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COMPARISON OF THE AMINO ACID SEQUENCE OF L-MANDELATE DEHYDROGENASE FROM RHODOTORULA GRAMINIS WITH OTHER L-2-HYDROXYACID DEHYDROGENASE ENZYME AND ITS PRIMARY STRUCTURE PREDICTION

ROSLI MD. ILLIAS[1] , GRAEME A. REID[2], & NADZARAH A. WAHAB[3]

Abstract. A comparison of the primary structure for L-mandelate dehydrogenase (L-MDH) from *Rhodotorula graminis* with other proteins from the protein databank suggests that there is similarity between this protein and L-2-hydroxyacid dehydrogenase enzymes. *R. graminis* LMDH exhibits 26–42% identity to L-lactate dehydrogenase from *Saccharomyces cerevisiae*, L-lactate dehydrogenase from *Hansenula anomala*, glycolate oxidase from spinach, L-lactate dehydrogenase from *Escherichia coli*, L-mandelate dehydrogenase from *Pseudomonas putida* and lactate-2-monooxygenase from *Mycobacterium smegmatis*. Structurally conserved amino acids are predicted from LMDH sequences corresponding to important regions of the cytochrome and FMN-binding domain defined from the known three-dimensional structure of the L-lactate dehydrogenase from *Saccharomyces cerevisiae*.

Key words: L-MDH, *Rhodotorula graminis*, L-mandelate dehydrogenase, amino acid, flavocytochrome b_2

Abstrak. Perbandingan struktur primer L(+)-mandalate dehydrogenase (L-MDH) daripada yis *Rhodotorula graminis* dengan protein lain di dalam bank data protein menunjukkan persamaan di antara protein ini dengan kumpulan enzim L-2-hidroksiasid dehidrogenase. LMDH daripada *R. graminis* mempamerkan kesamaan antara 26–42% kepada L-lactate dehidrogenase daripada *Saccharomyces cerevisiae*, L-lactate dehidrogenase daripada *Hansenula anomala*, glikolat oksida daripada bayam, L-laktat dehidrogenase daripada *Escherichia coli*, LMDH daripada *Pseudomonas putida* dan laktat-2-monooksigenase daripada *Mycobakterium smegmatis*. Asid amino yang penting secara strukturnya bagi LMDH diramalkan secara perbandingan dengan bahagian penting domain sitokrom dan domain perlekatan FMN yang diperolehi daripada struktur tiga dimensi L-laktat dehidrogenase daripada *Saccharomyces cerevisiae*.

Kata kunci: L-MDH, *Rhodotorula gramisis*, L(+)-mandalate dehydrogenase, asid amino, flavocytochrome b_2

[1, 3] Department of Bioprocess Engineering, Faculty of Chemical Engineering and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor Darul Takzim, Malaysia.
[2] Institute of Cell and Molecular Biology and Department of Chemistry, University of Edinburg, Scotland, UK.

1.0 INTRODUCTION

Flavocytochrome b_2 catalyses the oxidation of L-lactate to pyruvate with subsequent transfer of electrons to cytochrome C. This enzyme is a soluble component of the intermembrane space of yeast mitochondria and enables yeast to use L-lactate as a carbon and energy source [1]. L-(+)-mandelate dehydrogenase (LMDH) from the yeast *Rhodotorula graminis* catalyses the oxidation of L(+)-mandalate to phenylglyoxylate [2]. The other type of mandelate dehydrogenase in *R. graminis* is D-mandelate dehydrogenase which belongs to the family of D-isomer specific 2-hydroxyacid dehydrogenase. The L(+)-xmandelate dehydrogenase from *R. graminis* is a totally different enzyme from the D(−)-mandelate dehydrogenase in terms of its origin and substrate specificity. There are many similarities between L-mandelate dehydrogenase from *R. graminis* and flavocytochrome b_2 (L-lactate dehydrogenase) from *Saccharomyces cerevisiae*. The crystal structure of flavocytochrome b_2 from *S. cerevisiae* shows that the enzyme is a tetramer of identical subunit, each consisting of two distinct domain [3]. The N-terminal cythochrome domain is related to cytochrome b_5 whereas the FMN-containing C-terminal domain has a separate evolutionary history with relatives found in plants, animals and bacteria. This substrate differs in size and chemical nature. In this study, computer analysis of the mature amino acid sequence from *R. graminis* is carried-out. Comparison of the L-MDH amino acids with other related proteins is discussed here and the prediction of its function based on the 3-D structure of L-LDH from *S. cerevisiae* has also been made.

2.0 METHODOLOGY

Amino acid sequences of L-mandelate dehydrogenase from *Rhodotorula graminis* was compared with the protein sequence database of the SWISSPROT using the program FASTA and PILEUP from the University of Wisconsin Genetics Computer Group (UWGCG) package. The hydropathic profile of LMDH from *R. graminis* was determined by means of Kyte-Doolitle method [4]. The plots obtained characterize its hydrophobic character, which may be used in predicting the proteins membrane-spanning domains, its potential antigenic sites and exposed region on the surface of the protein.

3.0 RESULTS AND DISCUSSION

3.1 Amino Acid Sequence Comparisons

The multiple alignment program PILEUP generates a dendogram representing the relatedness of protein in a family. In this dendrogram (Figure 1) it can be seen that L(+)-mandelate dehydrogenase is very closely related to L(+)-lactate dehydrogenases from *Saccharomyces cerevisiae* and *Hansenula anomala*, which are flavocytochromes b_2. L(+)-mandelate dehydrogenase from *R. graminis* represents a new type of micro-

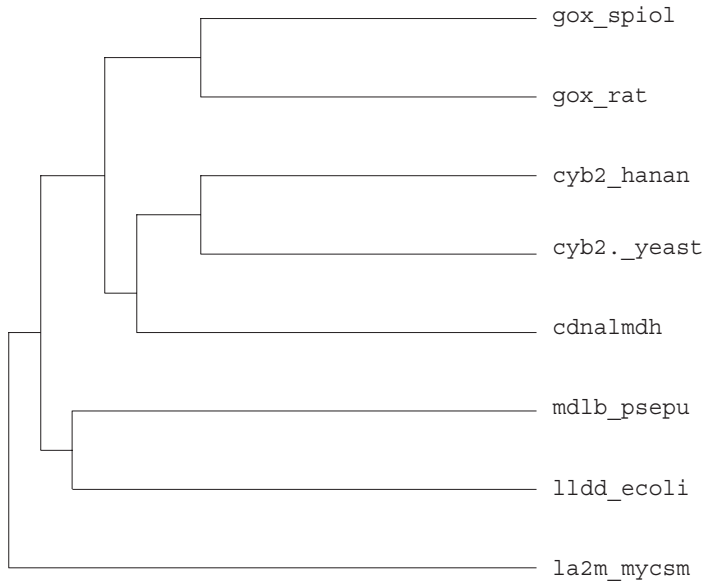

Figure 1 Family tree of the FMN-dependent a-hydroxy acid-oxidizing enzymes. The dendrogram shows the output of the UWGCG programme PILEUP.The deadrogram indicates a clustering order from a cluster of sequences based on the similarity. gox_spiol. glycolate oxidase from spinach, gox_rat: rat kidney hydroxy-acid oxidase, cyb2_hanan: L(+)-lactate dehydrogenase from *Hansenula anomala*, cyh2_yeast: L(+)-lactate dchdrogenase from *Saccharomyces cerevisiae*, ednalmdh: L(+)-mandelate dehydrogenase from *Rhodotorula graminis*, mdlb_psepu. mandelate clehydrogenase from *Pseudomonas putida*, *l lldd*_ecoli: lactate dehydrogenase from *E. coli*, 1a2m_myesm: lactate mono-oxygenase from *Mycobacterium smegmatis*. The diagram shows that L(+)-mandelate dehydrogenase is more closely related to L(+)-lactate dehydrogenase from *S. cerevisiae* and *H. anomala* which is a flavocytochrome b_2

bial mandelate dehydrogenase which is a flavocytochrome b_2. Since LMDH belongs to the family of flavocytochromes b_2, it is predicted that it will have a similar structure to flavocytochrome b_2 from *S. cerevisiae* and *H. anomala*.

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 gox_spiol   .......... .......... .......... .......... ..........
   gox_rat   .......... .......... .......... .......... ..........
cyb2_hanan   .......... .......... .......... .DVPHWKDIE LTPEIVSQHN  19
cyb2_yeast   .......... .......... .......... EPKLDMNKQK ISPAEVAKHN  20
  cdnalmdh   .......... .......... ......DAQL PVKQRGRARS ISAAEVAKHN  24
mdlb_psepu   .......... .......... .......... .......... ..........
lldd_ecoli   .......... .......... .......... .......... ..........
la2m_mycsm   .......... .......... .......... .......... ..........
                                               *  **
                                    f                        f
 gox_spiol   .......... .......... .......... .......... ..........
   gox_rat   .......... .......... .......... .......... ..........
cyb2_hanan   KKDDLWVVLN GQVYDLTDFL PNHPGGQKII IRYAGKDATK IFVPIHPPDT  69
cyb2_yeast   KPDDCWVVIN GYVYDLTRFL PNHPGGQDVI KFNAGKDVTA IFEPLHAPNV  70
  cdnalmdh   SRDSMWVCID DEVWDITNFV ELHPGGAKVL EQNAGKDVTK VFKSIHPPKT  74
mdlb_psepu   .......... .......... .......... .......... ..........
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lldd_ecoli   .......... .......... .......... .......... ..........
la2m_mycsm   .......... .......... .......... .......... ..........
               *  **      * * * *    ****        **** *       * *

 gox_spiol   .......... .......... .......... .......... .........M  1
   gox_rat   .......... .......... .......... .......... ..........
cyb2_hanan   IEKFIPPEKH LGPLVGEFEQ E......EEE LSDEEIDRLE RIER.KPPLS  112
cyb2_yeast   IDKYIAPEKK LGPLQGSMPP ELVCPPYAPG ETKEDIARKE QLKSLLPPLD  120
  cdnalmdh   LEKFLTDDNF VGRIDVDEVT KIGGGKNAED L......RIE QARKELRNVE  118
mdlb_psepu   .......... .......... .......... .......... .......MSQ  3
lldd_ecoli   .......... .......... .......... .......... .........M  1
la2m_mycsm   .......... .......... .........S NWGDYENEIY GQGLVGVAPT  21

                                      f
 gox_spiol   EITNVNEYEA IAKQKLPKMV YDYYASGAED QWTLAENRNA FSRILFRPRI  51
   gox_rat   PLVCLADFKA HAQKQLSKTS WDFIEGEADD GITYSENIAA FKRIRLRPRY  50
cyb2_hanan   QMINLHDFET IARQILPPPA LAYYCSAADD EVTLRENHNA YHRIFFNPKI  162
cyb2_yeast   NIINLYDFEY LASQTLTKQA WAYYSSGAND EVTHRENHNA YHRIFFKPKI  170
  cdnalmdh   TVVCLDEFEE ISQKILSEMA MAYYGTGAET EQTLRDEREA WQRVRFRPRV  168
mdlb_psepu   NLFNVEDYRK LRQKRLPKMV YDYLEGGAED EYGVKHNRDV FQQWRFKPKR  53
lldd_ecoli   IISAASDYRA AAQRILPPFL FHYMDGGAYS EYTLRRNVED LSEVALRQRI  51
la2m_mycsm   LPMSYADWEA HAQQALPPGV LSYVAGGSGD EHTQRANVEA FKHWGLMPRM  71
                         *       +   ++ + +     +           +
                                       f
 gox_spiol   LIDVTNIDMT TTILGFKISM PIMIAPTAMQ KMAHP.EGEY ATARAASAA.  99
   gox_rat   LRDMSKVDTR TTIQGQEISA PICISPTAFH SIAWP.DGEK STARAAQEA.  98
cyb2_hanan   LIDVKDVDIS TEFFGEKTSA PFYISATALA KLGHP.EGEV AIAKGAGRE.  210
cyb2_yeast   LVDVRKVDIS TDMLGSHVDV PFYVSATALC KLGNPLEGEK DVARGCGQGV  220
  cdnalmdh   LRKMRHIDTN TTFLGIPTPL PIFVAPAGLA RLGHP.DGEQ NIVRGVAKH.  216
mdlb_psepu   LVDVSRRSLQ AEVLGKRQSM PLLIGPTGLN GALWP.KGDL ALARAATKA.  101
lldd_ecoli   LKNMSDLSLE TTLFNEKLSM PVALAPVGLC GMYAR.RGEV QAAKAADAH.  99
la2m_mycsm   LMAATERDLS VELWGKTWAA PMFFAPIGVI ALC.AQDGHG DAASAQASAR  120
             *      +       +      *             +  *+
                    f                      f
 gox_spiol   .GTIMTLSSW ATSSVEEVAS TGP.G..IRF FQLYVYKDRN VVAQLVRRAE  145
   gox_rat   .NICYVISSY ASYSLEDIVA AAPEG..FRW FQLYMKSDWD FNKQMVQRAE  145
cyb2_hanan   .DVVQMISTL ASCSFDEIAD ARIPGQQ.QW YQLYVNADRS ITEKAVRHAE  258
cyb2_yeast   TKVPQMISTL ASCSPEEIIE AAPSDKQIQW YQLYVNSDRK ITDDLVKNVE  270
  cdnalmdh   .DILQVVSSG ASCSIDEIFE VKEPDQNLAW .QFYVHSDKK IAEEKLKRAL  264
mdlb_psepu   .GIPFVLSTA SNMSIEDLAR QCDGDL...W FQLYVIH.RE IAQGMVLKAL  146
lldd_ecoli   .GIPFTLSTV SVCPIEEVAP AIKRPM...W FQLYVLRDRG FMRNALERAK  145
la2m_mycsm   TGVPYITSTL AVSSLEDI.. RKHAGDTPAY FQLYYPEDRD LAESFIRRAE  168
                    *   +  + +              +  *+*+  *+          +
                        f
 gox_spiol   RAGFKAIALT VDTPRLGRRE ADIKNRFVL. ...PPFLTLK N.........  182
   gox_rat   ALGFKALVIT IDTPVLGNRR RDKRNQLNL. ...EANILLK D.........  182
cyb2_hanan   ERGMKGLFIT VDAPSLGRRE KDMKMK.... .FEADSDVQG ..........  293
cyb2_yeast   KLGVKALFVT VDAPSLGQRE KDMKLK.... .FSNTKAGPK A.........  306
  cdnalmdh   ALGAKAIFVT VDVPVLGKRE RDLKLKARSQ NYEHPIAAQW K.........  305
mdlb_psepu   HTGYTTLVLT TDVAVNGYRE RDLHNRFKIP MSYSAKVVLD GCLHPRWSLD  196
lldd_ecoli   AAGCSTLVFT VDMPTPGARY RDAHSGMSGP NA.AMRRYLQ AVTHPQWAWD  194
la2m_mycsm   EAGYDGLVIT LDTWIFGWRP RDLTI..... ...SNFPFLR GLCLTNYVTD  210
               *   +  *  * +  * *   *

 gox_spiol   .........F EGIDLGKMDK ANDSGLSSYV AGQIDRSLSW KDVAWLQTIT  223
   gox_rat   .........L RAL...KEEK PTQSVPVSFP KA....SFCW NDLSLLQSIT  216
cyb2_hanan   .......... DDEDIDRSQG ASRALSSF.. ...IDPSLSW KDIAFIKSIT  398
cyb2_yeast   .........M KKTNVEESQG ASRALSKF.. ...IDPSLTW KDIEELKKKT  342
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  cdnalmdh  .........A AGSKVEET.I AKRGVSDIPD TAHIDANLNW DDIAWIKERA  345
mdlb_psepu  FVRHGMPQLA NFVS...SQT SSLEMQAALM SRQMDASFNW EALRWLRDL.  242
lldd_ecoli  VGLNGRPHDL GNISAYLGKP TGLEDYIGWL GNNFDPSISW KDLEWIRDF.  243
la2m_mycsm  PVFQKKFKAH SGVEAEGLRD NPR.LAADFW HGLFGHSVTW EDIDWVRSIT  259
                                                + +  *  +
                                              f  f
 gox_spiol  S.LPILVKGV ITAEDARLAV QHGAAGIIVS NHGARQLDYV PATIMALEEV  272
   gox_rat  R.LPIILKGI LTKEDAELAM KHNVQGIVVS NHGGRQLDEV SASIDALREV  265
cyb2_hanan  K.MPIVIKGV QRKEDVLLAA EHGLQGVVLS NHGGRQLDYT RAPVEVLAEV  377
cyb2_yeast  K.LPIVIKGV QRTEDVIKAA EIGVSGVVLS NHGGRQLDFS RAPIEVLAET  391
  cdnalmdh  PGVPIVIKGV GCVEDVELAK QYGADGVVLS THGARQLDGA RAPLDVLIEV  395
mdlb_psepu  WPHKLLVKGL LSAEDADRCI AEGADGVILS NHGGRQLDCA IS...PM...  286
lldd_ecoli  WDGPMVIKGI LDPEDARDAV RFGADGIVVS NHGGRQLDGV LSSARAL...  290
la2m_mycsm  K.MPVILKGI QHPDDARRAV DSGVDGIYCS NHGGRQANGG LPALDCLPEV  308
               +   **     +*       +  *   * +**+**++

 gox_spiol  VK.....AAQ GRIPVFLDGG VRRGTDVFKA LALGAAGVFI GRPVVFSLAA  317
   gox_rat  VA.....AVK GKIEVYMDGG VRTGTDVLKA LALGARCIFL GRPILWGLAC  310
cyb2_hanan  MPILKERGLD QKIDIFVDGG VRRGTDVLKA LCLGAKGVGL GRPFLYAMSS  427
cyb2_yeast  MPILEQRNLK DKLEVFVDGG VRRGTDVLKA LCLGAKGVGL GRPFLYANSC  441
  cdnalmdh  RR..KNPALL KEIEVYVDGQ ARRGTDVLKA LCLGARGVGF GRGFLYAQSA  443
mdlb_psepu  .EVLAQSVAK TGKPVLIDSG FRRGSDIVKA LALGAEAVLL GRATLYGLAA  335
lldd_ecoli  .PAIADAV.K GDIAILADSG IRNGLDVVRM IALGADTVLL GRAFLYALAT  338
la2m_mycsm  VK......AS GDTPVLFDSG IRTGADVVKA LAMGASAVGI GRPYAWGAAL  352
                       *++  * * *+ ++ + +**  +   **  +

 gox_spiol  EGEAGVKKVL QMMRDEFELT MALSGCRSLK EISRSHIAAD WDGPSSRAVA  367
   gox_rat  KGEDGVKEVL DILTAELHRC MTLSGCQSVA EISPDLIQFS RL........  352
cyb2_hanan  YGDKGVTKAI QLLKDEIEMN MRLLGVNKIE ELTPELLDTR SIHNRAVPVA  477
cyb2_yeast  YGRNGVEKAI EILRDEIEMS MRLLGVTSIA ELKPDLLDLS TLKARTVGVP  491
  cdnalmdh  YGADGVDKAI RILENEIQNA MRLLGANTLA DLKPEMVE.C SFPERWVPE.  491
mdlb_psepu  RGETGVDEVL TLLKADIDRT LAQIGCPDIT SLSPDYLQNE GVTNTAPVDH  385
lldd_ecoli  AGQAGVANLL NLIEKEMKVA MTLTGAKSIS EITQDSLVQG LGKELPAALA  388
la2m_mycsm  GGSKGIEHVA RSLLAEADLI MAVDGYRNLK ELTIDALRPT R.........  393
             *  *+          +     +   *       +

 gox_spiol  RL........ .......... ... 369
   gox_rat  .......... .......... ...
cyb2_hanan  KDYLYEQNYQ RMSGAEFRPG IED 500
cyb2_yeast  NDVLYNEVYE GPTLTEFEDA ... 511
  cdnalmdh  .......... .......... ...
mdlb_psepu  LIGKGTHA.. .......... ... 393
lldd_ecoli  PMAKGNAA.. .......... ... 396
la2m_mycsm  .......... .......... ...
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Figure 2 Conserved residues (identical in all sequences) are marked with an asterisk (*) and the semi invariant residues (allowing two mismatches) are marked with (+) below the alignment. Flavocytochrome b_2 from *Saccharomyces cerevisiae* hinge region and proteinase sensitive loop are in bold. Amino acids, which are known to be functionally important, are marked with f on top. The sequences are: gox_spiol: glycolate oxidase from spinach [5]; gox_rat: hydroxy-acid oxidase from rat [6]; cyb2_hanan: L(+)-lactate dehydrogenase from *H.anomala* [7]; cyb2_yeast: L(+)-lactate dehydrogenase from *S.cerevisiae* [8]; cdnalmdh: L-mandelate dehydrogenase from *R.graminis* [9]; mdlb_psepu:mandelate dehydrogenase from *P.putida* [10]; lldd_ecoli: lactate dehydrogenase from *E.coli* [11]; la2m_mycsm: lactate mono-oxygenase from *M.smegmatis* [12].

A computer search of the Swissprot protein sequence data bank with the program FASTA, using the L(+)-mandelate dehydrogenase as the query sequence indicates amino acid sequence similarity with other L-2-hydroxy acid dehydrogenases. Alignment with other protein sequences in the database using the PILEUP program (Figure 2) demonstrates that *Rhodotorula graminis* L(+)-mandelate dehydrogenase exhibits 26-42 % identity to each of: L(+)-lactate dehydrogenase from *Saccharomyces cerevisiae*, L(+)-lactate dehydrogenase from *Hansenula anomala*, glycolate oxidase from spinach, L-lactate dehydrogenase from *E. coli*, L(+)-mandelate dehydrogenase from *Pseudomonas putida* and lactate-2-monooxygenase from *Mycobacterium smegmatis*. All these enzymes are members of the family of FMN-dependent 2-hydroxyacid-oxidising enzymes [6].

Saccharomyces cerevisiae flavocytochrome b_2 L (+)-lactate dehydrogenase has been crystallized and its structure determined [3]. The flavocytochrome b_2 polypeptide consists of two different regions, which form the haem binding domain (cytochrome domain) and the flavin-binding domain (flavodehydrogenase domain). The cytochrome domain is located at the N-terminus of the flavocytochrome b_2 polypeptide chain from residue 1 to 99 [3]. Based on a comparison with the sequence of flavocytochrome b_2 from *S. cerevisiae*, the cytochrome domain of L (+)-mandelate dehydrogenase from *R. graminis* consists approximately of residues 1 to 103. There are 21 invariant residues conserved in this region (Figure 2). The amino acid sequence of the predicted cytochrome domain from L(+)-mandelate dehydrogenase from *Rhodotorula graminis* also shows extensive similarity with the sequence of bovine microsomal cytochrome b_5 [13] as other flavocytochrome b_2.

Two conserved histidine residue found in flavocytochrome b_2 from *S. cerevisiae* are also conserved in L-MDH from *R. graminis* (Figure 3). The two histidine residues are involved in the ligation of haem iron via their Ne atoms. Tyrosine 141 and

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                                                        *
cdnalm  DAQLPVKQRGRARSISAAEVAKHNSRDSMWVCIDDEVWDITNFVELHPGGAKVLEQNAGK    60
                           |::|||:::| |: :: :|:|:|:|:| ||||::|| ::||
cyb5_b  AEESSKAVKYYTLEEIQKHNNSKSTWLILHYKVYDLTKFLEEHPGGEEVLREQAGG         50

                  *
cdnalm  DVTKVFKSI.HPPKTLEKFLTDDNFVGRIDVDEVTKIGGGKNAEDLRIEQARKELRNVETV   120
        |:|: |::: |:    :: |::: ::| :: |: :||:
cyb5_b  DATENFEDVGHSTD..ARELSKTFIIGELHPDDRSKITKPSESIITTIDSNPSWWTNWLIP   110


cdnalm  VCLDEFEEISQKILSEMAMAYYGTGAETEQTLRDEREAWQRVRFRPRVLRKMRHIDTNTT    180

cyb5_b  AISALFVALIYHLYTSEN 130
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Figure 3 Sequence alignment of the first 120 residues of L(+)-mandelate dehydrogenase from *R. graminis* (cdnalm) with amino acid sequence from bovine microsomal cytochrome b_5 (cyb5_b) [14]. Asterisks mark the two histidine, which may be the ligands to the haem iron.

Lysine 290 (Figure 2) in *R. graminis* L-mandelate dehydrogenase are predicted to make hydrogen bonds to a haem propionate group. In L-mandelate dehydrogenase from *R. graminis*, Tyr 97 (in flavocytochrome b_2) which is hydrogen bonded to the other haem propionate group is replaced by asparagine. In flavocytochrome b_2 from *S. cerevisiae*, the cytochrome domain is connected to the flavodehydrogenase domain through a huge region from residue 92 to 103. In L-MDH this region could be from residue 96 to 105 predicted from amino acid sequence comparison with flavocytochrome b_2 from *S. cerevisiae* (Figure 2). This region is believed to be involved in facilitating intramolecular electron transfer. Alignment of the L(+)-mandelate dehydrogenase from *R. graminis* with flavocytochrome b_2 from *S. cerevisiae* indicated that the flavin binding domain of LMDH consists of residues 104 to 487. The flavodehydrogenase domain of flavocytochrome b_2 has been shown to be structurally related to other FMN-containing enzymes as described above (Figure 2).

About 35 residues are conserved throughout all of the aligned sequences at this region of the polypeptide (Figure 2). Almost all of the residues are identified as functionally important by Lederer and Mathews (1987) are found to be identical except for Ala196 and Ala198 in LLDH from *S. cerevisiae* known to be in contact with the FMN, are replaced by Pro194 and Gly196 in LMDH from *R. graminis*.

The crystal structure of flavocytochrome b_2 from *S. cerevisiae* have been determined and all functional amino acids involved at the active site have been identified. Based on the amino acid comparison of L-mandelate dehydrogenase from *R. graminis* with the amino acid sequence of LLDH (flavocytochrome b_2) from *S. cerevisiae*, several conserved amino acids in LMDH are predicted to have the same catalytic function.

3.2 Active Site Residues

In the crystal structure of flavocytochrome b_2 from *S. cerevisiae* [3] Arg376 is well positioned to interact with the substrate carboxylate both electrostatically and formed a hydrogen bond between Nε of Arg376 and one of the carboxylate oxygen atoms. This residue is conserved in LMDH from *R. graminis* (Arg380) and throughout the aligned sequences (Figure 2) and apparently plays an important role to bind and orient the substrate along with Tyr143 [15] (Tyr 141 in LMDH). Tyr141 in LMDH from *R. graminis* (Tyr143 in LLDH from *S. cerevisiae*) is predicted to make a hydrogen bond with the oxygen at the carboxylate end of the substrate and play an important role in stabilising the Michaelis complex as in LLDH from *S. cerevisiae* [16]. The three dimensional structure also reveals that Tyr143 in LLDH is hydrogen bonded to a haem propionate [3]. Mutation of Tyr143 to phenylalanine resulted in a larger K_m value than the wild type, indicating a decrease in substrate binding affinity and it also disrupt electron transfer between FMN and haem [10]. Tyr254 (Tyr 248 in LMDH from *R.graminis*) in flavocytochrome b_2 from *S. cerevisiae* which

is also conserved throughout the aligned sequences, was predicted to act by making a hydrogen bond with the substrate OH at all stages of the reaction and facilitate electron departure to the flavin by deprotonating the substrate hydroxyl [15]. Mutation of this residue in flavocytochrome b_2 from *S. cerevisiae* to phenylalanine showed that Tyr254 takes part in transition state stabilization but is not essential for electron transfer [17].

His373 is important in catalysis by acting as a general base. It has been reported that mutation of His373 to glutamine reduced the catalytic activity by a factor of at least 5×10^5 compared to the wild type [18]. Mutation of His290 in lactate monooxygenase from Mycobacterium smegmatis which is equivalent to His373 in LLDH from *S. cerevisiae* has also been made. The mutant enzyme gave 10^7-10^8 fold less activity then the wild type enzyme [19]. It appears that replacement of His290 by glutamine has not resulted in a conformational disruption since substrate and inhibitors bind to the mutant enzyme in a similar fashion to their binding to wild type enzyme [19]. L (+)-mandelate dehydrogenase has the identical histidine residue at position 377 which could have the same function as His373 in LLDH from *S. cerevisiae*.

Asp282 has been shown in the crystal structure of flavocytochrome b_2 [3] to make a hydrogen bond with His373 through one of the carboxylate oxygens and it plays an important role in stabilizing the imidazolium ion of His373 [20]. Identical interactions are also formed by the active-site aspartate (Asp157) in glycolate oxidase [21–22]. Mutation of Asp282 to asparagine has been shown to cause a decrease in the activity of L (+)-lactate dehydrogenase from *S. cerevisiae* [23]. Asp282 is also conserved in LMDH from *R. graminis*.

Finally Lys349 in flavocytochrome b_2 from *S. cerevisiae* is believed to facilitate electron transfer by stabilising the N1 anion of the reduced flavin. Mutation of this residue to arginine caused a complete loss of activity in lactate dehydrogenase. L(+)-mandelate dehydrogenase from *R. graminis* contains an equivalent lysine at position 353.

A comparison of the amino acids which make contact with FMN in L(+)-lactate dehydrogenase from *S. cerevisiae* [20] to the corresponding amino acids in L(+)-mandelate dehydrogenase from *R. graminis* indicates that all the important residues are also conserved. In particular, Lys349 which is important in the catalytic mechanism of LLDH from *S. cerevisiae* and makes contact with the isoalloxazine ring and ribose moiety of FMN, is conserved in LMDH (Lys353) as well as throughout the family of FMN-dependent 2-hydroxyacid dehydrogenases.

Figure 4 shows the results of hydrophilicity calculations via Kyte-Doolitle method. It appears that most of the amino acid in the upper region (sequence from 1 until 178) and lower region (sequence from 280 until 492) of the LMDH polypeptide possess higher hydrophobic character relative to the middle region. This could be due to most of the amino acids which are involved in the formation of $\alpha_8\beta_8$ barrel structure in the FMN binding domain and the formation of hydrophobic crevice in

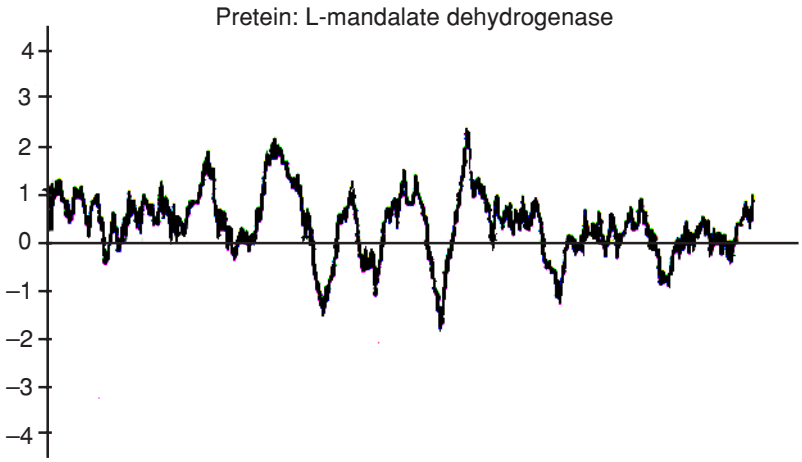

Figure 4 The hydropathic profile of L(+)-mandelate dehydrogenase from *Rhodotorula graminis*

the cytochrome domain, for the binding of haem and for amino acids which are involved at the active sites, are also located at these two regions.

4.0 CONCLUSION

In conclusion, the amino acid sequence comparison data of L(+)-mandelate dehydrogenase from *Rhodotorula graminis* with other L-2-hydroxyacid dehydrogenase enzyme especially L(+)-laclate dehydrogenase from *Saccharomyces cerevisiae* suggests that LMDH could share similar protein function and structure with the group of flavocytochrome b_2. This will assist further three dimensional structure prediction of LMDH from *R. graminis* through protein modelling.

REFERENCES

[1] Pajot, P. and M. Claisse, (1974). Utilization by yeast of D-lactate and L-lactate as sources of energy in the presence of antimycin. *Eur. J. Biochem.* 49: 275 – 285.

[2] Fewson, C. A. (1988). Microbiol metabolism of mandelate: a microcosm of diversity. *FEMS Microbiol. Rev.* 54: 85 – 110.

[3] Xia, Z-X. and F. S. Mathews, (1990). Molecular structure of flavocytochrome b_2 at 2.4 Å resolution. *J. Mol. Biol.* 212: 837 – 863.

[4] Kyte, J and R. F. Doolittle (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157: 105 – 132.

[5] Volokita, M. and C. R. Somerville, (1987). The primary structure of spinach glycolate oxidases deduced from the sequence of a cDNA clone. *J. Biol. Chem.* 262: 15825 – 15828.

[6] Lê, K. H. D. and F. Lederer, (1991). Amino acid sequence of long chain α-hydroxy acid oxidase from rat kidney, a member of the family of FMN-dependent α-hydroxy acid oxidising enzymes. *J. Biol. Chem.* 266: 20877 – 20881.

[7] Black, M. T., F. J. Gunn, S. K. Chapman, and G. A. Reid, (1989a). Structural Basis for the Kinetic Differences between flavocytochrome b_2 from the yeasts *Hansenula anomala* and *Saccharomyces cerevisiae*. *Biochem. J.* 263: 973 – 976.

[8] Guiard, B. (1985). Structure, expression and regulation of a nuclear gene encoding a mitochondrial protein: the yeast L(+)-lactate cytochrome c oxidoreductese (cytochrome b_2). *EMBO, J.* 4: 3265 – 3272.

[9] Rosli, M. I. R. Sinclair, D. Robertson, A. Neu, S. K. Chapman and G. A. Reid (1998). L-mandelate dehydrogenase from Rhodotorula graminis : cloning, sequencing and kinetic characterization of the recombinant enzyme and its independently expressed flavin domain. *Biochem. J.* 333: 107 – 115.

[10] Tsou, A. Y., S. C. Ransom, and J. A. Gerlt, (1990). Mandelate pathway of *Pseudomonas putida*: sequence relationshi ps involving mandelate racemase, (S)-mandelate dehydrogenase, and benzoylformate decarboxylase in *Escherichia coli. Biochemistry*. 29: 9856 – 9862.

[11] Dong, J. M., J. S. Taylor, Latour., Iuchi, S. and E. C. C. Lin, (1993). Three overlapping *lct* genes involved in lactate utilization by *Escherichia coli. J. Bacteriol.* 175: 6671 – 6678.

[12] Giegel, D. A., C. H. Williams, and V. Massey, (1990). L-lactate 2-monooxygenase from *Mycobacterium smegmatis*: cloning, nucleotide sequence and primary structure homology within an enzyme family. *J. Biol. Chem.* 265: 6626 – 6632.

[13] Ghrir, R. and, F. Lederer (1981). Study of a zone highly sensitive to proteases in flavocytochrome b_2 from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 120: 279 – 287.

[14] Cristiano, R. J. and A. W. Steggles, (1989). The complete nucleotide sequence of bovine liver cytochrome b_5 mRNA. *Nucl. Acids. Res. 17*, 799.

[15] Reid, G. A., S. White, M.T. Black, F. Lederer, F S. Mathews and S. K. Chapman, (1988). Probing the active site of flavocytochrome b_2 by site-directed mutagenesis. *Eur. J. Biochem.* 178: 329 – 333.

[16] Rouvière-Fourmy, N., C. Capeillère-Blandin, and F. Lederer, (1994). Role of Tyrosine 143 in Lactate dehydrogenation by flavoctochrome b_2. Primary kinetic isotope effect studies with a phenylalanine mutant. *Biochemistry*. 33: 798 – 806.

[17] Dubois, J., S. K. Chapman, F. S. Mathews, G. A. Reid, and, F. Lederer (1990). Substitution of Tyr254 with Phe at the active site of flavocytochrome b_2: consequences on catalytic of lactate dehydrogenation. *Biochemistry*. 29: 6393 – 6400.

[18] Gaume, B., R. E. Sharp, F. D. C. Manson, S. K. Chapman, G. A. Reid, and F. Lederer, (1995). Mutation to glutamine of histidine373, the catalytic base of flavocytochrome b_2 (L-lactate dehydrogenase). *Biochimie*. 77: 621 – 630.

[19] Müh, U., C. H. Williams and V. Massey, (1994b). Lactate monooxygenase. Site-directed mutagenesis of the postulated active site base histidine 290. *J. Biol. Chem.* 269: 7989 – 7993.

[20] Lederer, F. and F. S. Mathews. 1987. *Mechanism of L-lactate dehydrogenation catalyzed by flavocytochrome b_2 from Baker's yeast*. In: *Flavin and Flavoproteins*, edited by Edmonson, D. E. and McCormick, D. B. Walter de Gruyter, Berlin, 1987. p. 133 – 142.

[21] Lindqvist, Y.and C-I. Brändén, (1989). The active site of spinach glycolate oxidase. *J. Biol. Chem.* 264: 3624 – 3628.

[22] Lindqvist, Y., C-I. Brändén, F. S. Mathews, and F. Lederer, (1991). Spinach glycolate oxidase and yeast flavocytochrome b_2 are structurally homologous and evolutionarily related enzymes with distinctly different function and flavin mononucleotide binding. *J. Biol.Chem.* 266: 3198 – 3207.

[23] Gondary, M. and F. Lederer, (1996). Functional properties of the histidine-aspartate ion pair of flavocytochrome b_2 (L-lactate dehydrogenase): substitution of Asp282 with asparagine. *Biochemistry*. 35: 8587 – 8594.