

SUPPLEMENTARY INFORMATION

The HMP-P synthase of *Legionella pneumophila* (Lpg1565) suggests a difference between the metabolic networks of Bacteria and Yeast

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Running Title: HMP-P synthase in *Legionella pneumophila*

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Table S1 – HMP requirements with different carbon sources

Carbon Source and Additions	Final Cell Yield (OD ₆₅₀)
Glucose	0.08 ± 0.01
Glucose + 100 nM THZ	0.09 ± 0.01
Glucose + 10 nM HMP	0.28 ± 0.01
Glucose + 20 nM HMP	0.47 ± 0.03
Glucose + 100 nM THZ + 10 nM HMP	0.53 ± 0.01
Ribose	0.08 ± 0.01
Ribose + 10 nM HMP	0.42 ± 0.01

Final cell yield (OD₆₅₀) was recorded after 15 hours of growth with shaking at 37 °C. Values are averages ± standard deviations of three biological replicates.

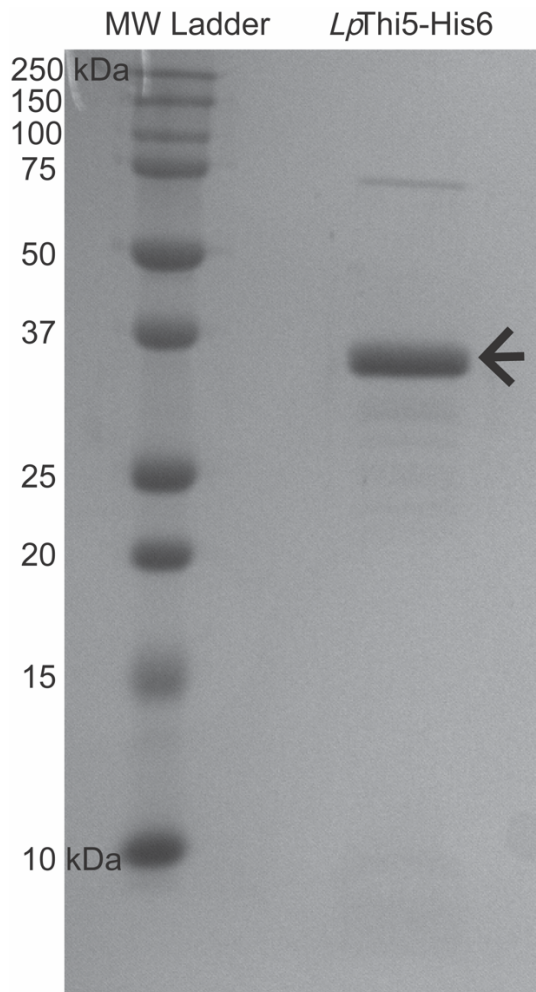


Figure S1 – Representative *LpThi5-His₆* protein purification

After purification by Ni-NTA chromatography, 1.5 μg of purified protein was denatured in SDS-PAGE loading dye (60 mM Tris pH 6.8, 0.1 M DTT, 2 % SDS, 10 % Glycerol) by incubating at 95 °C for 10 minutes, separated by SDS-PAGE using a 12 % acrylamide gel, stained with Coomassie Brilliant Blue, and purity was determined by TotalLab Quant v11 densitometry software. *LpThi5* was enriched to > 85 % purity.

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L. pneumophila      1 MAMSSLSKSRVTL LLNWTNPYHTPTLVAAQLGFEYSEEDIKLAILEPAPDPSDVTEIVGLGT
L. lansingensis    1 --MSTLSSRTTLLLNWYANPYHTPIFVAAQLGFEYQDEGIKLAILEPNDPSDVTEIVGLGR
L. longbeachae     1 --MNALSTRTLLLNWYANPYHTPIFVAAQLGYYQDEGIKLAILEPSPDPSDVTEIVGRGH
L. cherrii         1 --MSALSTRTLLLNWYANPYHTPIFVAAHSLGYYQDEGIKLAILEPSPDPSDVTEIVGMGH
L. anisa           1 --MSALSTRTLLLNWYANPYHTPIFVAHSLGYYQDEGIKLAILEPSPDPSDVTEIVGMGH
S. pombe           1 ----MSTNKITFLTNWEATPYHLPIFLAQTRGYEREGIEVAILEPNTNPSDVTALIGSGK
B. maydis          1 ----MSTDKITFLTNWHATPYHAPVYLAQSKGYFKDEGIKVAILEPNDPSDVTEIIGSGK
C. albicans        1 ----MSTNKITFLTNWEAAPYHPIVYLANIKGYFKDENLDTATLEPSPNPSDVTTELIGSGK
S. cerevisiae      1 ----MSTDKITFLTNWQPTPYHPIFLAQTKGYFKEQGLDMAILEPNTNPSDVTTELIGSGK
M. guilliermondii 1 ----MSTDIISFLLNWEAAPYHLPITVLAQIKGYEQEGIRLSILEPSPNPSDVTTELIGSGK

L. pneumophila      61 VDFGVKAMIHTVAAKAKGYPVTSIGTLLDEFPPTGLIALKSSGI-NSFQDIVGKRVGYIGE
L. lansingensis    59 VDFGVKAMIHTMAARAKGYPVTSIGTLLDEFPPTGLIALKSSGI-TSFQDIIGKRVGYIGE
L. longbeachae     59 VDFGVKAMIHTLAARAKGYPVTSIGTLLDEFPPTGLIALKSSGI-NSFQDIVGKRVGYIGE
L. cherrii         59 VDFGVKAMIHTLAARAKGYPVTSIGTLLDEFPPTGLIALKSSGI-NSFQDIVGKRVGYIGE
L. anisa           59 VDFGVKAMIHTLAARAKGYPVTSIGTLLDEFPPTGLIALKSSGI-SSFQDIVGKRVGYIGE
S. pombe           57 VDMGLKAMIHTLAAKARGYPVTSIGSLLNEFPPTGLITLKGNGI-NDFKDIKGRIGYVGE
B. maydis          57 VDLGFKAMIHTLAAARGFVQSIGSLLNEFPPTGVVYLTSSGITSDFSTLKGKIGYVGE
C. albicans        57 VDMGLKAMVHTLAAKARGLPVTSGSLLDEFPPTGICYLEGSGITSDFQSLKGRIGYVGE
S. cerevisiae      57 VDMGLKAMIHTLAAKARGFPVTSVASLLDEFPPTGVLYLKGSGITEDFQSLKGGKIGYVGE
M. guilliermondii 57 VDMGLKAMVHTLAAKARGFPVTSVASLLDEFPPTGILYLKASGITGDHSLKGGKIGYVGE

L. pneumophila      120 FGKKIIDDLASLAGIDPITSYKTVRIGMNVTDAIYRDVIDTGIGFITNFQKVELEHLIC----
L. lansingensis    118 FGKKIIDNLATLAGIDTNSYETVKIGMNVTDACRDIIDTGIGFITNFQKVELEHLR----
L. longbeachae     118 FGKKIIDNLANLAGIDTSSYETVRIGMNVTDACRDLIDTGIGFITNFQKVELEHLR----
L. cherrii         118 FGKKIIDNLAQLAGIDSASYETVRIGMNVTDACRDLIDTGIGFITNFQKVELEHLR----
L. anisa           118 FGKKIIDNLAQLAGIEPTSSETVRIGMNVTDACRDIIDTGIGFITNFQKVELEHLR----
S. pombe           116 FGKIQLDLCSKFGLSPSDYTAIRCGMNLAPAIINGEIDGGIGIECMQOQVELEERWCVSQG
B. maydis          117 FGKIQLDLTAHYGMSPSDYQAVRVGMNVTRSIITGEIDAGIGLEIVQOQVELEEWLVLQK
C. albicans        117 FGKIQVDELTKHYGMTDDYVAVRCGMNVAKYILEGTIDCGIGIECIQOQVELEEALKEQG
S. cerevisiae      117 FGKIQVDELTKHYGMKPEDYTAVRCGMNVAKYILEGKIDAGIGIECMQOQVELEEYLAKQG
M. guilliermondii 117 FGKIQVDELTKHYGMTDDYTAVRCGMNVAKYILEGKIDAGIGIECIQOQVELEDYLKKGQ

L. pneumophila      176 ---GETVFLRIDQLAGLGCCCFCSIQFIVPEITL-QQPELVKGFNRATORGAAYTTEKPE
L. lansingensis    174 ---GETVFLRIDQLAGLGCCCFCSIQFIVPEOTL-QQPALVKGFNRATORGAAYTTEQPD
L. longbeachae     174 ---GETVFLRIDQLAGLGCCCFCSIQFIVPERML-AQPQLIQGFNRATORGAAYTTEQPD
L. cherrii         174 ---GETVFLRIDQLAGLGCCCFCSIQFIVPERML-KNPETIKGFLKATORGAAYTTEQPD
L. anisa           174 ---GETVFLRIDQLAGLGCCCFCSIQFIVPERML-TNPETIKGFLKATORGAAYTTEQPD
S. pombe           176 RPRSDVQMLRIDRLANLGCCCFCTILYIAHDEFIAKHPDKIKAFRLRAHSATLDMKLDPV
B. maydis          177 RARDVQMLRIDELAQLGCCCFCSIYIYGNNAFIERHPDAVRAFLRACKRATDFVLAQPE
C. albicans        177 KDSNDAKMLRIDKLAELGCCCFCTILYIANDKFAIENSQAVKFLKAIKRATDYMLAHP
S. cerevisiae      177 RPASDAKMLRIDKLACLGCCCFCTVLYICNDEFLLKKNPEKVRKFLKAIKATDYVLADPV
M. guilliermondii 177 RPIEDAQMLRIDQLAELGCCCFCTILYICNDEFLLQANPKVKKFLKAVKRATDDMLANPQ

L. pneumophila      232 EAYELLCAKPKQLRTPLYQKIFTRITLPEFSRTLINVDRDWDKVGRTKHLKIIDEHFDIS
L. lansingensis    230 EAYELLCAQPGRLRTPMYKTIIFTRITLPEFSRNLLNVERDWDKVGSKHLGIIDDSFNVA
L. longbeachae     230 EAYELLCAKPKQLRTPMYHTIFIRSLPEFSRTLINVDRDWDKVGRTKHLGIIDDSFVH
L. cherrii         230 EAYELLCKTKPKQLRTPMYHTIFIRSLPEFSRTLINVERDWDKVGREKHLGVIDDSFVH
L. anisa           230 EAYELLCRMKPKQLRTPMYHTIFIRSLPEFSRTLINVERDWDKVGREKHLGVIDESFAVN
S. pombe           236 QTYKEYIHFKREMGSELHREQFERCFAYFSHDISNVPRDWNKVNTYKRLGIIIPQDFE--
B. maydis          237 QAWAEFCAAKTAMDTPNTRKIFERCFAYFSPDLONVORDWEKVTYKRLGLVLEAFT--
C. albicans        237 EAWAEYGNFKPTMQTDLNTKKEFORCYAYFSESLYNVHRDWRKVNNGYKRLDILPENYV--
S. cerevisiae      237 KAWKEYIDFKPQLNNDLSYKQYQRCYAYFSSLYNVHRDWRKVNNGYKRLAILPPDYV--
M. guilliermondii 237 ESWAQFCDFKPKQLANEVNHKKFORCYAYFSESLYNVHRDWRKVTAYGKRLAILPSDYV--

L. pneumophila      292 QCYTNRFLPDTFYSDDL-----KPIACCLLEN-----
L. lansingensis    290 SCYTNEWLPTKPHSDL-----KPIACCVDE-----
L. longbeachae     290 SCYTNEWLPTKPHSDL-----EPIACCVSE-----
L. cherrii         290 SCYTNEWLPTKPHSDL-----EPIACCVGE-----
L. anisa           290 ACYTNEWLPTKPHSDL-----EPIACCTEE-----
S. pombe           294 PNCTNGYLTWELDPDEKDPMGKQEALEIODEIKQKGGVFSGNSLRY-----VEPANL
B. maydis          295 PNYTNEYLSWGLQAEAEADPVGDQKKMVELQEGVKQNGGFKRLESMAGKTVVGAAPASA
C. albicans        295 PNYTNEYLSWPEPEVDDPEKAQDLMLKHQEECKTCCGGYKRLVLA-----
S. cerevisiae      295 SNYTNEYLSWPEPEVSDPLEAQRMLAIHQEKCRQEGTFKRLALPA-----
M. guilliermondii 295 PNYTNEYLSWKEPEETADPLEAQRQMAHQEECRACGGYRRLVV-----

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Figure S2 – CLUSTAL Omega alignment of diverse *Legionella* and Eukaryotic Thi5

Diverse Thi5 orthologs from the Eukaryotic clade and the *Legionella* clade of Bacteria were aligned using the CLUSTAL Omega algorithm (Sievers *et al.*, 2011) to highlight conserved, similar, and non-conserved structural features across these two domains. Strictly conserved residues are highlighted in black, similar residues are highlighted in gray, and non-conserved residues are annotated with a white background.

LITERATURE CITED

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., Thompson, J.D., and Higgins, D.G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7: 539.