Supplementary file

Table S1. Primers used in RT-PCR analysis of proteins identified by 2-DE

Gene	Forward	Reverse	Product size (bp)
16S rRNA	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG	103
pthA	GAGGCGATACTCTTACTGTTGATG	TGGTGCTTTTGGTACTGGACG	128
ecfA1	TGTTGGTGCTACTGTAGGTGA	ACCCGTTGCTTCTGTCCTC	116
LBA0908	TGAAACCCTAAAAGGTGGCG	CTCTTTTTTTGCTCATCCCTCC	149
LBA0745	CAGGTAAGACTGAAAGCTGGTATG	AATTGTGCCTGCTGGAACATAG	182

pthA: phosphotransferase system enzyme II; ecfA1: ABC ECF transporter, ATP-binding protein; LBA0908: fumarate reductase flavoprotein; LBA0745: mannose-6-phosphate isomerase

Table S2. The growth characteristics of *L.acidophilus* CICC22162 in staMRS or gluMRS

Medium	Fitted curves of growth curves	R^2	The maximum specific growth rate
staMRS	$y = -0.0015x^2 + 0.1399x$	0.9625	0.529
gluMRS	$y = -0.0015x^2 + 0.1483x$	0.9618	0.538

Table S3. Energy Components Contributing to Binding Free Energies in stachyose-LaEcfS

Contribution	**21st (Mode I)**	**24th (Mode II)**	**26th (Mode III)**	**28th (Mode IV)**
$\triangle E_{ele}$	-73.14	-7.69	-34.46	-25.26
$\triangle E_{vdW}$	-66.48	-28.58	-59.87	-47.9
$\triangle E_{gas}$	-139.63	-36.27	-94.33	-73.16
$\triangle G_{GB}$	100.68	28.57	65.85	52.09
$\triangle G_{sol\text{-}np,GB}$	-9.41	-4.87	-8.2	-7.17
$\triangle G_{sol,GB}$	91.27	23.7	57.64	44.91
$\triangle G_{ele,GB}$	27.54	20.88	31.38	26.83
$\triangle G_{bind,GB}$	-48.35	-12.57	-36.69	-28.25

All energies are in kcal mol-1.

Table S4. Energy Components Contributing to Binding Free Energies in various ECF transporters

Ligand	**Stachyose**	**Vitamin B9**	**Vitamin B2**	**biotin**	**Vitamin B1**
Receptor	**LaEcf_mode21**	**LdEcf**	**TmRibU**	**LlBioY**	**LlThiT**
$\triangle E_{ele}$	-73.14	-75.67	-89.14	-202.59	-43.84
$\triangle E_{vdW}$	-66.48	-61.39	-49.68	-38.31	-35.72
$\triangle E_{gas}$	-139.63	-137.06	-138.83	-240.9	-79.56
$\triangle G_{GB}$	100.68	96.85	91.87	202.24	69.23
$\triangle G_{sol\text{-}np,GB}$	-9.41	-6.92	-5.99	-4.51	-4.96
$\triangle G_{sol,GB}$	91.27	89.92	85.88	197.73	64.27
$\triangle G_{ele,GB}$	27.54	21.18	2.73	-0.34	25.38
$\triangle G_{bind,GB}$	-48.35	-47.14	-52.95	-43.17	-15.29

All energies are in kcal mol-1.

Figure S1. Two-dimensional gel electrophoresis profile of proteins from *L. acidophilus* CICC 22162 in staMRS (A) or gluMRS (B).

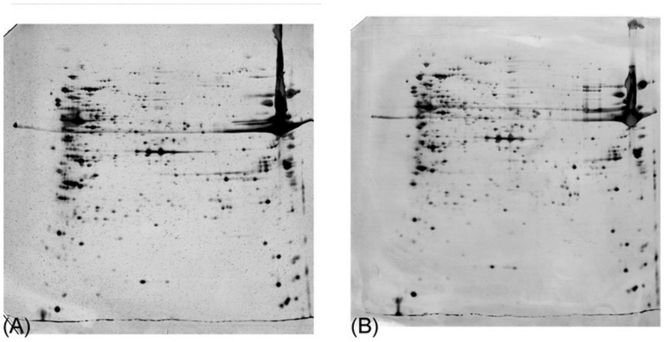
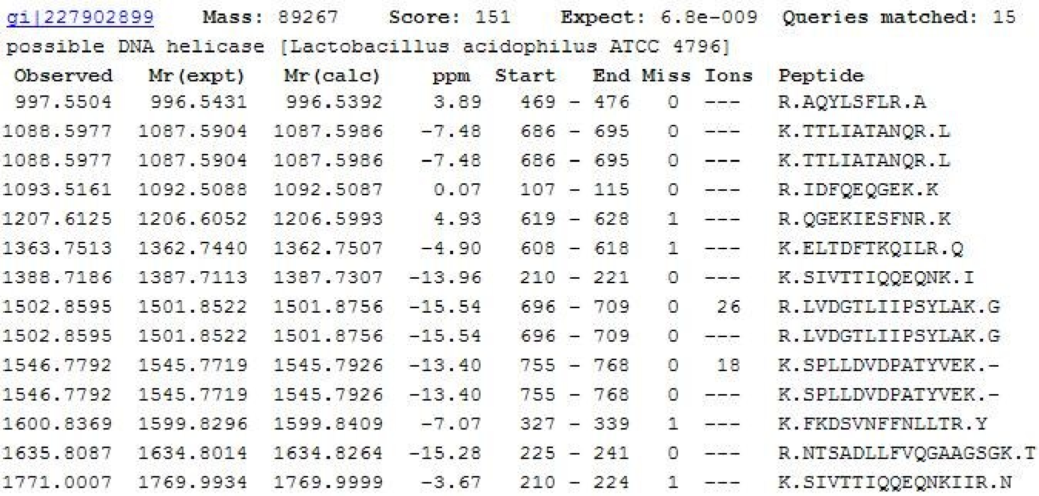


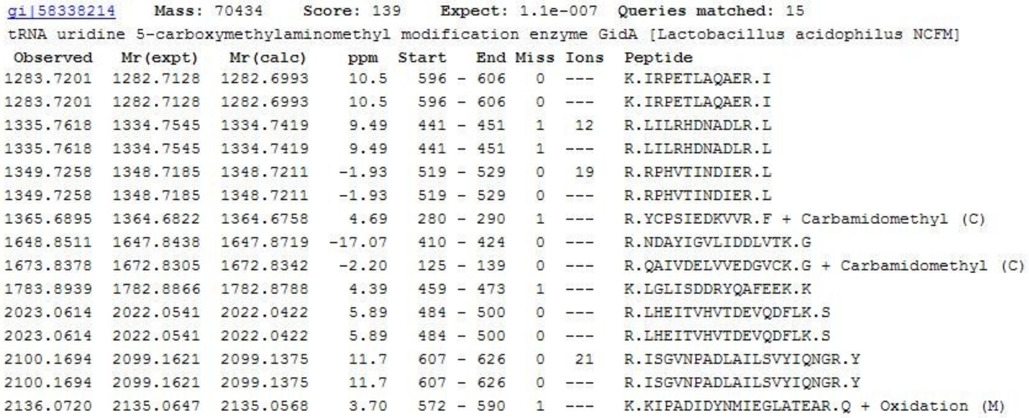
Figure S2. Peptide mass fingerprint spectrum of proteins identified from reference 2D-gel (pH 3−10) of L. acidophilus grown on staMRS or gluMRS medium. Protein identifications were confirmed with a Mascot score of 80 for peptide mass fingerprint.

Spot 4 ATP-dependent helicase



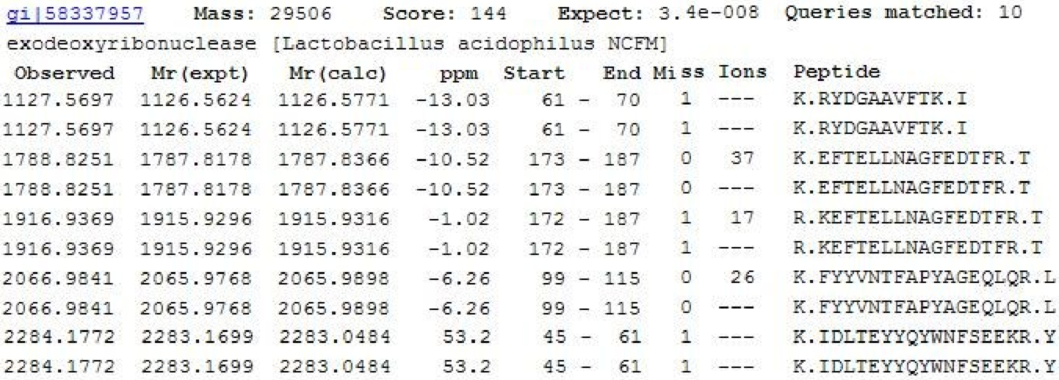
```
gi|227902899    Mass: 89267    Score: 151    Expect: 6.8e-009  Queries matched: 15
possible DNA helicase [Lactobacillus acidophilus ATCC 4796]
 Observed   Mr(expt)   Mr(calc)     ppm  Start    End Miss Ions   Peptide
 997.5504   996.5431   996.5392    3.89    469 - 476   0  ---    R.AQYLSFLR.A
1088.5977  1087.5904  1087.5986   -7.48    686 - 695   0  ---    K.TTLIATANQR.L
1088.5977  1087.5904  1087.5986   -7.48    686 - 695   0  ---    K.TTLIATANQR.L
1093.5161  1092.5088  1092.5087    0.07    107 - 115   0  ---    R.IDFQEQGEK.K
1207.6125  1206.6052  1206.5993    4.93    619 - 628   1  ---    R.QGEKIESFNR.K
1363.7513  1362.7440  1362.7507   -4.90    608 - 618   1  ---    K.ELTDFTKQILR.Q
1388.7186  1387.7113  1387.7307  -13.96    210 - 221   0  ---    K.SIVTTIQQEQNK.I
1502.8595  1501.8522  1501.8756  -15.54    696 - 709   0   26    R.LVDGTLIIPSYLAK.G
1502.8595  1501.8522  1501.8756  -15.54    696 - 709   0  ---    R.LVDGTLIIPSYLAK.G
1546.7792  1545.7719  1545.7926  -13.40    755 - 768   0   18    K.SPLLDVDPATYVEK.-
1546.7792  1545.7719  1545.7926  -13.40    755 - 768   0  ---    K.SPLLDVDPATYVEK.-
1600.8369  1599.8296  1599.8409   -7.07    327 - 339   1  ---    K.FKDSVNFFNLLTR.Y
1635.8087  1634.8014  1634.8264  -15.28    225 - 241   0  ---    R.NTSADLLFVQGAAGSGK.T
1771.0007  1769.9934  1769.9999   -3.67    210 - 224   1  ---    K.SIVTTIQQEQNKIIR.N
```

Spot 5: tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA



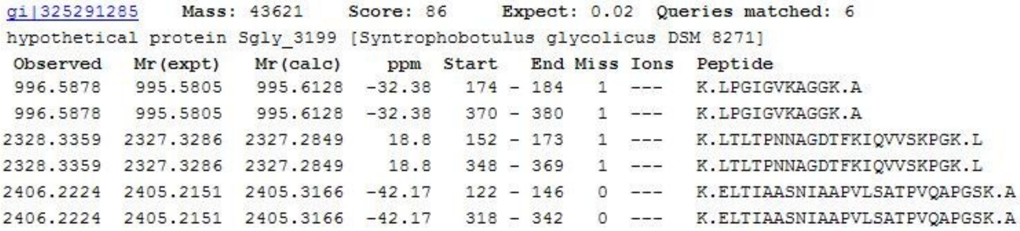
```
gi|58338214     Mass: 70434    Score: 139    Expect: 1.1e-007  Queries matched: 15
tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)     ppm  Start    End Miss Ions   Peptide
1283.7201  1282.7128  1282.6993    10.5   596 - 606    0  ---    K.IRPETLAQAER.I
1283.7201  1282.7128  1282.6993    10.5   596 - 606    0  ---    K.IRPETLAQAER.I
1335.7618  1334.7545  1334.7419    9.49   441 - 451    1   12    R.LILRHDNADLR.L
1335.7618  1334.7545  1334.7419    9.49   441 - 451    1  ---    R.LILRHDNADLR.L
1349.7258  1348.7185  1348.7211   -1.93   519 - 529    0   19    R.RPHVTINDIER.L
1349.7258  1348.7185  1348.7211   -1.93   519 - 529    0  ---    R.RPHVTINDIER.L
1365.6895  1364.6822  1364.6758    4.69   280 - 290    1  ---    R.YCPSIEDKVVR.F + Carbamidomethyl (C)
1648.8511  1647.8438  1647.8719  -17.07   410 - 424    0  ---    R.NDAYIGVLIDDLVTK.G
1673.8378  1672.8305  1672.8342   -2.20   125 - 139    0  ---    R.QAIVDELVVEDGVCK.G + Carbamidomethyl (C)
1783.8939  1782.8866  1782.8788    4.39   459 - 473    1  ---    K.LGLISDDRYQAFEEK.K
2023.0614  2022.0541  2022.0422    5.89   484 - 500    0  ---    R.LHEITVHVTDEVQDFLK.S
2023.0614  2022.0541  2022.0422    5.89   484 - 500    0  ---    R.LHEITVHVTDEVQDFLK.S
2100.1694  2099.1621  2099.1375    11.7   607 - 626    0   21    R.ISGVNPADLAILSVYIQNGR.Y
2100.1694  2099.1621  2099.1375    11.7   607 - 626    0  ---    R.ISGVNPADLAILSVYIQNGR.Y
2136.0720  2135.0647  2135.0568    3.70   572 - 590    1  ---    K.KIPADIDYNMIEGLATEAR.Q + Oxidation (M)
```

Spot 6: exodeoxyribonuclease



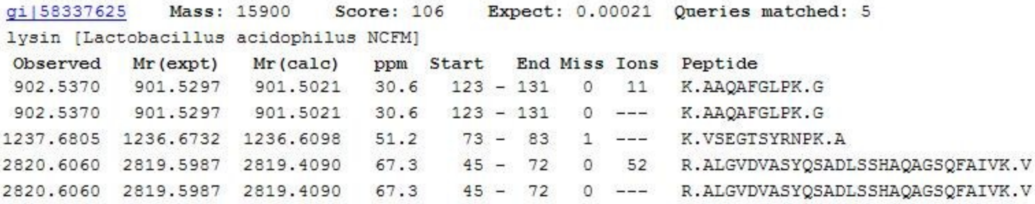
```
gi|58337957    Mass: 29506    Score: 144    Expect: 3.4e-008  Queries matched: 10
exodeoxyribonuclease [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)     ppm  Start    End Miss Ions   Peptide
1127.5697  1126.5624  1126.5771  -13.03    61 -  70    1  ---    K.RYDGAAVFTK.I
1127.5697  1126.5624  1126.5771  -13.03    61 -  70    1  ---    K.RYDGAAVFTK.I
1788.8251  1787.8178  1787.8366  -10.52   173 - 187    0   37    K.EFTELLNAGFEDTFR.T
1788.8251  1787.8178  1787.8366  -10.52   173 - 187    0  ---    K.EFTELLNAGFEDTFR.T
1916.9369  1915.9296  1915.9316   -1.02   172 - 187    1   17    R.KEFTELLNAGFEDTFR.T
1916.9369  1915.9296  1915.9316   -1.02   172 - 187    1  ---    R.KEFTELLNAGFEDTFR.T
2066.9841  2065.9768  2065.9898   -6.26    99 - 115    0   26    K.FYYVNTFAPYAGEQLQR.L
2066.9841  2065.9768  2065.9898   -6.26    99 - 115    0  ---    K.FYYVNTFAPYAGEQLQR.L
2284.1772  2283.1699  2283.0484    53.2    45 -  61    1  ---    K.IDLTEYYQYWNFSEEKR.Y
2284.1772  2283.1699  2283.0484    53.2    45 -  61    1  ---    K.IDLTEYYQYWNFSEEKR.Y
```

Spot 7 hypothetical protein Sgly_3199



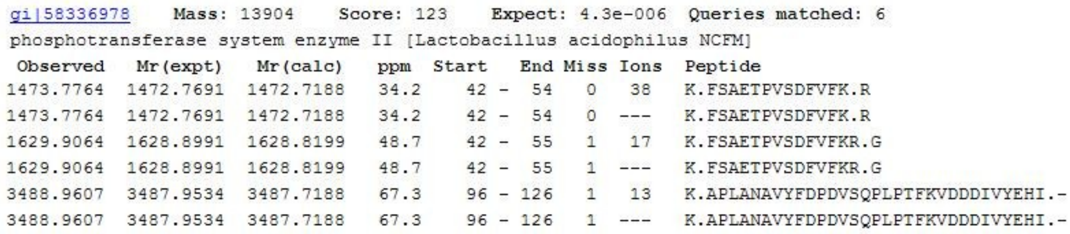
```
gi|325291285    Mass: 43621    Score: 86    Expect: 0.02   Queries matched: 6
hypothetical protein Sgly_3199 [Syntrophobotulus glycolicus DSM 8271]
 Observed   Mr(expt)   Mr(calc)     ppm  Start    End Miss Ions   Peptide
 996.5878   995.5805   995.6128  -32.38   174 - 184    1  ---    K.LPGIGVKAGGK.A
 996.5878   995.5805   995.6128  -32.38   370 - 380    1  ---    K.LPGIGVKAGGK.A
2328.3359  2327.3286  2327.2849    18.8   152 - 173    1  ---    K.LTLTPNNAGDTFKIQVVSKPGK.L
2328.3359  2327.3286  2327.2849    18.8   348 - 369    1  ---    K.LTLTPNNAGDTFKIQVVSKPGK.L
2406.2224  2405.2151  2405.3166  -42.17   122 - 146    0  ---    K.ELTIAASNIAAPVLSATPVQAPGSK.A
2406.2224  2405.2151  2405.3166  -42.17   318 - 342    0  ---    K.ELTIAASNIAAPVLSATPVQAPGSK.A
```

Spot 8 lysin



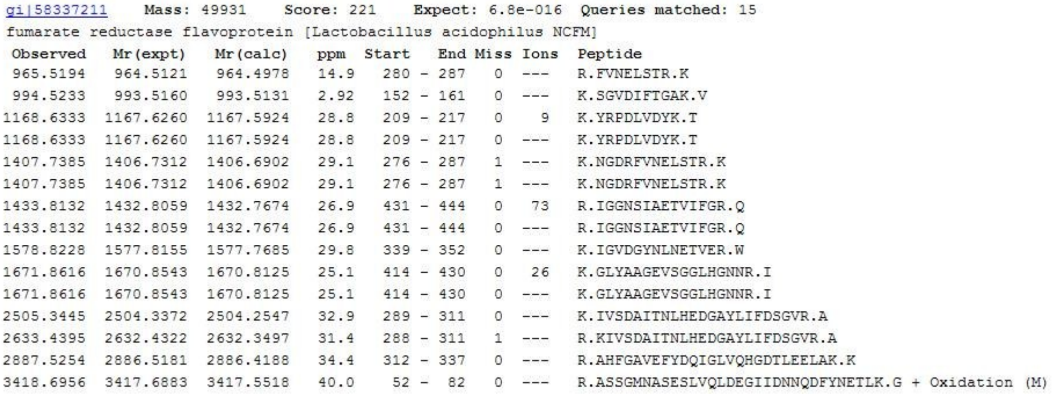
```
gi|58337625    Mass: 15900    Score: 106    Expect: 0.00021  Queries matched: 5
lysin [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)    ppm  Start    End Miss Ions   Peptide
 902.5370   901.5297   901.5021   30.6   123 - 131    0   11    K.AAQAFGLPK.G
 902.5370   901.5297   901.5021   30.6   123 - 131    0  ---    K.AAQAFGLPK.G
1237.6805  1236.6732  1236.6098   51.2    73 -  83    1  ---    K.VSEGTSYRNPK.A
2820.6060  2819.5987  2819.4090   67.3    45 -  72    0   52    R.ALGVDVASYQSADLSSHAQAGSQFAIVK.V
2820.6060  2819.5987  2819.4090   67.3    45 -  72    0  ---    R.ALGVDVASYQSADLSSHAQAGSQFAIVK.V
```

Spot 9 phosphotransferase system enzyme II (PTS)



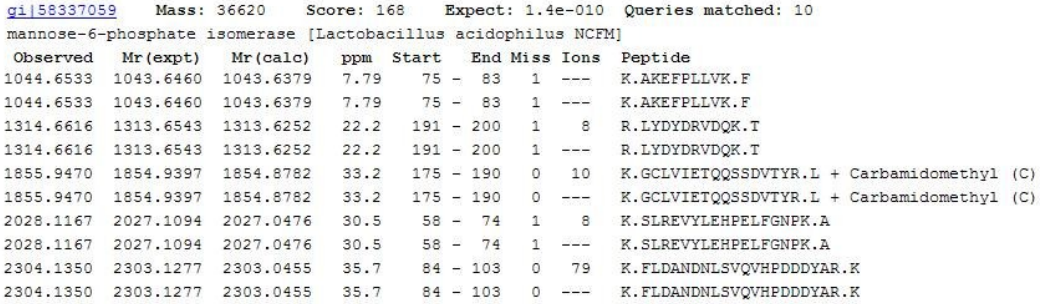
```
gi|58336978     Mass: 13904     Score: 123     Expect: 4.3e-006  Queries matched: 6
phosphotransferase system enzyme II [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)    ppm  Start    End Miss Ions   Peptide
1473.7764  1472.7691  1472.7188   34.2     42 -   54    0   38   K.FSAETPVSDFVFK.R
1473.7764  1472.7691  1472.7188   34.2     42 -   54    0  ---   K.FSAETPVSDFVFK.R
1629.9064  1628.8991  1628.8199   48.7     42 -   55    1   17   K.FSAETPVSDFVFKR.G
1629.9064  1628.8991  1628.8199   48.7     42 -   55    1  ---   K.FSAETPVSDFVFKR.G
3488.9607  3487.9534  3487.7188   67.3     96 - 126    1   13   K.APLANAVYFDPDVSQPLPTFKVDDDIVYEHI.-
3488.9607  3487.9534  3487.7188   67.3     96 - 126    1  ---   K.APLANAVYFDPDVSQPLPTFKVDDDIVYEHI.-
```

Spot 13 fumarate reductase flavoprotein



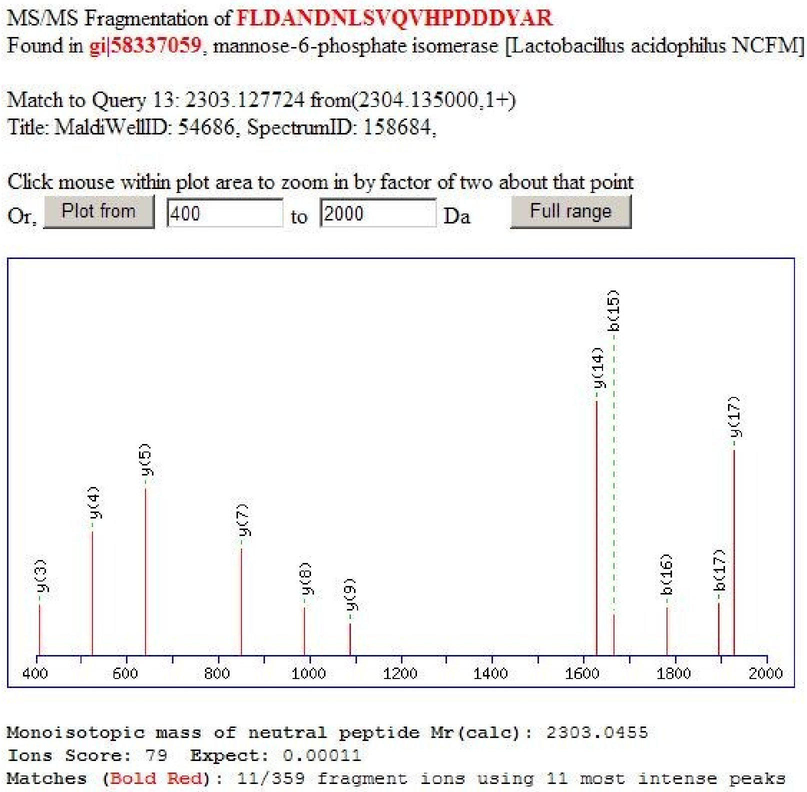
```
gi|58337211     Mass: 49931     Score: 221     Expect: 6.8e-016  Queries matched: 15
fumarate reductase flavoprotein [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)    ppm  Start    End Miss Ions   Peptide
 965.5194   964.5121   964.4978   14.9    280 - 287    0  ---   R.FVNELSTR.K
 994.5233   993.5160   993.5131   2.92    152 - 161    0  ---   K.SGVDIFTGAK.V
1168.6333  1167.6260  1167.5924   28.8    209 - 217    0    9   K.YRPDLVDYK.T
1168.6333  1167.6260  1167.5924   28.8    209 - 217    0  ---   K.YRPDLVDYK.T
1407.7385  1406.7312  1406.6902   29.1    276 - 287    1  ---   K.NGDRFVNELSTR.K
1407.7385  1406.7312  1406.6902   29.1    276 - 287    1  ---   K.NGDRFVNELSTR.K
1433.8132  1432.8059  1432.7674   26.9    431 - 444    0   73   R.IGGNSIAETVIFGR.Q
1433.8132  1432.8059  1432.7674   26.9    431 - 444    0  ---   R.IGGNSIAETVIFGR.Q
1578.8228  1577.8155  1577.7685   29.8    339 - 352    0  ---   K.IGVDGYNLNETVER.W
1671.8616  1670.8543  1670.8125   25.1    414 - 430    0   26   K.GLYAAGEVSGGLHGNNR.I
1671.8616  1670.8543  1670.8125   25.1    414 - 430    0  ---   K.GLYAAGEVSGGLHGNNR.I
2505.3445  2504.3372  2504.2547   32.9    289 - 311    0  ---   K.IVSDAITNLHEDGAYLIFDSGVR.A
2633.4395  2632.4322  2632.3497   31.4    288 - 311    1  ---   R.KIVSDAITNLHEDGAYLIFDSGVR.A
2887.5254  2886.5181  2886.4188   34.4    312 - 337    0  ---   R.AHFGAVEFYDQIGLVQHGDTLEELAK.K
3418.6956  3417.6883  3417.5518   40.0     52 -  82    0  ---   R.ASSGMNASESLVQLDEGIIDNNQDFYNETLK.G + Oxidation (M)
```

Spot 15 mannose-6-phosphate isomerase



```
gi|58337059     Mass: 36620     Score: 168     Expect: 1.4e-010  Queries matched: 10
mannose-6-phosphate isomerase [Lactobacillus acidophilus NCFM]
 Observed   Mr(expt)   Mr(calc)    ppm  Start    End Miss Ions   Peptide
1044.6533  1043.6460  1043.6379   7.79     75 -  83    1  ---   K.AKEFPLLVK.F
1044.6533  1043.6460  1043.6379   7.79     75 -  83    1  ---   K.AKEFPLLVK.F
1314.6616  1313.6543  1313.6252   22.2    191 - 200    1    8   R.LYDYDRVDQK.T
1314.6616  1313.6543  1313.6252   22.2    191 - 200    1  ---   R.LYDYDRVDQK.T
1855.9470  1854.9397  1854.8782   33.2    175 - 190    0   10   K.GCLVIETQQSSDVTYR.L + Carbamidomethyl (C)
1855.9470  1854.9397  1854.8782   33.2    175 - 190    0  ---   K.GCLVIETQQSSDVTYR.L + Carbamidomethyl (C)
2028.1167  2027.1094  2027.0476   30.5     58 -  74    1    8   K.SLREVYLEHPELFGNPK.A
2028.1167  2027.1094  2027.0476   30.5     58 -  74    1  ---   K.SLREVYLEHPELFGNPK.A
2304.1350  2303.1277  2303.0455   35.7     84 - 103    0   79   K.FLDANDNLSVQVHPDDDYAR.K
2304.1350  2303.1277  2303.0455   35.7     84 - 103    0  ---   K.FLDANDNLSVQVHPDDDYAR.K
```

The MS/MS fragmentation of mannose-6-phosphate isomerase. Single peptide based protein identifications by MS/MS analysis were confirmed with a Mascot score of 79.



MS/MS Fragmentation of **FLDANDNLSVQVHPDDDYAR**
Found in **gi|58337059**, mannose-6-phosphate isomerase [Lactobacillus acidophilus NCFM]

Match to Query 13: 2303.127724 from(2304.135000,1+)
Title: MaldiWellID: 54686, SpectrumID: 158684,

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 400 to 2000 Da Full range

```
Monoisotopic mass of neutral peptide Mr(calc): 2303.0455
Ions Score: 79  Expect: 0.00011
Matches (Bold Red): 11/359 fragment ions using 11 most intense peaks
```

Figure S3. Structure features of ECF and MSM transporters

(A) The ECF transporter in cartoon representation (LaEcfS in yellow, LaEcfT in magenta, LaEcfA1 in red and LaEcfA2 in silver), and the MSM transporter in cartoon representation (LaMsmG in yellow, LaMsmF in magenta, the LaMsmK dimer in green and orange) (B) System setup for MD simulations. The ECF transporter was inserted in the POPC membrane and solvated in the water solvent. (C) The number of basic residues of LaEcfS and LaEcfT binding to the lipids along simulations.

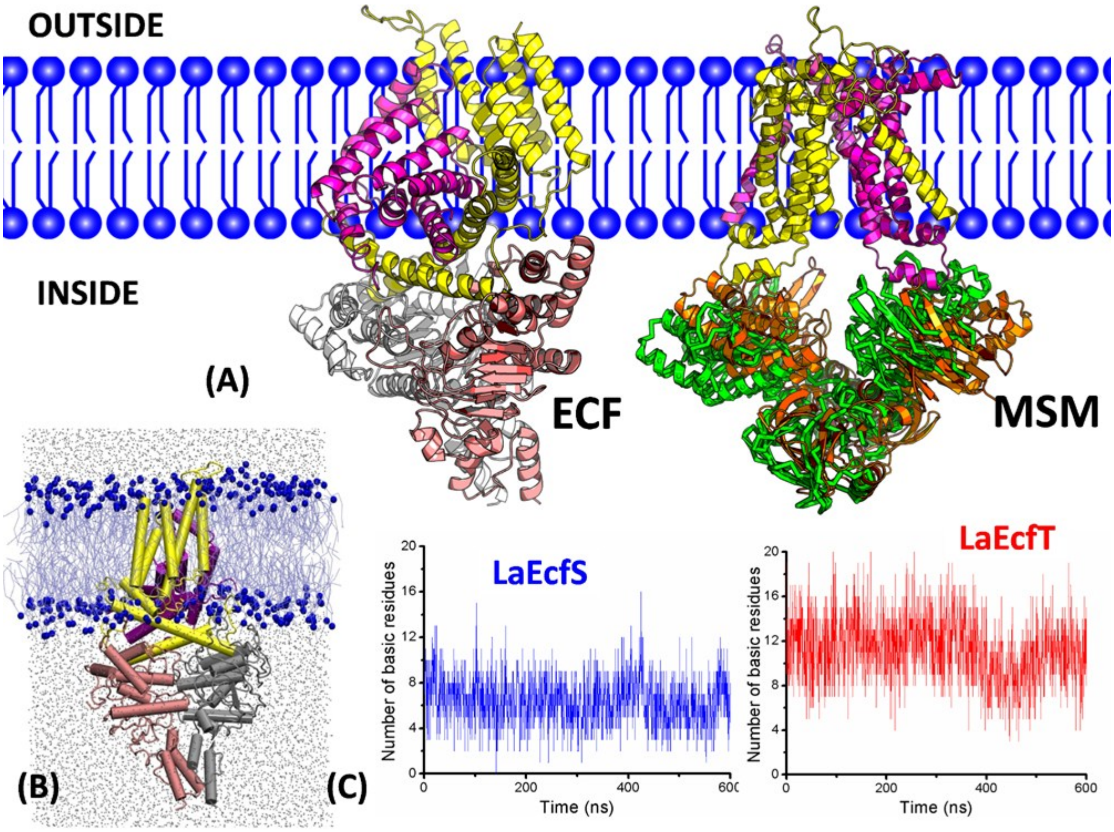


Figure S4. The number of lipids binding to LaEcfS/T along simulations

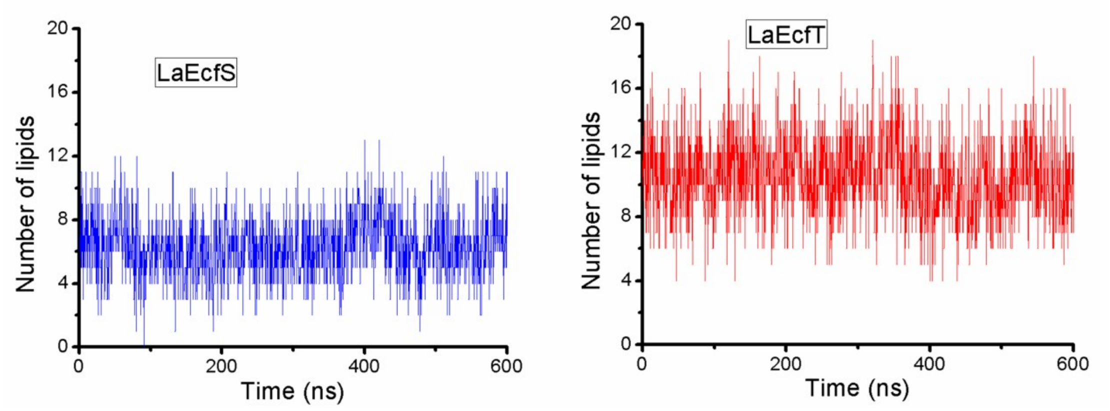


Figure S5. Binding modes of docked stachyose by SwissDock

(A) Superposition of 250 docking hints, colored by their scores. (B) Representations of the four binding modes that were identified. These modes correspond to different spatial orientations of stachyose in complex with LaEcfS (magenta).

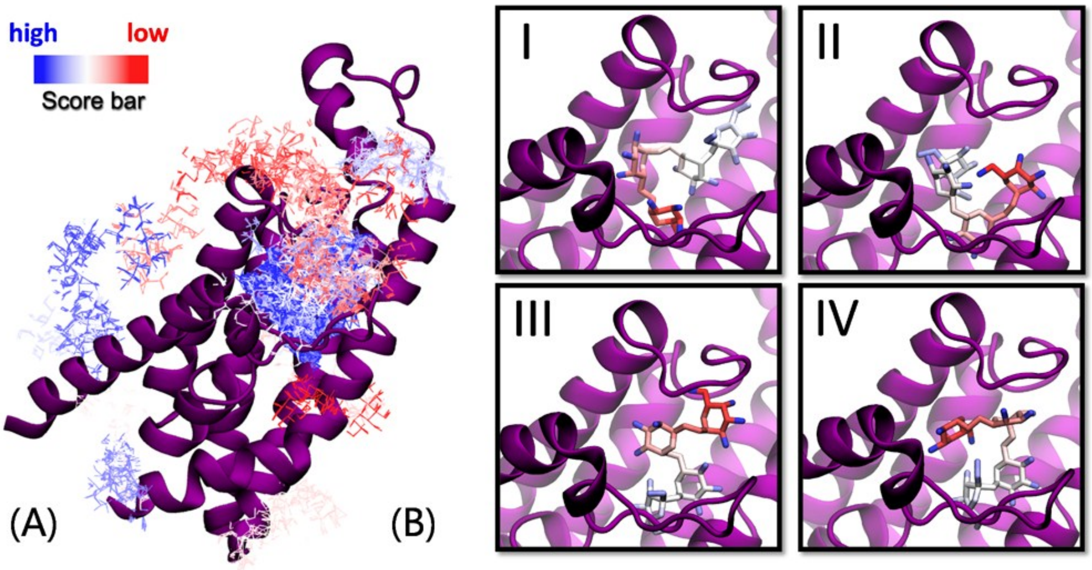


Figure S6. A close view of interactions in the stachyose-LaEcfS and vitaminB9-LdEcfS complexes. Residues on EcfS responsible for its binding to stachyose (A) and vitamin B9 (B). The per-residue interaction spectrum of the residues of EcfS with stachyose (C) and vitamin B9 (D) according to MM-GBSA free energy decomposition analysis.

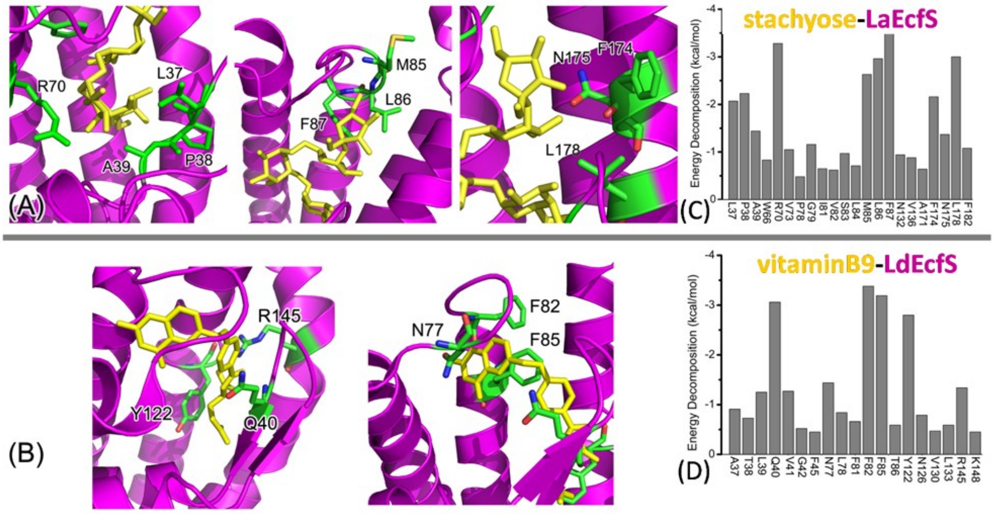
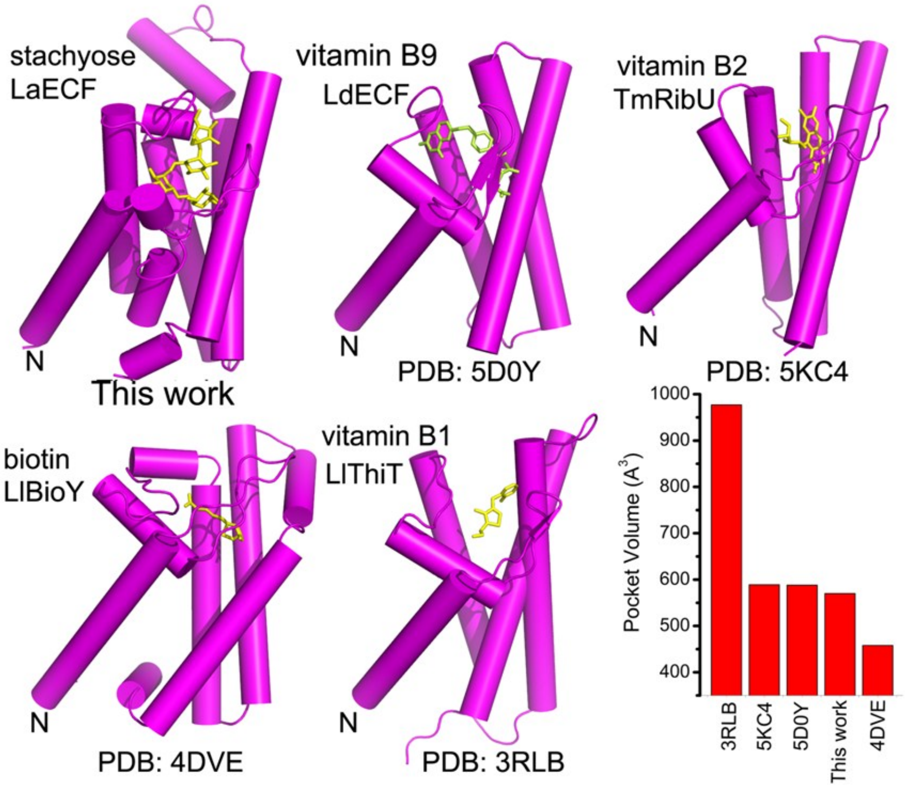


Figure S7. Representations of the substrate-load ECF extracted from the X-ray structures. The pocket volumes from these EcfS structures were calculated by CastP.


stachyose
LaECF
N
This work
vitamin B9
LdECF
N
PDB: 5D0Y
vitamin B2
TmRibU
N
PDB: 5KC4
biotin
LlBioY
N
PDB: 4DVE
vitamin B1
LlThiT
N
PDB: 3RLB
Pocket Volume (A^3)
1000
900
800
700
600
500
400
3RLB
5KC4
5D0Y
This work
4DVE