DNA sequence determination and biochemical analysis of the immunogenic protein P36, the lactate dehydrogenase (LDH) of *Mycoplasma hyopneumoniae*

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The DNA sequence of the gene encoding the early and specific immunogenic protein P36 of *Mycoplasma hyopneumoniae* has been determined. Comparison of the DNA sequence and the deduced amino acid sequence of P36 with known genes and proteins in data banks indicated that P36 is a L-lactate dehydrogenase (LDH) (EC 1.1.1.27). Biochemical analysis of protein P36 expressed from the cloned gene in *Escherichia coli* confirmed that P36 has L-lactate dehydrogenase activity. Protein P36 of *M. hyopneumoniae* therefore is termed LDH and its gene *ldh*. *M. hyopneumoniae* LDH was shown to contain the typical domains of LDH of other bacterial species. Immunologically however, we have shown that polyclonal antibodies against *M. hyopneumoniae* LDH do not cross-react with related LDH and show high specificity for *M. hyopneumoniae*. The *ldh* gene is preceded by several typical –10 sequences found in promoters of prokaryotes, but lacks the –35 sequence. Sequences rich in A + T, however, precede the –10 boxes, suggesting that factors involved in transcription initiation and their regulation may be different in *M. hyopneumoniae* compared to other bacterial species, but the putative ribosome binding site seems to be conserved.

Introduction

Mycoplasma hyopneumoniae is the primary agent of enzootic porcine pneumonia one of the most important direct or indirect causes of porcine respiratory infectious disease (Ross, 1992). It is spread worldwide and causes large economic losses in swine production units through retarded growth, poor food conversion and susceptibility of swine to infection by other organisms (Ross, 1992). The mechanisms of pathogenicity and the virulence factors involved in *M. hyopneumoniae* infections are very poorly understood due to the tedious growth procedures and lack of biochemical and genetic data on the organism. Potent immunogenic proteins that might be involved in pathogenicity were detected by immunoblot analysis using convalescent pig serum from naturally or experimentally infected pigs (Young & Ross, 1987; Mori

et al., 1988; Strasser *et al.*, 1991) and were named according to their molecular masses because the biochemical functions and structures of these proteins were initially unknown. Protein P54, a 54 kDa protein, was shown to be a cytopathic factor (Geary & Walczak, 1985), P74, a 74.5 kDa protein, was claimed to be a heat-shock protein (Brooks & Faulds, 1989), P65 was identified as a 65 kDa lipoprotein (Kim *et al.*, 1990) and P36, an early and strongly immunogenic 36 kDa intracellular protein which was cloned and expressed in *E. coli* (Strasser *et al.*, 1991) was shown to be highly species-specific (Stipkovits *et al.*, 1991). No sequence data of these proteins or their genes and no knowledge of the sequences involved in their expression are known. The only *M. hyopneumoniae* sequence data available up to now have been of rRNA genes which indicate that promoters of *M. hyopneumoniae* seem to be different from other mycoplasmas (Taschke & Herrmann, 1986; Taschke *et al.*, 1986, 1987), and two repeated sequences (Ferrell *et al.*, 1989; Harasawa *et al.*, 1991).

A better knowledge of the functions and nature of predominant antigens of *M. hyopneumoniae* and the regulation of their expression are required if their functions in pathogenicity are to be understood. This knowledge is also relevant for the use of such antigens in

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Abbreviation: LDH, lactate dehydrogenase.

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serological differentiation of porcine *Mycoplasma* species and for the evaluation of their use as ingredients in vaccines. In this report we describe the DNA sequence of the gene encoding the early immunogenic protein P36 and the enzymic, biochemical and immunogenic properties of P36 and compare these properties with those of related proteins of other bacterial species.

Methods

Organisms and growth conditions. *M. hyopneumoniae* NCTC 10110 (J^T strain) (Rose *et al.*, 1979) was grown in liquid Friis medium (Friis, 1971) and on solid Friis medium supplemented with 0.8% Ionagar (Oxoid no. 2).

Plasmid pJFF631, which contains a 1.4 kbp chromosomal fragment of *M. hyopneumoniae* in the vector pBluescript II SK⁻ (Stratagene) expresses the full length *M. hyopneumoniae* P36 protein (Strasser *et al.*, 1991). Transcription of the P36 gene occurs from the inducible *lac* promoter of the vector; P36 is translated from the normal ribosome binding site of the gene (Strasser *et al.*, 1991). The 1.4 kbp *M. hyopneumoniae* DNA insert of pJFF631 was subcloned with suitable restriction enzymes into pBluescript II SK⁻ to generate clones for sequencing. *E. coli* XL1-blue *endA hsdR17 supE44* lambda⁻ *recA1* Δ(*proAB-lac*) [*F'* *proAB lacP*ΔM15 Tn10 (Tc^r)] (Bullock *et al.*, 1987) containing different subclones of the P36 gene were grown in LB broth (Sambrook *et al.*, 1989) supplemented with 25 µg ampicillin ml⁻¹. Induction of the *lac* promoter was obtained by adding 0.1 mM (final concentration) isopropyl-β-D-thiogalactoside (IPTG) to cultures at an OD₆₅₀ of 0.2 and subsequent growth to an OD₆₅₀ of 0.7. *Bacillus stearothermophilus* NCTC 10339 (NCIB 8923^T) was cultured on blood agar plates at 50 °C. The cells were collected from the surface by resuspension in 0.9% NaCl.

Growth and metabolism inhibition tests. The growth inhibition test was performed essentially as described by Clyde (1983) on solid Friis medium. After 7 d of incubation the distance between the antiserum disk and the nearest colonies was measured. Zones of inhibition larger than 1.5 mm were considered significant.

The metabolism inhibition test was performed according to Taylor-Robinson (1983). *M. hyopneumoniae* cells (10³ colour-changing units per well) were incubated in 200 µl Friis medium supplemented with test serum at various dilutions. After 3 d at 37 °C the highest dilution without colour change was determined and compared to wells containing control sera.

Immunoblot analysis. SDS-PAGE, transfer of proteins to nitrocellulose filters and immunological detection of proteins using polyclonal, monospecific anti-P36 antibodies (Stipkovits *et al.*, 1991) were performed using standard protocols (Sambrook *et al.*, 1989).

LDH activity. Cell extracts were obtained by sonication with a Branson sonifier and subsequent centrifugation for 20 min at 20000 g and 4 °C. LDH activity was measured with a commercial test (Merck 3349). Cell extracts (10 µl) containing 0.5 to 7 µg protein were mixed with 500 µl reaction solution (0.6 mM-sodium pyruvate; 0.18 mM-NADH; 50.0 mM-phosphate buffer pH 7.5) and the decrease in NADH was followed at room temperature by measuring the change in OD₃₄₀.

Protein concentration was determined by the Bio-Rad protein assay. Enzyme activities are given in units (mg protein)⁻¹ (1 U = 1 µmol NAD produced min⁻¹).

DNA and protein sequence analysis. The dideoxy chain-termination method (Sanger *et al.*, 1977) was performed using [³⁵S]dATP and T₇ DNA polymerase with Sequenase sequencing kit (United States

Biochemical) for the direct sequencing of double-stranded plasmid DNA (Zhang *et al.*, 1988). T₃ primer 5'-AATTAACCCTCACTAAAGGG-3', and T₇ primer 5'-TAATACGACTCACTATAGGG-3' were used, in order to ensure that both strands were completely sequenced.

The computer programs PC/Gene (IntelliGenetics, University of Geneva) and the GCG sequence analysis software package (University of Wisconsin, Biotechnology Center) were used for assembly of the DNA sequence and analysis of DNA and protein sequences as well as for comparison of the sequences with the Swiss-Prot and the EMBL data banks.

Sequencing of the 12 N-terminal amino-acid residues of purified recombinant P36 was achieved with a gas-phase sequencer (Applied Biosystems 477 A). For this procedure, 200 pmol protein P36 were attached to ProBlot membrane (Applied Biosystems).

Results

Sequence analysis of the gene encoding protein P36

The DNA insert of plasmid pJFF631 specifying P36 (Strasser *et al.*, 1991) was sequenced by subcloning the restriction enzyme fragments (Fig. 1) into vector pBluescript II SK⁻. The 1394 bp DNA sequence is shown in Fig. 2. It contains two open reading frames (ORF) in the direction of transcription of P36 (Strasser *et al.*, 1991), one encoding a complete protein of 315 amino acids (ORF1), and a second (ORF2) encoding a 49 amino acid C-terminal polypeptide fragment. ORF1 encodes a polypeptide with a computed molecular mass of 34.2 kDa, pI = 8.12, and a charge of 1.11 at pH 7.0, which represents the P36 protein. It is preceded by a consensus sequence for a putative ribosome binding site (Shine-Dalgarno sequence) (Shine & Dalgarno, 1974) 11 bp upstream from the ATG initiation codon. To confirm the identity of the translated polypeptide of ORF1 with P36, protein P36 was expressed from plasmid pJFF631 by induction with IPTG in *E. coli* strain XL1-blue and purified from a polyacrylamide gel after electrophoresis for amino acid sequence analysis. The sequence of the first 12 amino acid residues of protein P36 were identical to the first 12 amino acid residues deduced from ORF1 (Fig. 2) confirming that ORF1 encodes P36. The G+C content of ORF1 (34.7%) is slightly higher than that of the complete chromosome which is 33% (Stemke *et al.*, 1985). ORF1 stops with codon UAA and does not contain UGA codons in the reading frame [specifying tryptophan in mycoplasmas, (Yamao *et al.*, 1985) but which is a translation stop signal (opal) in *E. coli*]. Upstream of ORF1, three consensus sequences for -10 boxes of transcription promoters are found preceded by A+T-rich segments. However, no consensus sequence for a -35 box is present. Both at the end of ORF1 and of ORF2, sequences that can encode mRNA structures very similar to *rho*-independent transcription stop signals of procaryotes were found (Fig. 2). The calculated stabilities of these RNA secondary structures have ΔG

Fig. 1 Restriction endonuclease map of the 1.4 kbp *M. hyopneumoniae* DNA insert in vector pJFF631. Filled box, *ldh* gene (P36); open box, C-terminus of ORF2; arrows, direction of transcription.

values of $-58.2 \text{ kJ mol}^{-1}$ for the 'hairpin' preceding the -10 boxes and $-71.6 \text{ kJ mol}^{-1}$ for the 'hairpin' downstream of ORF1 as calculated by the method of Tinoco *et al.* (1973).

Computer-assisted searches for homologous proteins using the Swiss-Prot and EMBL databases showed significant sequence identities and similarities between protein P36 and all known L-lactate dehydrogenases (LDH) (EC 1.1.1.27) from various species (Table 1) indicating that P36 is the LDH of *Mycoplasma hyopneumoniae*. In particular we have found 70% identity of amino acid residues of P36 to the sites involved in enzymic activity of LDH from prokaryotes (Hensel *et al.*, 1983).

Biochemical and immunological properties of *M. hyopneumoniae* LDH

To confirm protein P36 as the L-lactate dehydrogenase of *M. hyopneumoniae*, LDH activity of the lysates from *E. coli* strains containing the cloned P36 gene or the vector alone were made using a commercial test measuring oxidation of NADH to NAD^+ in the enzymic reduction of pyruvate to L-lactate. *E. coli* strain XL1-blue containing the pBluescript II SK⁻ vector alone had an LDH activity of $0.05 \pm 0.03 \text{ U (mg protein)}^{-1}$ independent of whether IPTG, the inducing agent of the *lac* promoter on the vector, was present in the culture or not. The same *E. coli* host strain containing plasmid pJFF631 (encoding P36) had a LDH activity of $0.38 \pm 0.05 \text{ U (mg protein)}^{-1}$ without induction, and $7.1 \pm 0.3 \text{ U (mg protein)}^{-1}$ after induction of the vector promoter with IPTG, which represents a 120-fold increase compared to the strain with the vector alone and which corresponds to the strong increase of the 36 kDa protein seen on SDS gels after IPTG induction. These results confirm that *M. hyopneumoniae* protein P36 is L-lactate dehydrogenase (LDH) and its corresponding gene is therefore termed *ldh*.

Protein P36 (LDH) was shown to be immunologically highly specific for the species *M. hyopneumoniae* and showed no serological cross-reactions with *Mycoplasma*

flocculare or other closely related mycoplasmas (Stipkovits *et al.*, 1991) which is surprising for a common (housekeeping) enzyme. In order to verify the species specificity of *M. hyopneumoniae* LDH, we used the immunoblot technique to analyse total proteins of *Bacillus stearothermophilus* (which is known to produce LDH with sequence similarity to LDH of *M. hyopneumoniae*) (Table 1), pure LDH type H from pig, total proteins of *M. hyopneumoniae* and recombinant P36 from *E. coli* containing plasmid pJFF631 with polyclonal anti-P36 antibodies (data not shown). On the immunoblots, a 36 kDa band appeared in *M. hyopneumoniae* and *E. coli* containing pJFF631; whereas no reaction was found with *E. coli* containing the vector alone, *Bacillus stearothermophilus*, or with pure LDH type H from pig.

To investigate whether antibodies directed against LDH can inhibit growth of *M. hyopneumoniae*, growth and metabolism inhibition tests of *M. hyopneumoniae* were performed. Polyclonal anti-LDH antibodies raised in rabbits did not inhibit growth and metabolic fermentation activity of *M. hyopneumoniae* in culture medium, while rabbit antibodies made against whole cells of *M. hyopneumoniae* showed inhibitory effects in both tests, indicating that anti-LDH antibodies cannot stop or reduce growth of *M. hyopneumoniae*.

Discussion

DNA sequence analysis of the gene encoding the early and strongly immunogenic protein P36 of *M. hyopneumoniae* revealed that this polypeptide is an L-lactate dehydrogenase (LDH). Amino acid sequence comparisons revealed that the most closely related LDH are those from the Bacilli (Table 1) known to be phylogenetically related to mycoplasmas (Weisburg *et al.*, 1989). Bacterial LDH, however, show distinct differences even between closely related species in contrast to LDH from vertebrates that are closely related (Table 1). We have analysed the amino acid sequence of the hexapeptides of the major putative immunogenic epitope of bacterial LDH. The results (Table 1) show that these domains, in contrast to the domains of the active centres (Hensel *et al.*, 1983), strongly differ among different bacterial species even when they are closely related. This supports earlier observations which showed that *M. hyopneumoniae* LDH, originally termed P36, is highly species-specific in its immunogenic reaction and can be used for immunological species identification (Stipkovits *et al.*, 1991). At the DNA sequence level, however, differences are less pronounced since cross-hybridization of the *M. hyopneumoniae* *ldh* gene could be observed with a fragment of chromosomal DNA supposed to contain the *ldh* gene of the related porcine *Mycoplasma flocculare*

20 40 60 80
HpaI | | | |
 GTTAACACGCCACCAGGAATTGATCTAAAAAATTAGAACTAAATAATGTCGATGGTTATGCTCTGGAGCTTATATTCT
 ----- ORF2 -----
 100 120 140 160 180 200
 | | *DraI* | | *SnaBI* | |
 CATTATTATTTCTCACTTGTTTCTCAATTGGTTTATGCCCTTTTAAAGGAACCAATCCAGAATATAAAAAATTATAAAAATACGTAGTTTTCTGAAATTGAACGAATCAAAAAAT
 ----- ORF2 ----- ***
 220 240 260 280 300 320
 | | <-10> | <-10> | <-10> | | < RBS > |
 AAAAAAATTTTCATTTTGATTCGTTTTAATTAATAAGAAAAAATTATCATTACTTGTTTTAATTAATAAAAAATTAATCTATCAATGAAAAAATTAACAAAAAGGAGAAATC
 → ←
 340 360 380 400 420 440
 | | | | | |
 AAACCTATGAAACCTATTAAATAGCTCTAATTGGTGTGGAAATGTCGGAAATTCCTTCCTTTATGCAGCAATGAATCAAGGACTTGCATCCGAGTATGGAATTATTGATTAATCCT
METLysProIleLysIleAlaLeuIleGlyAlaGlyAsnValGlyAsnSerPheLeuTyrAlaAlaMetAsnGlnGlyLeuAlaSerGluTyrGlyIleIleAspIleAsnPro
 460 480 500 520 540 560
 | | | | *BglII* | |
 GATTTTGCCGATGGTAATGCTTTTGATTTGAAGATGCCTCAGCTTCTTTCCTTTTCCGATTAGTGCTCCGTTATGAATATAAAGATCTAAAGATGCTGATTTTGTGAATTACA
 AspPheAlaAspGlyAsnAlaPheAspPheGluAspAlaSerAlaSerLeuProPheProIleSerValSerArgTyrGluTyrLysAspLeuLysAspAlaAspPheIleValIleThr
 580 600 620 640 660 680
 | | | | | |
 GCGGGAAGACCACAAAAACCGGGTGAACCTCGGCTTGAATTAGTAGCTGATAACATCCGAATTATCCGGAAATGCACTAAAAGTCAAAGAAAGTGCTTTAGTGAATAAGTATTATT
 AlaGlyArgProGlnLysProGlyGluThrArgLeuGluLeuValAlaAspAsnIleArgIleIleArgGluIleAlaLeuLysValLysGluSerGlyPheSerGlyIleSerIleIle
 700 720 740 760 780 800
 | | *NsiI* | | | |
 GTTGCTAATCCTGTTGATATAATTACAAGGGCTTACCGGGATGCATCTGGATTTTCCGATCAAAAGTTATCGGTAGTGAACCTGTTTATGATACAGCAAGGCTTCAATTTGCAATCGCA
 ValAlaAsnProValAspIleIleThrArgAlaTyrArgAspAlaSerGlyPheSerAspGlnLysValIleGlySerGlyThrValLeuAspThrAlaArgLeuGlnPheAlaIleAla
 820 840 860 880 900 920
 | | *StuI* | | | |
 AAAAGAGCAAAGTATCGCCTAATTCGGTTCAGGCTACGTGATGGGTGAACATGGTGATTCATCTTTTGTGCTTATTCAAATATTAATGCGGGTGAATGTTCTGTGCTTATTCT
 LysArgAlaLysValSerProAsnSerValGlnAlaTyrValMetGlyGluHisGlyAspSerSerPheValAlaTyrSerAsnIleLysIleAlaGlyGluCysPheCysAlaTyrSer
 940 960 980 1000 1020 1040
 | | | | | |
 AAACCTAACCGAATTGATAGCTCAAATTACGAAAAAGAACTTGAATATCCAGTTTCTCGCCGGCTTATGAAATTATTAAATCGTAAAGGGCAACATTTTATGGAATTGGTGCAGCTATT
 LysLeuThrGlyIleAspSerSerAsnTyrGluLysGluLeuGluTyrProValSerArgArgAlaTyrGluIleIleAsnArgLysArgAlaThrPheTyrGlyIleGlyAlaAlaIle
 1060 1080 1100 1120 1140 1160
 | | | | | |
 GCCAAAATAGTTTCTAATATTATCAAGATACAAAAATATTATGATTGCCGGAGCAAAATTCAGGAGAGAAATACGGATTTACGGAGTAAATATCGGAGTTCAGTTGTTTATGGAGCA
 AlaLysIleValSerAsnIleIleLysAspThrLysAsnIleMetIleAlaGlyAlaAsnLeuArgGlyGluTyrGlyPheHisGlyValAsnIleGlyValProValValLeuGlyAla
 1180 1200 1220 1240 1260 1280
 | | | | | |
 AACGGAATTGAAAAATATTGAGATTAGTCTTAATGATAAGAAAAAGAAAAATTTGCCAAATCAGTTGCAATCATTGATAAAATTTATCAGGATGCAATAAAAATTTAATTTTTT
 AsnGlyIleGluLysIleIleGluIleSerLeuAsnAspLysGluLysGluLysPheAlaLysSerValAlaIleIleAspLysIleTyrGlnAspAlaIleLysAsnIle***
 1300 1320 1340 1360 1380 1394
DraI | | *DraI* | | *HindIII*
 AGGAAAAACAGTTAAAAATCTCTATAAATTTAATTTTTATCTATGATTTTAAAAAGTACTATAAATTTATAGTACTTTTTTAATTTTTAGCAAAAAATTTGAAGCTT
 → ←

Table 1. Sequence similarities and major immunogenic epitopes of L-lactate dehydrogenase from various species

Numbers 1–10 in the top line indicate the same species as the bold numbers on the vertical line right. The values are given in % identity (upper figures) or % similarity (lower figures) of the protein sequence calculated according to Needleman & Wunsch (1970). The hexapeptide of the putative major immunogenic epitope (shown in the right column in the amino acid one letter code) was computed from the amino acid sequences using the method of Hopp & Woods (1981).

1	2	3	4	5	6	7	8	9	10	No.	Species (Ref.)	Epitope
41	39	39	41	33	34	28	28	24	23	0	<i>M. hyopneumoniae</i>	DKEKEK
55	54	53	54	53	52	44	44	38	38	1	<i>B. stearothermophilus</i> (Wirz <i>et al.</i> , 1983)	DDEKNR
	90	67	48	29	30	31	31	34	34		<i>B. caldopenax</i> (Züllig <i>et al.</i> , 1987)	DEEEKK
	93	78	59	42	43	48	48	52	52	2	<i>B. subtilis</i> (Hediger <i>et al.</i> , 1986)	EKEKEQ
		67	48	28	29	31	32	34	35		<i>L. casei</i> (Hensel <i>et al.</i> , 1983)	DHEEES
		78	59	41	41	50	50	53	53	3	<i>T. aquaticus</i> (Ono <i>et al.</i> , 1990)	NEEERQ
			46	26	27	33	33	35	33		<i>T. caldophilus</i> (Kunai <i>et al.</i> , 1986)	SPEERE
			58	42	42	50	49	51	51	4	Human H (Sakai <i>et al.</i> , 1987)	KLKDDE*
				40	39	38	37	38	40		Pig H (Kiltz <i>et al.</i> , 1977)	KLKDDE*
				60	59	57	56	57	58	5	Human M (Tsujibo <i>et al.</i> , 1985)	DKDKKEQ*
					87	28	28	33	33		Pig M (Kiltz <i>et al.</i> , 1977)	TDADKE*
					91	48	47	52	51	6		
						29	28	33	34			
						47	47	51	51	7		
							96	76	76			
							98	86	85	8		
								75	75			
								86	85	9		
								92	96			
								100	100	10		

* Epitope not located congruently with those of prokaryotic LDHs.

(J. Frey, unpublished observations). The *ldh* gene is therefore not suitable as a gene probe for detection of *M. hyopneumoniae*.

LDH activity seems to be common to mycoplasmas including *M. hyopneumoniae* (Manolukas *et al.*, 1988) which produces in culture conditions a LDH activity of 217 ± 2 U (mg protein)⁻¹. At first glance, it seems surprising that *M. hyopneumoniae* LDH is an early and strongly immunogenic protein in naturally or experimentally infected pigs. LDH is not generally known to be directly involved in pathogenicity mechanisms. Its function is the enzymic conversion of pyruvate to lactate which is the major step for the regeneration of NAD (Garvie, 1980). In *E. coli* and other enteric bacteria LDH is induced at low pH (Slonczewski *et al.*, 1987; Fairouz *et al.*, 1989). This regulation seems to be very similar to the regulation of expression of stress proteins which are thought to be strongly induced in pathogenic bacteria at certain critical steps of infection (Slonczewski *et al.*, 1987; Foster, 1991). Although LDH is not known to be a bacterial stress protein, one can speculate that the

presence of relatively large amounts of this protein might be crucial for *M. hyopneumoniae* for a specific step during infection. To achieve this, the *ldh* gene might be induced under certain conditions. The relatively strong immunogenic reaction of this protein in the infected animal could be a response to such an induction of LDH in *M. hyopneumoniae* at an early stage of infection. The LDH of *M. hyopneumoniae* formerly known as P36, has been shown to be located intracellularly (Strasser *et al.*, 1991), as is LDH of other bacterial species. It is therefore expected that antibodies raised against LDH do not inhibit growth or metabolic activity of *M. hyopneumoniae* as was confirmed experimentally. The protein therefore is most unlikely to induce protective immunity. It is, however, an interesting tool for further studies of bacteria–host interactions and is expected to be useful for serological analysis of early stages of *M. hyopneumoniae* infection.

The *ldh* gene is the first gene encoding a protein with a known function that has been sequenced in *M. hyopneumoniae*. As expected from previous results, *M.*

Fig. 2. DNA sequence of the *ldh* gene of *M. hyopneumoniae* and the deduced amino acid sequence of the encoded protein. The restriction enzyme sites used for subcloning are given. Possible –10 regions are indicated by < –10 >, and the ribosome binding site by < RBS >. The putative transcription termination signals are shown by arrows. Symbols: ---, open reading frame 2; ***, translation stop. The 12 amino acid residues sequenced from protein P36 at the N-terminus are underlined.

hyopneumoniae LDH is expressed as a full length polypeptide in *E. coli* hosts confirming that no internal UGA codons which are known in mycoplasmas to encode *trp* (Yamao *et al.*, 1985) are used. In addition, the coding sequence of *ldh* is preceded by a RBS which shows good complementarity to the 3' end of the 16S RNA from *E. coli* (Shine & Dalgarno, 1974) and from *M. hyopneumoniae* (Taschke *et al.*, 1987), and which seems to be conserved not only in mycoplasmas (Klinkert *et al.*, 1986) but in all bacterial species. The putative transcription promoter of the *M. hyopneumoniae* *ldh* gene, however, seems to be different from promoters found in other bacteria. The sequence data revealed three possible –10 consensus sequences (Pribnow box) which are not preceded by the typical –35 sequences which are commonly found in bacterial promoters (Rosenberg & Court, 1979). Characteristic for the region upstream of the –10 boxes is an A+T-rich stretch. A similar finding has been made for the promoter of an unknown *M. hyopneumoniae* gene (Klinkert *et al.*, 1986) and for the promoter of the operon encoding the 16S and 23S rRNA genes of *M. hyopneumoniae* (Taschke & Herrmann, 1986). This new type of promoter, however, is not common to mycoplasmas or other bacteria and has been found thus far only in *M. hyopneumoniae*. It remains still to prove whether the A+T-rich sequences replace the –35 region in *M. hyopneumoniae* promoters. The DNA sequence data currently available are limited and do not yet allow description of a consensus sequence for *M. hyopneumoniae* specific promoters. The sequence data in Fig. 2 show that the *ldh* gene and its putative promoter sequences are flanked by inverted repeat sequences followed by T residues. These 'hairpin' sequences can encode mRNA structures very similar to *rho*-independent transcription stop signals of other prokaryotes and suggest that the *ldh* gene in *M. hyopneumoniae* is encoded on a monocistronic operon. In *E. coli*, one of these putative transcription stop signals, located upstream of *ldh* is not recognized as such since the *ldh* gene on plasmid pJFF631 is transcribed from the vector's *lac* promoter (Strasser *et al.*, 1991) which is located upstream from the 'hairpin' sequences found to precede the *ldh* gene.

In summary, we have shown that P36 of *M. hyopneumoniae* is an L-lactate dehydrogenase. The role of LDH in pathogenicity of *M. hyopneumoniae* is not known, but the strong immunogenic character of this 'housekeeping' enzyme indicates that LDH might be needed by *M. hyopneumoniae* to achieve certain steps in infection.

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