVGS MVVSTPRGV VPIVLRDVDTL
odp2-staur	MRAIAKAMV NSXHTAPHRV LMDEIDVQAL WDHRKKFKE, IAEEQ. GTKL TFLPYVVKAL	TSFNEEAGE IVHKHYWNIG IADTDRCGII VPIVVKHADR
odp2-neucr	MRTKIIARL ESVTENPHF VSTNLNSVSKL LKL7QALNNS ADGR .. YKL SVNDLFLKAM GIASKRKPVY	NSSM.. RDGV IROFETVDS VVATPNCGIV TPIVVKYGEVK
odp2-yeast	MRSII1GERL OSTQGIPSYI VSSKSISISSLK LKLROSLNAT ANDK .. YKL SINDLVLKA	NAFLPNPENV IRKFKNVUDVS VVATPTGCLL IVVKNEAK
odp2-rat	RSVIAQORLM OSKOTI PHYI LSVDNVNGEV LLVRLKELNLBKG .. . GKI SVNDLFLKAS	ALACLKVPKA NSSM.. DTW IRONHVVDVS VVATPAGI TPIVNAHIC
odo2-rat	MQRQIAQRL BEAQNTAMLT TFNEVDMDSN1 QEMBRARKHDL FLKHNH LKL GLMSAVFKAS AFALQEPVIL	NVIDADTIVDID VVATPAGCIV VPIVIRNVTM
odb2-bovin	FHKAVKMTMS AA_LKIPHFG YCDEVDTLTEL VKLREELKPI AFAR.. . GIKL SMFMPFLKAA SLCLLQFLPIL	MSAVDENCN ITYKASHNIG IADTDTEGQII VPIVVKVNOIC
odc2-human	FOKAVKMTMS AA_LKIPHFG YCDEVDTLTEL VKLREELKPI AFAR.. . GIKL SMFMPFLKAA SLCLLQFLPIL	MSAVDENCN ITYKASHNIG IADTDTEGQII VPIVVKVNOIC
odc2-yeast	MQRQIAQRL BEAQNTAMLT TFNEVDMDSN1 QEMBRARKHDL FLKHNH LKL GMFSAVFKAS AFALQEPVIL	MSAVDENCN ITYKASHNIG IADTDTEGQII VPIVVKVNOIC
odp2-entfa	MRLRIAREBL ESQNTAMLT TFNEVDMDSN1 MEMRKKYKDE	IIKK.. TGTKF GFMSFPVKAQ TLAAKDPVAP
odc2-human	TRKAIAKAMV NSXHTAPHRV LHDVEEVSKL NDHRKKFKD	NAIGAE.. DO IVYRDYDID IS VVATPAGCIV TPIVVRNAEFL
odp2-achla	LRKAIAKAMV NSXHTAPHRV LMDEIDVQAL VNFRRNEAKG	ESLKS.. GIKL TMYMAFIKAV LIALKEFPMI
odp2-akleu	INKISGANLH RNWVMPHIV NHDEADTIL EAFRFLNLNEK NEXS.. . GIKV TMLAFMIFAT	MSASNHNIDTE VYIKKFINLUS MAVTDIDCGII VPIVKNADRL
odp2-azovi	INKISGANLH RNWVMPHIV NHDEADTIL EAFRFLNLNEK NEXS.. . GIKV TMLAFMIFAT	MSALDGNLW.. . LKKYFNIG FADTPNGCIV VPIVKAADKK
odp2-haein	TFKRAQOEIN LKPNEMTGGT FTITNQCMF	NVIDADTIVDID VVATPAGCIV VPIVIRNVTM
odp2-pssep	LEVEGAANLH RSWLNVPHVT QFDQSDITDM EAFRVAQAKA AEKA.. . GVKL TVPLILLKAC AHLLKELPD	LLKQVHNG VVATPAGCIV VPIVIRNVTM
odp2-dicdi	IRKVTAAARTL QSKQTIPHRY LTMECRVDSLK LLLAFLRQKELQE .. . NHG.. . VKV SVNDVIFKAS	MSLAPSQKA LIRKKVHNG FADTPNGCIV VPIVIRNVTM
odp2-arath	QIRKTAIRL LESKQKIPHL YLQSDQVULDP LLAFLRQKELQE .. . NHG.. . VKV SVNDVIFKAS	PAALRNQVKAQ NDAEWAEGD VVATPAGCIV TPIIKNADQK
odp2-human	IRVIAQORM LSQKIPHL YLSDQVNLGRV LLVRKELNKI LLLAFLRQKELQE .. . SKV SVNDVIFKAS	IRVHNVVDVS VVATPAGCIV TPIVNAHIC
odp2-ecoli	QSKQISANLH RSWLNVPHVT HFDKTDITEL AFRKQONEE AAKRKLQDNL VTFVFMFVKAQ	MSLSESDQR LTLLKYYINIG VVATPAGCIV VPIVFKDWNKX
odp2-azovi	LMOQIGATNLH RSWLNVPHVT QFESADITEL EAFRVAQAKV AEKA.. . GVKL TVPLILLKAC	MSLAPSQKA LIRKKVHNG FADTPNGCIV VPIVIRNVTM
	661	
SVE1AIKIS	ELANKAKDGK LTRAEMTEAT FTVSNFQCSV. GLDYATPII SDE.SAILGV GTMSQPLYI NGELOKRF.. IMPLSMT CIIHRIIDGADAGRFLKQV
odp2-mycca	SVE1AIQAVI DLANKARTK IKLTLNLNGK TISVNFQCSV. GAAVGTPIQ YEE.. . MCIVAT	.. . TILPLTIA AIIHWRVIGAD VGRGKEIAK
odp2-mycge	TF11EAEQIN ELAEKARDKG LTPGEMKGAS CTITNIGCA	GNLNEERIVK VV.. . GNEI.AVH.. .
odp2-bacsu	SVE1SEDEIN GLATKAREKG LAPAEMKGAS CTITNIGCA	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odc2-bacsu	TFAEIKEIG ELAKKARAKNN LTSELEEGGS FTITNGCFT.	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odp2-entfa	TIGKIAKDT GLAKKVRDQK LTADMDQGQT FTITNGCFT.	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odp2-achla	GLAKKVRDQK LTADMDQGQT FTITNGCFT.	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
acoc-cloma	GLKESTEIVK LSLAKKASNO LKPNEMTGGT FTITNQCMF	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
acoc-klepn	SISDISENIEH SLVTRAKAGK LKPNEMTGGT FTITNQCMF	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
acoc-pelca	SELEVASAASR AVIDVKVAGT CGPAEMAGGN FAISNLCM	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odb2-psepu	SLWANAGEIS RLARAANRNK ASREELSGST ITLTSVLPV	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odc2-azovi	SLEAIEGGK EFGKGRDQK TLIEEMTGTT FTISNGCFV.	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odp2-ecoli	GMADIEKKIK ELAVKGRDQK LTVEDLTTGGN FTITNGCFT.	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odp2-staur	SIFQISDEIN ELAVKARDKG LTADEMKGAT CTITNIGCA	GGWQFTPVIN HEE.. . VAILGI GRIAEPKIV DGEIVAAP.. .
odp2-neucr	GLEISIAAVK LPEKKEVYOGGS ISISNCHMNP OASQFTAII	GGWQFTPVIN HEE.. . VAILGV GAPOKVAVFPV EMEDGTVGS WDEQIIIVTAS FPIKVKVIGAV GAEWIRELK
odp2-arath	GLSISQNEIK ELVKARARIK LPAEEFOGGT ICISNCHMNP AVMFTPSJIN PRO.. . STILAI ATVERV.. . V	.. . LMMNLSL AMBRVIEGSV AAQFLSKVKE
odp2-yeast	GLETIASDVG SLASKARSK LQHEFOGGT FTISNLCM	DDAEEANGFS FDNOVITGCF BPERTIDGAK GAEMKELKT
odp2-achla	YLS.. . KPEL LML.. IIELWM
odp2-azovi	NYADIERTIN ELGEKARNEK LAIEDMDGDT FTISNLCM	YLS.. . KPEL LML.. .
odp2-bovin	SIEFATEILN RLQKLQSGAC LSTNDLIGGT FTISNLCM	YLS.. . KPEL LML.. .
odc2-human	SIFDIAETLN RLQKLQSGVQ LSTTDLTGGT FTISNLCM	YLS.. . KPEL LML.. .
odc2-yeast	NFADIETLN ELGEKARNEK LAIEDMDGDT FTISNLCM	YLS.. . KPEL LML.. .
odp2-entfa	SLVDIENEIV RLSHARKDKG LTLEDMTGGT FTISNLCM	YLS.. . KPEL LML.. .
odp2-achla	SMFAIDEIN EKAALAIKSL LTQADMRTGGT ITISNIGSV.	YLS.. . KPEL LML.. .
odp2-akleu	SLFELASOVS SLADDITIARK ISMDOQCTNGT FTITNFCSA	YLS.. . KPEL LML.. .
odp2-haein	GLIESELQMS ELAKLARDKG LKPDQMGCGC FTISNLCM	YLS.. . KPEL LML.. .
odp2-pssep	YLS.. . KPEL LML.. .	YLS.. . KPEL LML.. .
odp2-dicdi	SLQLOAAEAA LSLADKARNK LSAADMQGAC FTISNLCM	YLS.. . KPEL LML.. .
odp2-arath	SLQLOAAEAA LSLADKARNK LSAADMQGAC FTISNLCM	YLS.. . KPEL LML.. .
odp2-human	SISIASELVK ELQAKRSGK LPHEFQGGT FTISNLCM	YLS.. . KPEL LML.. .
odp2-yeast	GVETIANDVU SLATKAREKG LQHEFOGGT FTISNLCM	YLS.. . KPEL LML.. .
odp2-achla	GIIESELRELM TISKSKARDKG LTAGEMQGCGC FTISNLCM	YLS.. . KPEL LML.. .
odp2-akleu	SLQLOAAEAA ELAEKARSKK LGADAMQGAC FTISNLCM	YLS.. . KPEL LML.. .
odp2-azovi	SLQLOAAEAA ELAEKARSKK LGADAMQGAC FTISNLCM	YLS.. . KPEL LML.. .
	771	
odp2-mycca	YLS.. . KPEL LML.. .	
odp2-mycge	QIEELIDLTV A.. .	
odp2-bacsu	LLS.. . DPEL LMLEA.. .	
odp2-bacsu	LLN.. . DPQI ILMEA.. .	
odc2-bacsu	LLEDPQ.. . LLLLEG.. .	
odp2-bacsu	LLEIDESPKT VY.. .	
acoc-cloma	YME.. . KPEL LML.. .	
acoc-klepn	
acoc-pelca	LLNEPEL.. .	
odb2-psepu	LLE.. . OPAC LFVE.. .	
odc2-azovi	LLEDPAR.. . LLLDV.. .	
odc2-ecoli	LLEDPTR.. . LLLDV.. .	
odp2-staur	LLN.. . NPCL LLMEG.. .	
odp2-bacsu	LLE.. . NPCL LLMEG.. .	
odp2-bacsu	VIENPLELL.. .	
odp2-yeast	VIENPLELL.. .	
odp2-achla	EQLEPSGL.. .	
odp2-azovi	AVEDPAV.. . LLLL.. .	
odp2-bovin	YLENPAMFL DLK.. .	
odc2-human	YLENPAMFL DLK.. .	
odc2-human	AVEDPVR.. . LLLL.. .	
odc2-yeast	LIEDPRKCY GDLKFAAHTN LIS	
odp2-entfa	LLA.. . DPEL LLLS.. .	
odp2-achla	LLT.. . NPLT LLLS.. .	
odp2-akleu	LLADFRILL.. .	
odp2-haein	VLADLRLRLL.. .	
odp2-pssep	LLADRTILL.. .	
odp2-dicdi	YVENPIKLL.. .	
odp2-arath	NFEDVRLRLL.. .	
odp2-human	YLERPITML.. .	
odp2-ecoli	TLSDIRRLRVM.. .	
odp2-azovi	LLADIRALL.. .	

Fig. 9. Alignment of sequences of members of the Enzyme II family. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed Enzyme II proteins are shown in reverse shading. Boxed shaded regions correspond to lipoyl domains. Abbreviations used and references to published sequences are: *M. capricolum* (odp2-mycca) (this work); *M. genitalium* (odp2-mycge) (MG272 from the TIGR database); *B. stearothermophilus* (odp2-bacst) (Borges et al., 1990); *B. subtilis* (odp2-bacsu) (Wang et al., 1993), (odb2-bacsu) (Wang et al., 1993), and (odc2-bacsu) (Carlsson & Hedstrand, 1989); *C. magnum* (acoc-cloma) (Kruger et al., 1994); *K. pneumoniae* (acoc-klepn) (Deg et al., 1994); *P. carbinolicus* (acoc-pelca) (Oppermann & Steinbuchel, 1994); *P. putida* (odb2-pssep) (Burns et al., 1988); *A. vinelandii* (odo2-azovi) (Westphal & de Kok, 1990) and (odp2-azovi) (Hanemaaijer et al., 1988); *E. coli* (odo2-ecoli) (Spencer et al., 1984) and (odp2-ecoli) (Guest, 1987); *Staphylococcus aureus* (odp2-staur) (Hermila, 1991); *Neurospora crassa* (odp2-neucr) (Kreader et al., 1989); yeast (odp2-yeast) (Niu et al., 1988) and (odo2-yeast) (Repetto & Tzagoloff, 1990); rat (odp2-rat) (Gershwin et al., 1987) and (odo2-rat) (Nakano et al., 1991); bovine (odb2-bovin) (Lau et al., 1988); human (odb2-human) (Lau et al., 1992), (odp2-human) (Thekkumkara et al., 1988), and (odo2-human) (Nakano et al., 1993); *E. faecalis* (odp2-entfa) (Allen & Perham, 1991); *A. laidlawii* (odp2-achla) (Wallbrandt et al., 1992); *A. eutrophus* (odp2-akleu) (Hein & Steinbuchel, 1994); *H. influenzae* (odp2-haein) (H1232 from the TIGR database); *P. aeruginosa* (odp2-pssep) (Genbank accession no. U47920); *D. discoideum* (odp2-dicdi) (Genbank accession no. u06634); *A. thaliana* (odp2-arath) (Guan et al., 1995). odp2, odb2, and odc2 refer to the genes encoding the Enzyme IIs of the pyruvate dehydrogenase; α -oxo acid dehydrogenase and 2-oxoglutarate dehydrogenase complexes, respectively; and acoc refers to acetooin dehydrogenase EII.

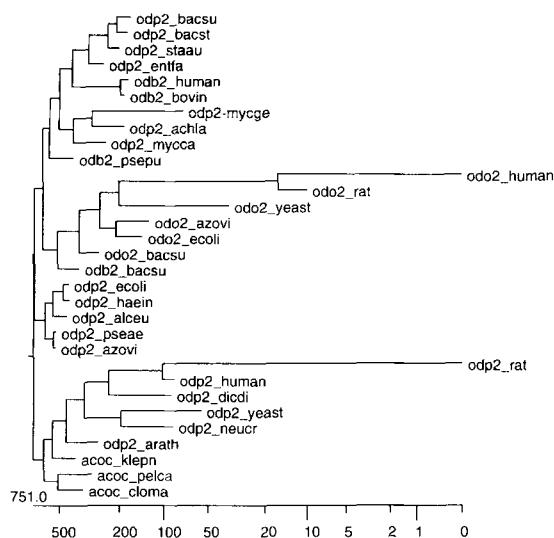


Fig. 10. Phylogenetic tree of sequenced proteins of the Enzyme II family. Relative evolutionary distances are shown on the numerical scale. Abbreviations are as in the legend to Figure 9.

Gene transcription analyses

The cloned sequence encodes 13 genes that might be transcribed in the same direction (see Fig. 1). The only significant gap between sequences occurs between the *ack* and *orfA* open reading frames. The program TERMINATOR was used to search the cloned sequence for potential transcription termination sequences (designated by diverging arrows in Fig. 2). Nineteen bases downstream of the translation termination codon for *ack*, there is a possible transcription termination region (from nucleotides 8886–8914). Using the program FOLDRNA, the stability of this stem-loop structure was calculated to be -11.8 kcal/mol . The stem-loop structure formed contains a perfectly matched stem 12 bases long. It is therefore possible that the region from *naox* to *ack* is transcribed as a single mRNA. Evidence was presented previously (Zhu et al., 1994) that the *ptsI* and *crr* genes constituted an operon. Consequently, it may be the case that the *orfA* and *kdtB* genes are cotranscribed.

Discussion

The sequence analysis presented here (see Figs. 1, 2) demonstrates the presence in *M. capricolum* of a unique arrangement of genes involved in the metabolism of pyruvate. In other bacterial species where gene mapping has been performed (*E. coli*, *H. influenzae*, *M. genitalium*), the genes are somewhat scattered throughout the genome. For example, in *E. coli*, the genes encoding the pyruvate dehydrogenase complex are located at 3 min on the genetic map, whereas the *ack*, *pta* and *ptsH*, *ptsI* and *crr* genes are in the 50–52-min region. In contrast, in *M. capricolum*, all the relevant genes are clustered in a single region. The positioning of these genes close to the *ptsI-crr* operon (whose products use PEP to form pyruvate) may also be of some regulatory significance. The scheme shown in Figure 17 indicates that all the enzymes required for the conversion of PEP to acetate and ATP, including those activities used for lipoylation of

the pyruvate dehydrogenase and regeneration of NAD from NADH, are accounted for in the region of the *M. capricolum* genome sequenced in this study (see Fig. 1).

We reported previously (Zhu et al., 1993) that the HPr protein from *M. capricolum* has an unusually high isoelectric point. Using the computer program PEPTIDESORT, we calculated the pIs of the protein sequences deduced in this study. The pIs of the E1 and EII proteins of the pyruvate dehydrogenase complex were in the range 5.3–6.75 and were similar for both *M. capricolum* and *M. genitalium*. However, several of the proteins from *M. genitalium* showed significantly higher pIs than the corresponding proteins from *M. capricolum*. For DLDH, the pIs for *M. capricolum* and *M. genitalium* were 5.35 and 7.08, respectively. For lipoate–protein ligase, the pIs for *M. capricolum* and *M. genitalium* were 6.97 and 9.29, respectively. For phosphotransacetylase, the pIs for *M. capricolum* and *M. genitalium* were 5.24 and 7.48, respectively. For acetate kinase, the pIs for *M. capricolum* and *M. genitalium* were 6.67 and 9.09, respectively. The significance of the widespread occurrence of proteins with high isoelectric points in *M. genitalium* remains to be clarified.

In order to evaluate the possibility that the genes encoding the enzymes involved in pyruvate metabolism are part of a polycistronic operon(s), northern blotting and primer extension experiments using probes derived from the plasmids described in Figure 1 were performed. No detectable mRNA species were found. Similar lack of success in detecting *ack*- or *pta*-specific mRNA species was reported for *Methanosaeca thermophila* (Latimer & Ferry, 1993) and it was suggested that the mRNAs may have a short half-life or be degraded rapidly during the RNA preparation. Consequently, the nature of the transcripts encoding the enzymes for pyruvate metabolism remains to be established.

The dihydrolipoamide dehydrogenase from *M. capricolum* is atypical, containing an amino-terminal lipoyl domain (see Fig. 11). This structure is also shared by the enzymes from *A. eutrophus* and *C. magnum*. This observation opens the possibility that effective function of the pyruvate dehydrogenase complex may be possible with the association of lipoate residues with either the E2 or E3 components. It is interesting to note that a recent description of an outer membrane protein from *Neisseria meningitidis* (de la Sierra et al., 1994) indicated that it contained an amino-terminal lipoyl domain and was otherwise homologous to lipoamide dehydrogenases.

The *M. capricolum* pyruvate dehydrogenase and *C. magnum* acetoin dehydrogenase complexes show a unique similarity. Each of these complexes contain an E2 with a single lipoyl domain, as well as an E3 with a single lipoyl domain. Comparison of the sequences of these lipoyl domains (see Figs. 9, 11) show that they are almost exact duplicates. This suggests that the lipoyl domains in the E3 proteins of these organisms arose by a duplication of the preexisting domain in the genes encoding the E2s.

In contrast, the pyruvate dehydrogenase complex of *A. eutrophus* is characterized by an E2 with two lipoyl domains, as well as an E3 with a single lipoyl domain. In this case, the two lipoyl domains of E2 are essentially identical, consistent with the idea that they arose by a duplication mechanism. However, the single lipoyl domain in the E3 diverges considerably from the sequences in the E2. Therefore, it seems unlikely that the lipoyl domain of the E2 in this organism arose by a simple duplication of the sequences in the gene encoding the E2.

Fig. 11A. (*Figure continues on following page.*)

	661	678
<i>dldh_mycce</i>	GKAIHP.....	
<i>dldh_alceu</i>	GTCCTDVPPR KR.....	
<i>acol_cloma</i>	NQAIHMNPK.....	
<i>dldh_achla</i>		
<i>dldh_mycke</i>	DVPS.....	
<i>acol_klepn</i>	DQPLHQ.....	
<i>dldh_pea</i>	DKPIHI.....	
<i>dldh_trybb</i>	AKTINF.....	
<i>dldh_pig</i>	GKAIFN.....	
<i>dldh_human</i>	GKSINF.....	
<i>dldh_yeast</i>	DKAIIC.....	
<i>dld3_psepu</i>	GMAMOI.....	
<i>dldh_psef1</i>	GHAIIHANRK KR.....	
<i>dldh_azovi</i>	GHAIIHVANRK K.....	
<i>dld2_psepu</i>	GGAIIHVANRK KR.....	
<i>acol_pelca</i>	GAAVHC.....	
<i>dldh_ecoli</i>	GSITDLPNPK AKKK.....	
<i>dldh_haein</i>	GSITDLPNAK AKEKIISI	
<i>dldh_bacst</i>	GTPIIHITK.....	
<i>dldh_bacsu</i>	GSPIIHVVK.....	
<i>dldh_staa</i>	GYPIHTM.....	
<i>dldh_halvo</i>	GQAIIHLNR.....	
<i>dld1_psepu</i>	GHALHI.....	

Fig. 11. Alignment of sequences of members of the dihydrolipoamide dehydrogenase family. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed Enzyme II proteins are shown in reverse shading. Boxed shaded region corresponds to a lipoyl domain. Abbreviations used and references to published sequences are: *M. capricolum* (*dldh-mycce*) (this work); *A. eutrophus* (*dldh-alceu*) (Hein & Steinbuchel, 1994); *C. magnum* (*acol-cloma*) (Kruger et al., 1994); *A. laidlawii* (*dldh-achla*) (Wallbrandt et al., 1992); *M. genitalium* (*dldh-mycke*) (MG271 from the TIGR database); *K. pneumoniae* (*acol-klepn*) (Genbank accession no. U30887); pea (*dldh-pea*) (Bourguignon et al., 1992); *Trypanosoma brucei* (*dldh-trybb*) (Else et al., 1993); pig (*dldh-pig*) (Otulakowski & Robinson, 1987); human (*dldh-human*) (Pons et al., 1988); yeast (*dldh-yeast*) (Browning et al., 1988); *P. putida* (*dld2-psepu*) (Palmer et al., 1991a), (*dld1-psepu*) (Burns et al., 1989), and (*dld3-psepu*) (Palmer et al., 1991b); *Pseudomonas fluorescens* (*dldh-psef1*) (Benen et al., 1989); *A. vinelandii* (*dldh-azovi*) (Westphal & de Kok, 1988); *P. carbinolicus* (*acol-pelca*) (Oppermann & Steinbuchel, 1994); *E. coli* (*dldh-ecoli*) (Guest, 1987); *H. influenzae* (*dldh-haein*) (HI1231 from the TIGR database); *B. stearothermophilus* (*dldh-bacst*) (Borges et al., 1990); *B. subtilis* (*dldh-bacsu*) (Hemila et al., 1990); *S. aureus* (*dldh-staa*) (Hemila, 1991); *Halofexax volcanii*, (*dldh-halvo*) (Vettakkorumakankav & Stevenson, 1992). *dldh*, *dld1*, and *dld2* refer to the genes encoding dihydrolipoamide dehydrogenases from the pyruvate, 2-oxoglutarate, and branched chain α -ketoadic dehydrogenase complexes, respectively. *dld3* refers to the third dehydrogenase isolated from *P. putida* and *acol* refers to the dihydrolipoamide dehydrogenase of the acetoin dehydrogenase complex.

Mycoplasmas are generally believed to be descendants of Gram-positive bacteria. All the *M. capricolum* proteins involved with the metabolism of pyruvate described here show phylogenetic relatedness to the homologous proteins from Gram-positive bacteria and *M. genitalium*.

The complete genome of *M. genitalium* has been reported recently (Fraser et al., 1995). Because it might be expected that these two species are closely related, the question was posed concerning the genomic locations of the genes encoding enzymes of pyruvate metabolism. Figure 18 shows a comparison of the location of the genes of interest in *M. capricolum* and *M. genitalium*.

genitalium. It is most surprising to see that, whereas all the genes involved with pyruvate metabolism are clustered in *M. capricolum*, this is not the case in *M. genitalium*. In both organisms, the *ptsI* genes are located approximately 500 kb from the replication origin. Further, the *gyrA,B* complex is separated from the *naox*, *odpA*, *odpB*, *odp2*, *dldH*, *lplA* complex by approximately 300 kb in both organisms. Clearly, there have been extensive rearrangements in the genomes of these organisms during evolution, resulting in resolution of *gyrA,B* from the replication origin in the case of *M. capricolum* and scattering of genes involved with pyruvate metabolism in *M. genitalium*.

In summary, the present work has demonstrated the unique arrangement of the genes encoding enzymes involved with pyruvate metabolism in *M. capricolum* and described some unique properties of the products of these genes.

Materials and methods

Growth of cells

M. capricolum (kid strain) were grown in modified Edwards medium at pH 8, as described previously (Mugharbil & Cirillo, 1978). After harvesting, cells were stored as frozen pellets for future use.

Nucleic acid preparations

DNA was prepared from frozen cells as previously described (Ausubel et al., 1990). RNA was prepared by suspension of cells (100 mg) in buffer (1 mL) containing 50 mM Tris-HCl, pH 6.8, 2 mM EDTA, and 1% SDS; the suspension was mixed with 5 mL of 4 M guanidium thiocyanate homogenization buffer

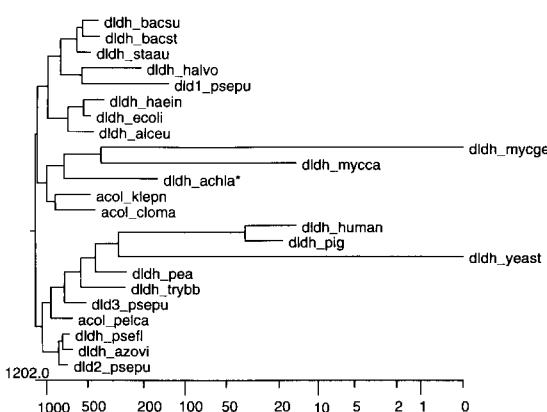


Fig. 12. Phylogenetic tree of sequenced proteins of the dihydrolipoamide dehydrogenase family. Relative evolutionary distances are shown on the numerical scale. Abbreviations are as in the legend to Figure 11. *, corresponds to a partial amino acid sequence (see Fig. 11).

Fig. 13. Alignment of sequences of members of the phosphotransacetylase family. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed phosphotransacetylase proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (pta-mycce) (this work); *M. genitalium* (pta-mycge) (MG299 from the TIGR database); *Paracoccus denitrificans* (pta-parde) (Van Spanning et al., 1995); *M. thermophila* (pta-mette) (Latimer & Ferry, 1993); *B. subtilis* (pta-bacsu) (Glaser et al., 1993); *H. influenzae* (pta-haein) (HII203 from the TIGR database); *E. coli* (pta-ecoli) (Kakuda et al., 1994).

(Sambrook et al., 1989). The cell suspension was frozen in dry ice-ethanol, then warmed briefly in a 64 °C water bath (Salser et al., 1967). Total RNA was purified by ultracentrifugation through 5.7 M CsCl/10 mM EDTA (Sambrook et al., 1989).

Cloning and screening

Genomic DNA fragments produced by digestion of *M. capricolum* DNA with *Hind* III (9,542-bp fragment), *Xba* I (6,654-bp

fragment), or *Spe* I (4,384-bp fragment) were cloned into the vector pBluescript II KS⁺ (pKSII⁺). Recombinant plasmids were used to transform Epicurean coli XLI-Blue Competent cells (Stratagene). Colonies were lifted onto nylon membranes (NEN Research Products, NEF-978). [³²P]5'-end-labeled oligonucleotide probes (1×10^6 cpm/mL of hybridization solution) were used for selecting positive clones. Oligonucleotide probes, synthesized as tritelyl-off derivatives on an Applied Biosystems 380B DNA synthesizer, were labeled with [γ ³²P]ATP by the DNA 5'-end-labeling method (Sambrook et al., 1989). Prehybridization was performed at 40 °C for 4 h in 6× SSPE/0.1% SDS/10× Denhardt's solution containing 20 mg/mL tRNA and 50 mg/mL of denatured heterologous DNA. Hybridization was performed at 42 °C for 16 h in 6× SSPE/10% SDS solution containing 1.4×10^6 cpm of [³²P]-labeled oligonucleotide/mL. The membrane was finally washed in 0.5× SSPE/1% SDS solution at 40 °C for 30 min. Positive clones were detected by autoradiography.

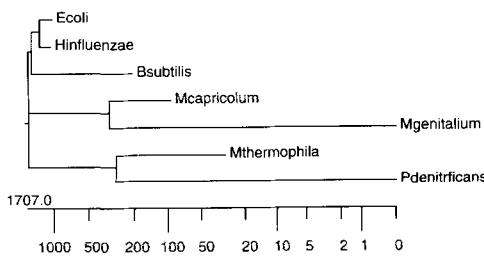


Fig. 14. Phylogenetic tree of sequenced proteins of the phosphotransacetylase family. Relative evolutionary distances are shown on the numerical scale. Abbreviations are as in the legend to Figure 13.

DNA sequencing

DNA sequencing on both strands of the DNA was performed by the dideoxy chain termination method of Sanger et al. (1977), with [α -³⁵S]dATP, using Sequenase 2.0 (United States Biochemicals) DNA sequencing kits. M13 forward or reverse primers or

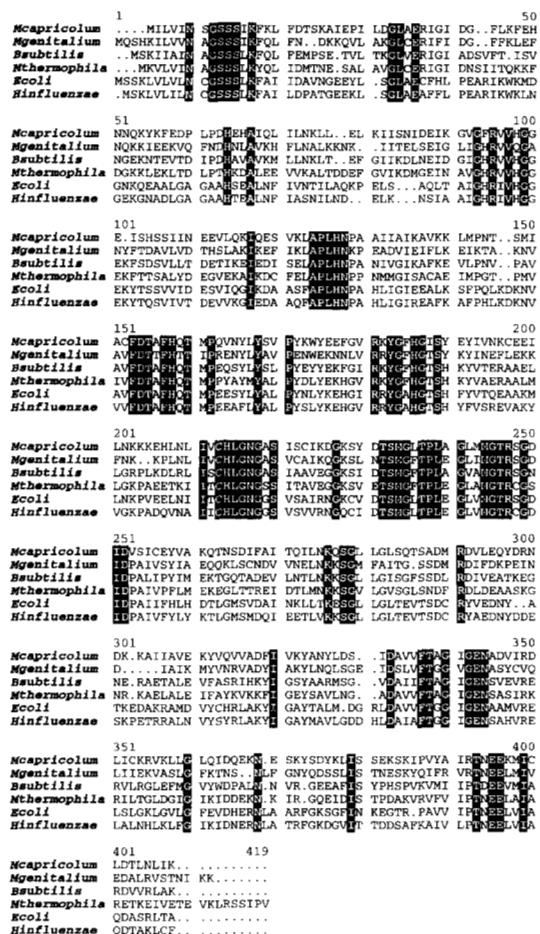


Fig. 15. Alignment of sequences of members of the acetate kinase family. Numbering above the aligned sequences corresponds only to the residue in the alignment rather than to a residue number in any of the aligned proteins. Residues that are conserved in the listed phosphotransacetylase proteins are shown in reverse shading. Abbreviations used and references to published sequences are: *M. capricolum* (*Mcapricolum*) (this work); *M. genitalium* (*Mgenitalium*) (MG357 from the TIGR database); *B. subtilis* (*Bsubtilis*) (Grundy et al., 1993); *M. thermophila* (*Mthermophila*) (Latimer & Ferry, 1993); *E. coli* (*Matsuyama et al., 1989*); *H. influenzae* (*Hinfluenzae*) (HI1204 from the TIGR database).

specific primers complementary to previously determined sequences were used.

Computer analyses

Analyses of DNA and protein sequence were performed using the GCG programs, version 7.2 (Devereux et al., 1984). Isoelectric points were calculated using the PEPTIDESORT program. A search for transcription termination sites used the TERMINATOR program. Stem-loop structures were analyzed using FOLDRNA. Translation frames were detected using the MAP program. Phylogenetic trees were constructed using the MEGALIGN module of the LaserGene program (DNAStar, Madison, Wisconsin) by the method of Hein (1990).

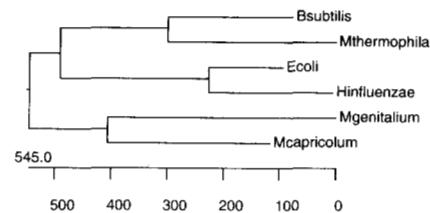


Fig. 16. Phylogenetic tree of sequenced proteins of the acetate kinase family. Relative evolutionary distances are shown on the numerical scale. Abbreviations are as in the legend to Figure 15.

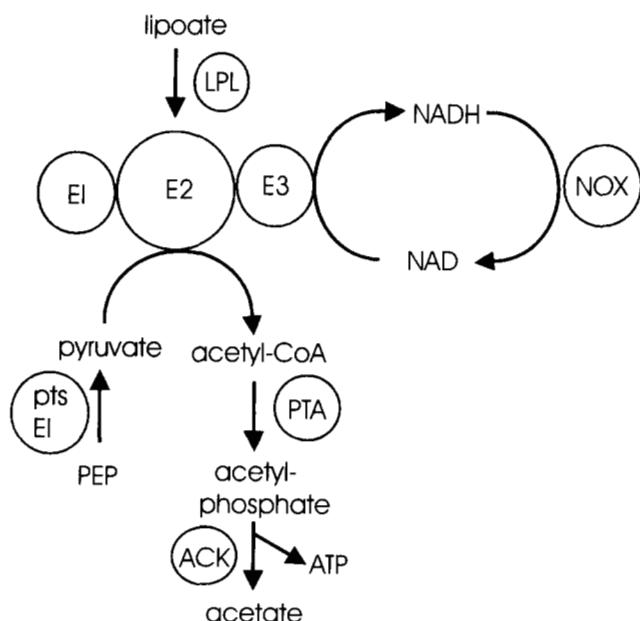


Fig. 17. Metabolic scheme for conversion of phosphoenolpyruvate to acetate in *M. capricolum*.

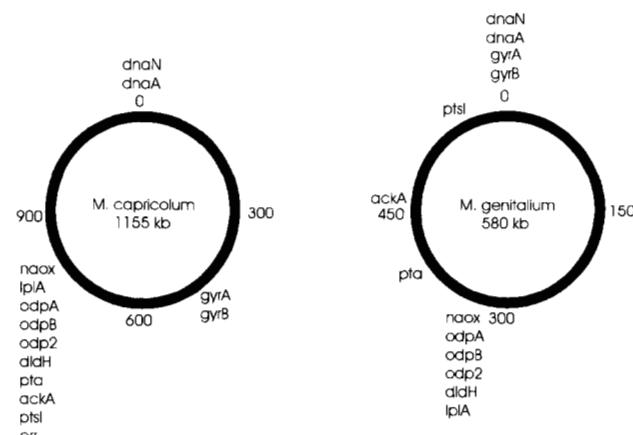


Fig. 18. Comparison of positions on the genetic map of various genes in *M. capricolum* and *M. genitalium*.

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