# Identification of a Major Glucose Transporter in *Flavobacterium johnsoniae*: Inhibition of *F. johnsoniae* Colony Spreading by Glucose Uptake

Keigo Imamura[1,2], Keiko Sato[1], Yuka Narita[1,*], Yoshio Kondo[2], Daisuke Nakane[3], Mariko Naito[1], Taku Fujiwara[2] and Koji Nakayama[1]

[1]Department of Microbiology and Oral Infection and [2]Department of Pediatric Dentistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588 and [3]Department of Physics, Gakushuin University, 1-5-1 Mejiro, Toshima-ku,Tokyo 171-8588, Japan

*Present Address: Section of Infection Biology, Department of Functional Bioscience, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka 814-0193, Japan

Short Running Title: MFS Glucose Transporter

Correspondence:
Koji Nakayama, Department of Microbiology and Oral Infection, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan.
Tel: +81 95 819 7648; fax: +81 95 819 7650; email address: knak@nagasaki-u.ac.jp

List of Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; CYE, casitone yeast extract; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol; Em, erythromycin; MFS, major facilitator superfamily; PBS, phosphate buffered saline; RT, room temperature; Sm, streptomycin; 2DG, 2-deoxy-D-glucose

**ABSTRACT**

Many members of the phylum *Bacteroidetes* such as *Flavobacterium johnsoniae* can glide over a solid surface: an ability called *gliding motility*. It can be usually observed on agar plates as thin, flat, spreading colonies with irregular, feathery edges; this phenomenon is called *colony spreading*. Colony spreading of *F. johnsoniae* on 1.5% agar plates containing poor nutrients is dose-dependently inhibited by addition of D-glucose, as previously reported. Accordingly, here, we created mutants (by transposon mutagenesis) that partially suppressed glucose-mediated inhibition of colony spreading. Among the isolates, we found that one had a transposon insertion in Fjoh_4565, tentatively named *mfsA*, which encodes a major facilitator superfamily (MFS) transporter previously shown to be required for growth on glucose, N-acetyl-glucosamine, and chitin. We constructed an *mfsA* deletion mutant and found that the mutant showed no glucose-mediated acceleration of growth or glucose uptake. The *mfsA* gene complemented the phenotype of a glucose-negative *Escherichia coli*. These results suggested that the *mfsA* gene encodes the sole MFS transporter of glucose in *F. johnsoniae* and that glucose uptake is partially required for the glucose-mediated inhibition of *F. johnsoniae* colony spreading.

Key words: *Bacteroidetes,* colony spreading, gliding motility, glucose uptake, major facilitator superfamily transporter

## INTRODUCTION

Many bacterial species can glide over solid surfaces: an ability that is called *gliding motility*. This ability can be observed in many members of the phylum *Bacteroidetes*, *Myxococcus xanthus*, *Mycoplasma mobile*, and many cyanobacteria, but these bacteria have their own unique motility machineries (1). *Flavobacterium johnsoniae* belonging to the phylum *Bacteroidetes* has been studied for many years to understand the motility mechanism. A large number of *F. johnsoniae* proteins have been found to be involved in gliding motility, which include Gld, Spr, and Rem (2). Some of these proteins are also components of the type IX protein secretion system (3, 4). We proposed a helical track model, where adhesive SprB filaments are propelled along a left-handed closed helical loop on the cell surface. Attachment of SprB to a substratum results in cell movement (5).

Gliding motility of *F. johnsoniae* is usually observed on agar plates as thin, flat, spreading colonies with irregular, feathery edges: this phenomenon is called *colony spreading* (6). This phenomenon requires gliding motility because *F. johnsoniae* mutants deficient in *gld* or *spr* genes show no colony spreading (3, 7-19). Colony spreading takes place on rather nutrient-poor plates, and when nutrients are added, the colonies tend to be raised and smooth-edged (6). Chang & Pate (20) first reported that sugars suppress colony spreading of *F. johnsoniae* on 1.5% agar plates. In their study, they found that metabolizable sugars including glucose, galactose, fructose, mannose, xylose, and maltose suppress colony spreading, whereas a nonmetabolizable sugar, lactose, does not. More extensive experiments revealed that a nonmetabolizable sugar, sucrose, suppresses colony spreading at a low concentration and minimal inhibitory concentrations for colony spreading vary among metabolizable sugars (21). Gorski et al.

(22) found that the inhibitory sugars have a common structural feature regardless of their metabolizable abilities.

In this study, we created *F. johnsoniae* mutants that showed colony spreading on glucose-containing agar plates using transposon mutagenesis to investigate which genes are involved in the inhibitory effect of glucose on colony spreading of the bacterium.

## MATERIALS AND METHODS

### Bacterial strains and culture conditions

Bacterial strains and plasmids used in this study are listed in Table 1 (23, 24). *F. johnsoniae* cells were grown aerobically in the enriched casitone yeast extract (CYE) medium and on enriched CYE agar. For selection and maintenance of antibiotic-resistant *F. johnsoniae* strains, antibiotics were added to the medium at the following concentrations: streptomycin (Sm) 100 μg/ml and erythromycin (Em) 100 μg/ml. To observe colony spreading, we grew *F. johnsoniae* on PY2 agar (7) at 25°C.

### Transposon mutagenesis and gene-directed mutagenesis

Transposon mutagenesis in *F. johnsoniae* strain UW101 by means of Tn*4351* was described previously (25). Gene-directed mutagenesis of *F. johnsoniae* was carried out as follows. After the mating of *E. coli* S17-1 λ*pir* (carrying a pRR51 derivative) with *F. johnsoniae* CJ1827, an $Em^r$ transconjugant was obtained to select for integration of the plasmid into the genome by homologous recombination. An erythromycin-resistant clone was grown overnight in CYE, and the loss of the plasmid via a second recombination event was selected by growth on CYE agar containing streptomycin

(18).

**Construction of plasmids and bacterial strains**

For construction of a targeting plasmid vector designed to create an *F. johnsoniae mfsA* deletion mutant, DNA regions upstream and downstream of *mfsA* were PCR-amplified from the chromosomal DNA of *F. johnsoniae* using pairs of primers (F4565-UF-BamHI plus F4565-UR-SalI and F4565-DF-SalI plus F4565-DR-SphI, respectively, where "U" indicates upstream, "F" indicates forward, "D" indicates downstream, and "R" indicates reverse). Primers used in this study are listed in Table S1. The amplified DNA upstream was double-digested with BamHI plus SalI. The DNA downstream was digested with SalI plus SphI. Both digested products were ligated with pRR51 that had been digested with BamHI and SphI. (Consequently, we obtained pDF1.)

For construction of shuttle vector pNS1 for *F. johnsoniae*, the multiple cloning site (MCS) region was 1st-PCR-amplified from pFj29 using the primer pair pFj29-1st-F and gfpmut3-R-SphI. Then, the MCS region was 2nd-PCR-amplified from the 1st-amplified DNA using the primer pair pFj29-2nd-F and gfpmut3-R-SphI. The amplified DNA was digested with BglII and SphI and inserted at the BamHI and SphI sites of pFj29, resulting in pNS1.

For construction of a complemented version of the *mfsA* strain DFJ, the gene encoding Fjoh_4565 was PCR-amplified from *F. johnsoniae* UW101 chromosomal DNA using the primer pair F4565-F-BamHI and F4565-stop-R-NotI. The amplified DNA was digested with BamHI and NotI and inserted into the corresponding region of pNS1, resulting in plasmid pNS1 containing *mfsA* (pDF2).

For construction of an *F. johnsoniae* strain expressing MfsA-Gfp, the gene

encoding Fjoh_4565 was PCR-amplified from *F. johnsoniae* UW101 chromosomal DNA using the primer pair F4565-F-BamHI and F4565-GR-NotI. The amplified DNA was digested with BamHI and NotI and inserted into the corresponding region of pNS1, resulting in plasmid pNS1 containing *mfsA-gfp* (pDF3).

For construction of a glucose-negative *E. coli* strain expressing *F. johnsoniae* MfsA and MfsA-Gfp, the *mfsA* gene DNA encoding Fjoh_4565 was PCR-amplified from *F. johnsoniae* UW101 chromosomal DNA and from the pDF2 plasmid DNA using the primer pairs F4565-22bF-NdeI and F4565-22bR-XhoI as well as F4565-22bF-NdeI and Gfpmut3-stopR-XhoI, respectively. The amplified DNAs were digested with NdeI and XhoI and inserted into the corresponding region of pET-22b (Novagen), resulting in plasmids pDF4 and pDF5, respectively. The glucose-negative *E. coli* strain LJ141 was then transformed with pDF4 and pDF5.

**Glucose uptake**

*F. johnsoniae* cells were grown in the CYE medium at 27°C with shaking (165 rpm) overnight to optical density of ~1.0 at 600 nm. The samples were washed two times with 10 mM Tris-HCl (pH 7.5). The cells were exposed to 1 mM 2,4-dinitrophenol (DNP), 10 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 50 μM *N*,*N*′-dicyclohexylcarbodiimide (DCCD), or 50 mM arsenate for 1 min at room temperature (RT) and incubated in 10 mM Tris-HCl (pH 7.5) supplemented with 2-deoxy-D-glucose (2DG) at RT for 2 h. Glucose uptake was determined by means of the 2DG uptake in an enzymatic photometric assay using the 2DG Uptake Measurement Kit (COSMO BIO Co.) (26, 27).

**RNA Isolation**

Total RNA from cells of the wild-type and the *mfsA* mutant at different growth conditions (1% PY2 and 1% PYG) from three independent cultures. After 24 h of culture, bacterial cells were collected by cell scraping in RNAlater® solution (Thermo Fisher Scientific) and centrifuged at 8,000 rpm for 10 min. Cell pellets were resuspended with Trizol, and RNA was extracted using an RNeasy Mini Kit (Qiagen) according to the manufacturer's recommendations. DNA was removed with RNase-free DNase.

**Gene Expression Microarrays**

According to manufacturers' instructions, the complementary RNA was amplified and labeled by Low Input Quick Amp Labeling Kit (Agilent Technologies), and hybridized to Agilent-based microarray platform with 4 x 44 K probes per slide (Agilent Technologies). The array contains probe sets to 5,113 open reading frames of *F. johnsoniae* UW101. Designing microarray probes was done with the Agilent eArraysystem with the following settings during the microarray probe design: Tm (70°C) matching methodology, 60-mer probe length, 8 probes/gene. All hybridized microarray slides were scanned using an Agilent scanner. Relative hybridization intensities and background hybridization values were calculated using Agilent Feature Extraction Software (ver. 9.5.1.1).

**Localization of MfsA**

Cells were examined by microscopy to identify MfsA on the cell membrane. Cells of *F. johnsoniae mfsA*/*mfsA-gfp* (200 μl) were placed on a slide glass for 3 min at RT and

were washed two times with PBS. DAPI (Invitrogen) and FM4-64 (Invitrogen) were used for detection of DNA and cell membranes, respectively. After two washes with PBS, the cells were incubated with a 1/500 dilution of DAPI (Invitrogen) and FM4-64 (Invitrogen) for 30 min and were washed two times with PBS. The coverslip was mounted on glass and examined under an inverted fluorescence microscope.

**Cell growth**

The growth curves of the wild-type, *mfsA* deletion mutant, and *mfsA*/*mfsA*$^{+}$ complemented strain were determined. The cells were incubated in CYE overnight to optical density of ~1.0 at 600 nm. The microorganisms were washed two times with 10 mM Tris-HCl (pH 7.5) and incubated in the mPY2 medium (0.05% peptone, 0.05% yeast extract) or mPY2 supplemented with D-glucose (15 mM) with shaking at 27°C. Cell growth was measured by optical density at 600 nm at indicated time points. Error bars show standard deviation.

**Statistical analysis**

The data of glucose uptake test were analysed using Student's *t*-test. Results were considered to be statistically significant with a *P* value <0.001.

**RESULTS**

**The inhibitory effect of glucose on colony spreading of *F. johnsoniae***

As previously found (20-22), glucose suppressed colony spreading of *F. johnsoniae* on PY2 plates in a concentration-dependent manner, and D-glucose with 10 mM completely inhibited colony spreading (Fig. 1A and B). A nonmetabolizable derivative

of glucose, 2-deoxy-D-glucose (15 mM), partially suppressed colony spreading (Fig. 1C), suggesting that there were two types of suppression: metabolism-dependent and metabolism-independent.

**Construction of a transposon insertion library and screening on the basis of colony spreading**

Transposon-containing suicide plasmid R751::Tn*4351*Ω4 was used for mutagenesis of *F. johnsoniae* strain UW101. Cells were grown on agar plates supplemented with 15 mM glucose and 100 mM Em. Seventeen colonies showing higher levels of colony spreading compared to the wild type were found among nearly 48,000 colonies. The transposon insertion sites in all the mutants were determined by DNA sequencing. Fjoh_4565, which encode a major facilitator superfamily (MFS) transporter, was present at the insertion site of one of the mutants (Fig. 2A). Fjoh_4565 was recently shown to be required for growth on glucose, N-acetyl-glucosamine, and chitin (28). We tentatively named this gene *mfsA*. We constructed an *mfsA* deletion mutant (DFJ1), which partially restored colony spreading on the 15 mM glucose-containing plate (Fig. 2B and Fig. S1). *E. coli–F. johnsoniae* shuttle vector plasmids containing the *mfsA*$^+$ and *mfsA-gfp* fusion genes were then introduced into strain DFJ1, resulting in *mfsA*/*mfsA*$^+$ and *mfsA*/*mfsA-gfp* complemented strains (DFJ1/pDF2 and DFJ1/pDF3). Complemented strains DFJ1/pDF2 and DFJ1/pDF3 showed no colony spreading on a 15 mM glucose-containing plate, just as the wild type did (Fig. 2B and Fig. S1). The other sixteen mutants had the transposon DNA in different genes and the results will be reported elsewhere.

**Growth of the *mfsA* mutant in media with or without glucose**

The *mfsA* mutant, the *mfsA*/*mfsA*$^+$ complemented strain, and the wild type were incubated in mPY2 with or without 15 mM glucose, and growth of the strains was determined via optical density at 600 nm. Addition of glucose resulted in increased growth of the wild-type and *mfsA*/*mfsA*$^+$ complemented strains, whereas the growth of the *mfsA* mutant was not changed by addition of glucose (Fig. 2C).

**Glucose uptake in the *mfsA* mutant**

Glucose uptake of the *mfsA* mutant, of the *mfsA*/*mfsA*$^+$ complemented strain, and of the wild type was determined. The *mfsA*/*mfsA*$^+$ complemented strain and the wild type showed glucose uptake, whereas the *mfsA* mutant showed no glucose uptake (Fig. 3A). Glucose uptake of the wild type was decreased by proton motive force inhibitors, CCCP and DNP, but not decreased by ATPase inhibitors, arsenate, and DCCD, indicating that the glucose uptake system in *F. johnsoniae* depends on proton motive force (Fig. 3B).

**Gene expression in the *mfsA* mutant**

To determine which genes are influenced by *mfsA*, microarray analysis of the *mfsA* mutant, which was grown in agar plates supplemented with 15 mM glucose, was performed, and the result was compared to that of the wild type grown in the glucose-supplemented agar plates. The ratio of expression of each gene in the *mfsA* mutant with glucose versus that in the wild type with glucose was compared with the ratio of expression of each gene in the wild type without glucose versus that in the wild type with glucose (Fig. 4). The result revealed that gene expression in the *mfsA* mutant correlated with that in the wild type without glucose. The 100 genes most upregulated

and downregulated by the disruption of *mfsA* (*mfsA* with glucose versus the wild type with glucose) were compared with those under the influence of depletion of glucose in the wild type (wild type without glucose versus wild type with glucose) (Tables S2 and S3). Seventy-seven and 87 of the 100 upregulated and downregulated genes, respectively, were common between the two comparisons. These results suggested that the *mfsA* mutant experienced glucose starvation even when glucose was added into the medium.

**Location of the MfsA protein**

To determine intracellular localization of MfsA, we used the *mfsA*/*mfsA-gfp* fusion strain. Using fluorescence microscopy, we found that green fluorescence was located around the cell (Fig. 5A), suggesting that MfsA is located in the cell surface membranes. FM4-64 (red) and DAPI (blue) were used to indicate the areas of lipid layers and cytoplasm, respectively (Fig. 5B, C, and D).

**Complementation of the glucose-negative phenotype in *E. coli* by the *mfsA* gene**

We tested whether *F. johnsoniae* MfsA can complement the glucose-negative phenotype in *E. coli.* The *mfsA* and *mfsA-gfp* genes were placed after the T7 promoter in plasmid pET-22b, resulting in vectors pDF4 and pDF5, respectively. The glucose-negative *E. coli* strain LJ141 that lacks detectable glucose transport activity was then transformed with pDF4 and pDF5. The transformed *E. coli* strains were streaked onto MacConkey agar plates supplemented with 50 mM glucose. Strains LJ141/pDF4 and LJ141/pDF5 formed red colonies because of the fermentation of glucose, whereas strains LJ141 and LJ141 carrying the vector plasmid pET-22b showed non-glucose-fermenting ocher

colonies, demonstrating that *F. johnsoniae* MfsA can function as a glucose transporter in *E. coli* (Fig. 6). Strains LJ141/pDF4 and LJ141/pDF5 did not form red colonies on MacConkey agar supplemented with 50 mM mannose or mannitol, suggesting that MfsA has no contribution to the uptake of mannose or mannitol (Fig. 6).

## DISCUSSION

The results presented here illustrate the role of *mfsA* in glucose inhibition of colony spreading. A recent study by Larsbrink et al (28) identified a locus containing *mfsA* and 10 other genes that were involved in *F. johnsoniae* chitin utilization. *mfsA* was shown to be required for growth on glucose, N-acetylglucosamine, and chitin. Our results confirm and extend these findings. Genome information on *F. johnsoniae* reveals that it has no phosphotransferase system but has 8 genes encoding putative major MFS transporters. The MFS is one of the largest groups of secondary active transporters conserved from bacteria to humans. In this study, we found that (i) the *mfsA* mutant showed no glucose uptake, (ii) the *mfsA* mutant did not utilize glucose for its growth, and (iii) the *mfsA* gene complemented the glucose-negative phenotype of *E. coli* LJ141. The present findings with the previous one (28) strongly indicate that *mfsA* (Fjoh_4565, which is one of the 8 above-mentioned genes) encodes the sole glucose transporter in *F. johnsoniae.* Comparison with proteins in the MFS family using the IUBMB-approved Transporter Classification Database (www.tcbd.org) revealed that a protein most similar to MfsA is glucose/galactose transporter Ggp (2.A.1.7.2) in *Brucella abortus*, which belongs to the fucose: H+ symporter (FHS) family (2.A.1.7) and that the top 13 proteins similar to MfsA belong to the FHS family. These 13 proteins including Ggp in *B. abortus* have 12 transmembrane segments (TMSs) except for one protein, which has 11

TMSs. On the other hand, MfsA appears to have 14 TMSs (Fig. S2). In this study, we found that MfsA requires proton motive force for its glucose uptake; this finding is consistent with the comparison result, which suggested that MfsA may belong to the FHS family.

In 1947, Stanier (6) reported that *F. johnsoniae* cells form spreading colonies on nutrient-poor plates. The cells formed rather small colonies with smooth edges on a plate with 2.0% tryptone, whereas they formed larger colonies with irregular, feathery edges on a plate containing 0.25% tryptone. Carbohydrates such as glucose, maltose, glucosamine, *N*-acetylglucosamine, sucrose, and trehalose can suppress colony spreading of *F. johnsoniae* (21). Most of the carbohydrates are metabolized by *F. johnsoniae*, but this bacterium cannot utilize sucrose as an energy source. Nevertheless, sucrose inhibits colony spreading. Similarly, a nonmetabolizable derivative of glucose, 2-deoxy-glucose, also inhibits colony spreading although the inhibitory effect was much weaker than that of D-glucose, suggesting that there may be two types of the carbohydrate-mediated inhibitory effect: metabolism-dependent and metabolism-independent. In this study in *F. johnsoniae*, we created mutations that suppress the effect of glucose on colony spreading. They included the mutant possessing the transposon DNA in the *mfsA* gene, which encode an MFS protein. These results suggest that the glucose-mediated inhibitory effect on colony spreading is at least partly attributable to glucose uptake. Further research is needed to find which metabolite(s) in the metabolic pathway inhibits colony spreading.

**ACKNOWLEDGMENT**

We thank Drs. K. Jahreis and S. Chen for generous gifts of *E. coli* LJ141 and the shuttle

plasmid pFj29, respectively. This work was supported by the Japan Society for the Promotion of Science Kakenhi Grants (Grant IDs 24117006 and 25293375 to KN).

## DISCLOSURE

The authors have no conflicting financial interests.

**REFERENCES**


1. Jarrell K.F., McBride M.J. (2008) The surprisingly diverse ways that prokaryotes move. *Nat Rev Microbiol* **6**: 466-76.
2. McBride M.J., Nakane D. (2015) Flavobacterium gliding motility and the type IX secretion system. *Curr Opin Microbiol* **28**: 72-7.
3. Sato K. Naito M., Yukitake H., Hirakawa H., Shoji M., McBride M.J., Rhodes R.G., Nakayama K. (2010) A protein secretion system linked to bacteroidete gliding motility and pathogenesis. *Proc Natl Acad Sci U S A* **107**: 276-81.
4. Nakayama K. (2015) *Porphyromonas gingivalis* and related bacteria: from colonial pigmentation to the type IX secretion system and gliding motility. *J Periodont Res* **50**: 1-8.
5. Nakane D., Sato K., Wada H., McBride M.J., Nakayama K. (2013) Helical flow of surface protein required for bacterial gliding motility. *Proc Natl Acad Sci U S A* **110**: 11145-50.
6. Stanier R.Y., (1947) Studies on nonfruiting myxobacteria I. *Cytophaga johnsoae* sp., a chitin-decomposing myxobacterium. *J Bacteriol* **53**: 297-315.
7. Agarwal S., Hunnicutt D.W., McBride M.J. (1997) Cloning and characterization of the *Flavobacterium johnsoniae* (*Cytophaga johnsonae*) gliding motility gene, *gldA*. *Proc Natl Acad Sci U S A* **94**: 12139-44.
8. Braun T.F., Khubbar M.K., Saffarini D.A., McBride M.J. (2005) *Flavobacterium johnsoniae* gliding motility genes identified by mariner mutagenesis. *J Bacteriol* **187**: 6943-52.
9. Braun T.F., McBride M.J. (2005) *Flavobacterium johnsoniae* GldJ is a lipoprotein that is required for gliding motility. *J Bacteriol* **187**: 2628-37.

10. Hunnicutt D.W., Kempf M.J., McBride M.J. (2002) Mutations in *Flavobacterium johnsoniae gldF* and *gldG* disrupt gliding motility and interfere with membrane localization of GldA. *J Bacteriol* **184**: 2370-8.
11. Hunnicutt D.W., McBride M.J. (2000) Cloning and characterization of the *Flavobacterium johnsoniae* gliding motility genes *gldB* and *gldC*. J Bacteriol 182:911-8.
12. Hunnicutt D.W., McBride M.J. (2001) Cloning and characterization of the *Flavobacterium johnsoniae* gliding motility genes *gldD* and *gldE*. *J Bacterial* **183**: 4167-75.
13. McBride M.J., Braun D.W. (2004) GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *J Bacteriol* **186**: 2295-302.
14. McBride M.J., Braun T.F., Brust J.L. (2003) *Flavobacterium johnsoniae* GldH is a lipoprotein that is required for gliding motility and chitin utilization. *J Bacteriol* **185**: 6648-57.
15. Rhodes R.G., Samarasam M.N., Shrivastava A. van Baaren J.M., Pochiraju S., Bollampalli S., McBride M.J. (2010) *Flavobacterium johnsoniae gldN* and *gldO* are partially redundant genes required for gliding motility and surface localization of SprB. *J Bacteriol* **192**: 1201-11.
16. Shrivastava A., Rhodes R.G., Pochiraju S., Nakane D., McBride M.J. (2012) *Flavobacterium johnsoniae* RemA is a mobile cell surface lectin involved in gliding. *J Bacteriol*. **194**: 3678-88.

17. Rhodes R.G., Samarasam M.N., Van Groll E.J., McBride M.J. (2011) Mutations in *Flavobacterium johnsoniae sprE* result in defects in gliding motility and protein secretion. *J Bacteriol*. **193**: 5322-7.

18. Rhodes R.G., Pucker H.G., McBride M.J. (2011) Development and use of a gene deletion strategy for *Flavobacterium johnsoniae* to identify the redundant gliding motility genes *remF, remG, remH,* and *remI*. *J Bacteriol*. **193**: 2418-28.

19. Rhodes R.G., Nelson S.S., Pochiraju S., McBride M.J. (2011) *Flavobacterium johnsoniae sprB* is part of an operon spanning the additional gliding motility genes *sprC, sprD*, and *sprF*.*J Bacteriol* **193**: 599-610.

20. Chang L.E. Pate J.L. (1981) Nutritional requirements of *Cytophaga johnsonae* and some of its auxotrophic mutants. *Curr Microbiol* **5**: 235-40.

21. Wolkin R.H., Pate J.L. (1984) Translocation of motile cells of the gliding bacterium *Cytophaga johnsonae* depends on a surface component that may be modified by sugars. *J Gen Microbiol* **130**: 2651-69.

22. Gorski L., Godchaux III W., Leadbetter E.R. (1993) Structural specificity of sugars that inhibit gliding motility of *Cytophaga johnsonae*. *Arch Microbiol* **60**: 121-5.

23. Simon R., Priefer U., Puhler A. (1983) A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram negative bacteria. *Bio/Technology* **2**:784-91.

24. Chen S., Bagdasarian M., Kaufman M.G., Bates A.K., Walker E.D. (2007) Mutational analysis of the *ompA* promoter from *Flavobacterium johnsoniae*. *J Bacteriol* **189**:5108–18.

25. McBride M.J., Baker S.A. (1996) Development of techniques to genetically manipulate members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and S*porocytophaga*. *Appl Environ Microbiol* **62**: 3017-22.
26. Monden M., Koyama H., Otsuka Y., Morioka T., Mori K., Shoji T., Mima Y., Motoyama K., Fukumoto S., Shioi A., Emoto M., Yamamoto Y., Yamamoto H., Nishizawa Y., Kurajoh M., Yamamoto T., Inaba M. (2013) Receptor for Advanced Glycation End Products Regulates Adipocyte Hypertrophy and Insulin Sensitivity in Mice. *Diabetes* **62**: 478-89.
27. Saito K., Lee S., Shiuchi T., Toda C., Kamijo M., Inagaki-Ohara K., Okamoto S., Minokoshi Y. (2011) An enzymatic photometric assay for 2-deoxyglucose uptake in insulin-responsive tissues and 3T3-L1 adipocytes. *Anal Biochem* **412**:9–17
28. Larsbrink J., Zhu Y., Kharade S.S., Kwiatkowski K.J., Eijsink V.G.H., Koropatkin N.M., McBride M.J., Pope P.B. (2016) A polysaccharide utilization locus from *Flavobacterium johnsoniae* enables conversion of recalcitrant chitin. *Biotechnol Biofuels* **9**: 260.

## FIGURE LEGENDS

**Fig. 1. The inhibitory effect of D-glucose on colony spreading.** (A) *F. johnsoniae* strains wild type (UW101) and *gldJ* mutant (UW102-55) were grown on PY2 agar with or without 15 mM D-glucose at 25°C for 96 h. (B) A concentration-dependent inhibitory effect of D-glucose on colony spreading. (C) Effects of 2-deoxy-D-glucose on colony spreading.

**Fig. 2. Insertion sites of transposon-mediated mutations, and the growth of the *mfsA* mutant on a PY2 plate with D-glucose and in mPY2 broth with or without D-glucose.** (A) Insertion site of Tn*4351* in Fjoh_4565 (*mfsA*). (B) Colonies of *F. johnsoniae* strains: wild type (CJ1827), *ΔmfsA* (DFJ1), *ΔmfsA* with a vector plasmid (DFJ1/pNS1), *ΔmfsA*/pNS1 containing $mfsA^+$ (DFJ1/pDF2), and *ΔmfsA*/pNS1 containing *mfsA-gfp* (DFJ1/pDF3) on PY2 agar with 15 mM D-glucose after 5 days incubation at 25ºC. (C) Growth of the wild type, *ΔmfsA*, and *ΔmfsA*/pNS1 containing $mfsA^+$ (pDF2) in mPY2 broth with (red) or without (blue) 15 mM D-glucose.

**Fig. 3. Glucose uptake of *F. johnsoniae* strains.** (A) *F. johnsoniae* strains—wild type, *ΔmfsA*/pNS1 containing $mfsA^+$ (pDF2), and *ΔmfsA*/pNS1 containing *mfsA-gfp* (pDF3)—were grown in the CYE medium at 27°C to optical density of ~1.0 at 600 nm. After two washes with 10 mM Tris-HCl buffer (pH 7.5), the cells were incubated in the buffer containing 2-deoxy-D-glucose at RT for 2 h. Glucose uptake was measured by an enzymatic photometric assay. (B) *F. johnsoniae* wild-type cells were treated with CCCP, DNP, arsenate, or DCCD. ＊: $P<0.001$.

**Fig. 4. Comparison of gene expression between the *mfsA* mutant with glucose and the wild type without glucose.** Ratio of expression of each gene in the *mfsA* mutant with glucose versus that in the wild type with glucose was compared with ratio of expression of each gene in the wild type without glucose versus that in the wild type with glucose.

**Fig. 5. Subcellular localization of MfsA.** Cells were examined by microscopy to identify the location of MfsA. To stain DNA and cell membranes, DAPI (Invitrogen) and FM4-64 (Invitrogen) were used, respectively. A, GFP fluorescence; B, FM4-64 fluorescence; C, DAPI fluorescence; D, Merging of A, B, and C. All the images were captured at 100× magnification.

**Fig. 6. Complementation of the glucose-negative phenotype of *E. coli*.** *E. coli* strains LJ141 (glucose-negative), LJ141 harboring pET-22b, LJ141 harboring pET-22b containing $mfsA^+$ (pDF4), and LJ141 harboring pET-22b containing *mfsA-gfp* (pDF5) were streaked on MacConkey agar plates supplemented with 50 mM glucose, mannose, and mannitol. Red colonies indicate the fermentation of sugars, whereas ocher colonies reflect a deficiency in sugar fermentation.

**Supporting Information**

**Table S1. Primers.**

**Table S2. Upregulated genes.** The 100 genes most upregulated by the disruption of

*mfsA* (*mfsA* with glucose versus the wild type with glucose) were compared with those under the influence of depletion of glucose in the wild type (wild type without glucose versus wild type with glucose).

**Table S3. Downregulated genes.** The 100 genes most downregulated by the disruption of *mfsA* (*mfsA* with glucose versus the wild type with glucose) were compared with those under the influence of depletion of glucose in the wild type (wild type without glucose versus wild type with glucose).

**Fig. S1. Colony spreading of *F. johnsoniae* strains on PY2 agar with or without glucose**. *F. johnsoniae* strains were incubated on PY2 agar with 5 mM glucose (A), with 15 mM glucose (B) and without glucose (C) for 5 days at 25ºC. Panel A: 1, wild type (CJ1827); 2, *ΔmfsA*/pNS1 containing *mfsA-gfp* (DFJ1/pDF3); 3, *ΔmfsA* with a vector plasmid (DFJ1/pNS1); 4, *ΔmfsA* (DFJ1); 5, *ΔmfsA*/pNS1 containing $mfsA^+$ (DFJ1/pDF2). Panel B: 1, wild type (CJ1827); 2, *ΔmfsA* with a vector plasmid (DFJ1/pNS1); 3, *ΔmfsA*/pNS1 containing *mfsA-gfp* (DFJ1/pDF3); 4, *ΔmfsA* (DFJ1); 5, *ΔmfsA*/pNS1 containing $mfsA^+$ (DFJ1/pDF2). Panel C: 1, wild type (CJ1827); 2, *ΔmfsA*/pNS1 containing $mfsA^+$ (DFJ1/pDF2); 3, *ΔmfsA*/pNS1 containing *mfsA-gfp* (DFJ1/pDF3); 4, *ΔmfsA* with a vector plasmid (DFJ1/pNS1).

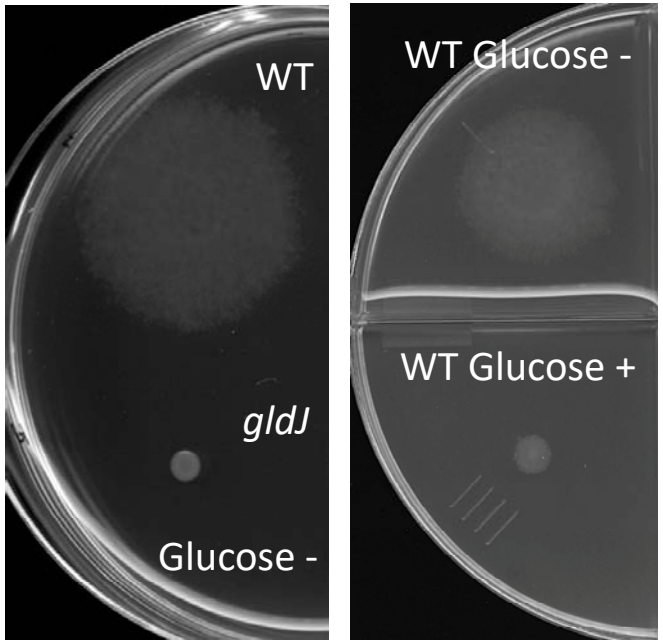
**Fig. S2. Transmembrane segments of the MfsA protein.**

**Table 1. Bacterial strains and plasmids used in this study**

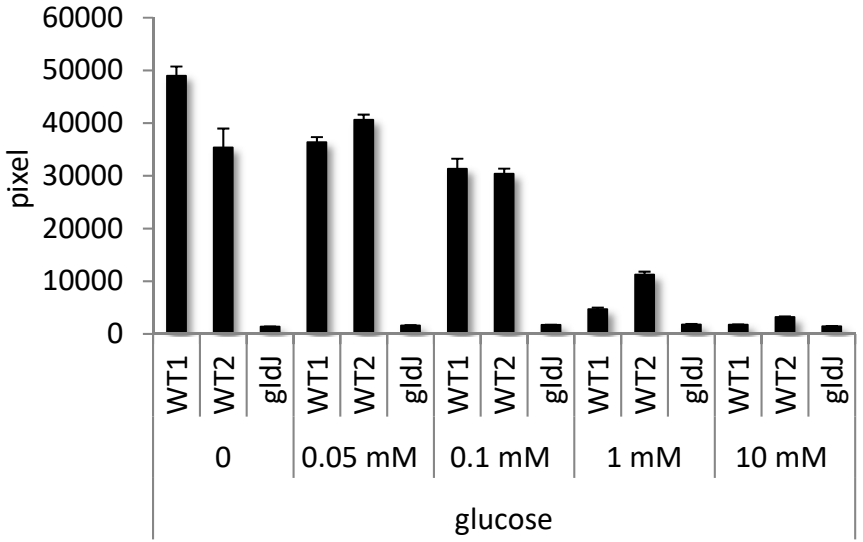
| Strain | Description | Reference or source |
|---|---|---|
| *E. coli* strain | | |
| S17-1 λ*pir* | *hsdR17* (rK$^-$ mK$^-$) *recA* RP4-2-Tc::Mu *aph* ::Tn7 λ*pir* lysogen, Sm$^r$ | 23 |
| LJ141 | W3110 Δ[*ptsHI crr* ]::*kan galP* ::*cam mgl500* ::Tn*10* | K. Jahreis |
| *F. johnsoniae* strain | | |
| UW101 | wild type | ATCC |
| CJ1827 | WT (*rps1* ), Background UW101 | 18 |
| UW102-55 | *gldJ* | 9 |
| DFJ1 | *Δ mfsA* | this study |
| *F. johnsoniae* plasmid | | |
| pFj29 | Ap$^r$ Em$^r$, *E. coli-F. johnsoniae* shuttle plasmid | 24 |
| pNS1 | Ap$^r$ Em$^r$, *E. coli-F. johnsoniae* shuttle plasmid | this study |
| pDF1 | Apr, pRR51 containing *mfsA* upstream and downstream regions | this study |
| pDF2 | Ap$^r$ Em$^r$, pNS1 containing *mfsA* $^+$ | this study |
| pDF3 | Ap$^r$ Em$^r$, pNS1 containing *mfsA-gfp* | this study |
| pRR51 | suicide vector | 18 |
| *E. coli* plasmid | | |
| pET-22b | Ap$^r$ , expression vector | Novagen |
| pDF4 | Ap$^r$, pET-22b containing *mfsA* $^+$ | this study |
| pDF5 | Ap$^r$, pET-22b containing *mfsA-gfp* | this study |

Fig. 1

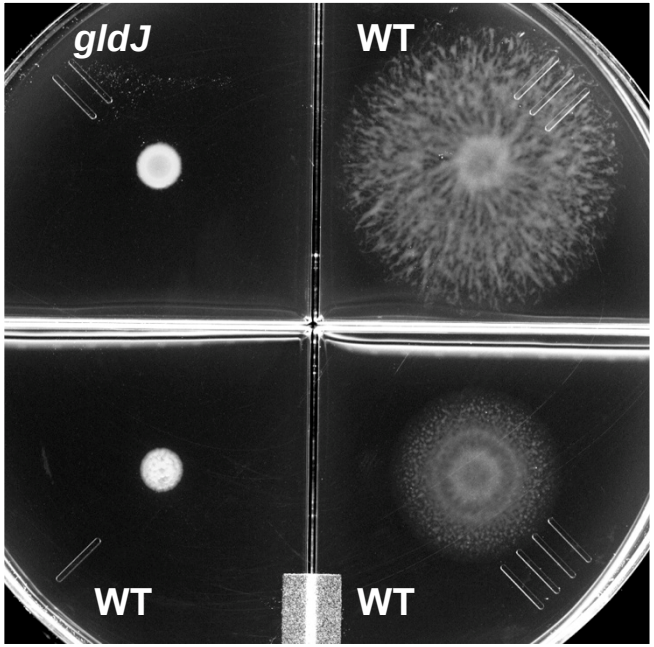
A




B




C

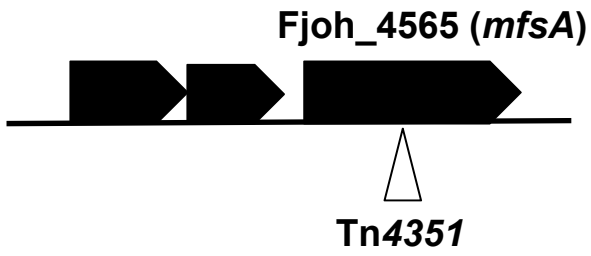



15 mM D-glucose

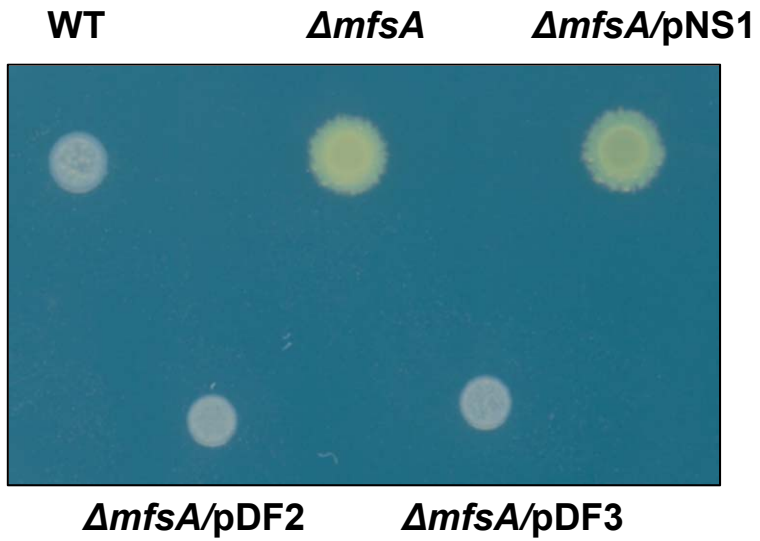
15 mM 2-Deoxy-D-Glucose

Fig. 2

A


Fjoh_4565 (*mfsA*)
Tn*4351*

B


WT
*ΔmfsA*
*ΔmfsA*/pNS1
*ΔmfsA*/pDF2
*ΔmfsA*/pDF3

C

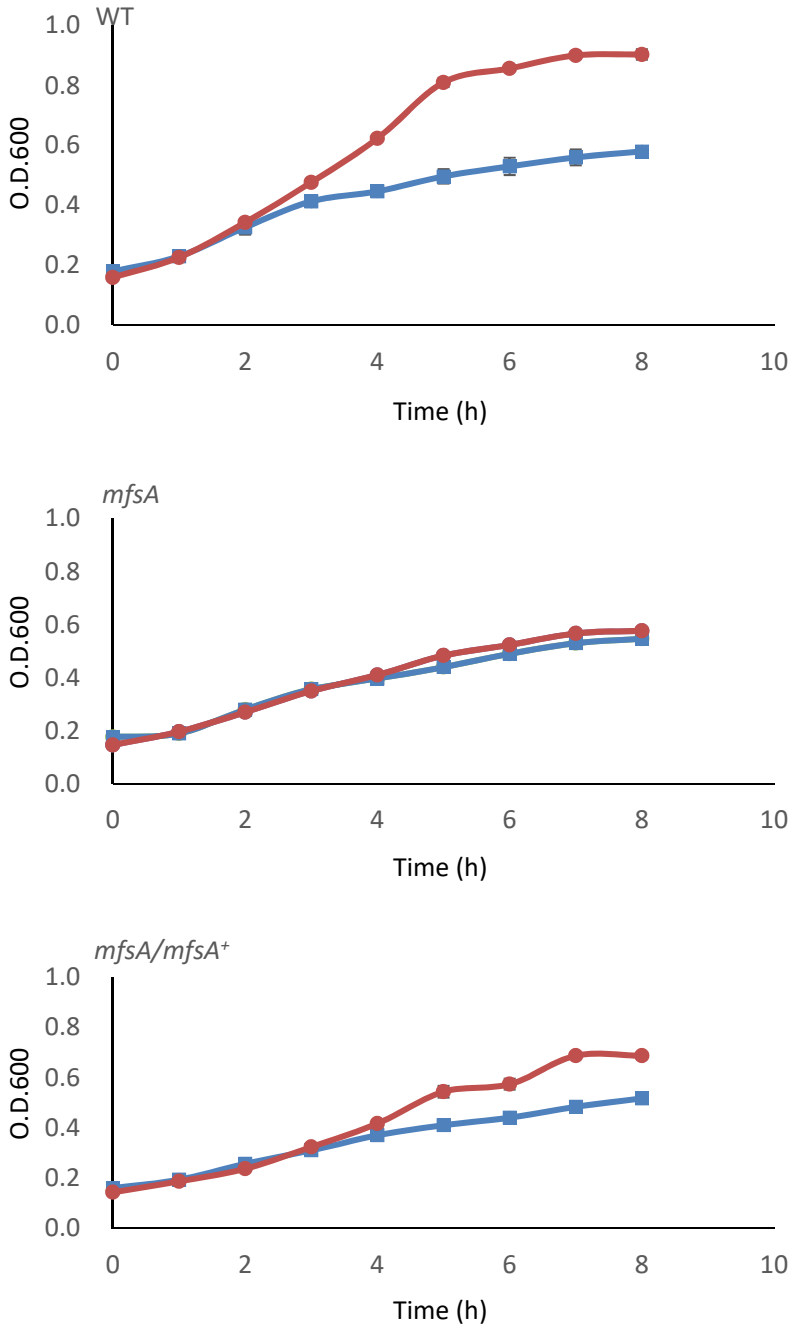

WT
*mfsA*
*mfsA/mfsA⁺*
O.D.600
Time (h)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10

Fig. 3

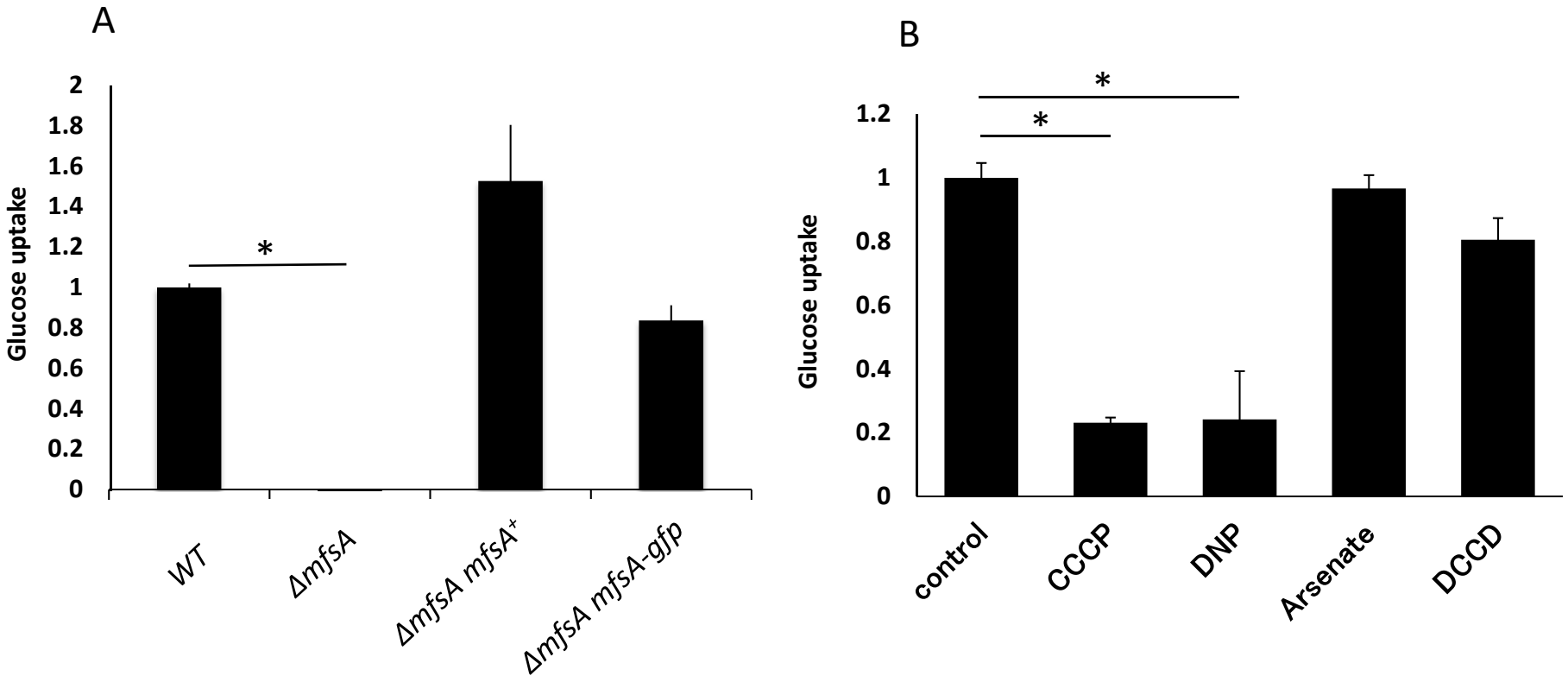

A
Glucose uptake
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
WT
ΔmfsA
ΔmfsA mfsA+
ΔmfsA mfsA-gfp
B
Glucose uptake
1.2
1
0.8
0.6
0.4
0.2
0
*
*
control
CCCP
DNP
Arsenate
DCCD

Fig. 4


y = 1.2639x
10.0000
9.0000
8.0000
7.0000
6.0000
5.0000
4.0000
3.0000
2.0000
1.0000
0.0000
0.0000
1.0000
2.0000
3.0000
4.0000
5.0000
6.0000
7.0000
8.0000
9.0000
10.0000
ΔmfsA with glucose / WT with glucose
WT without glucose / WT with glucose

Fig. 5

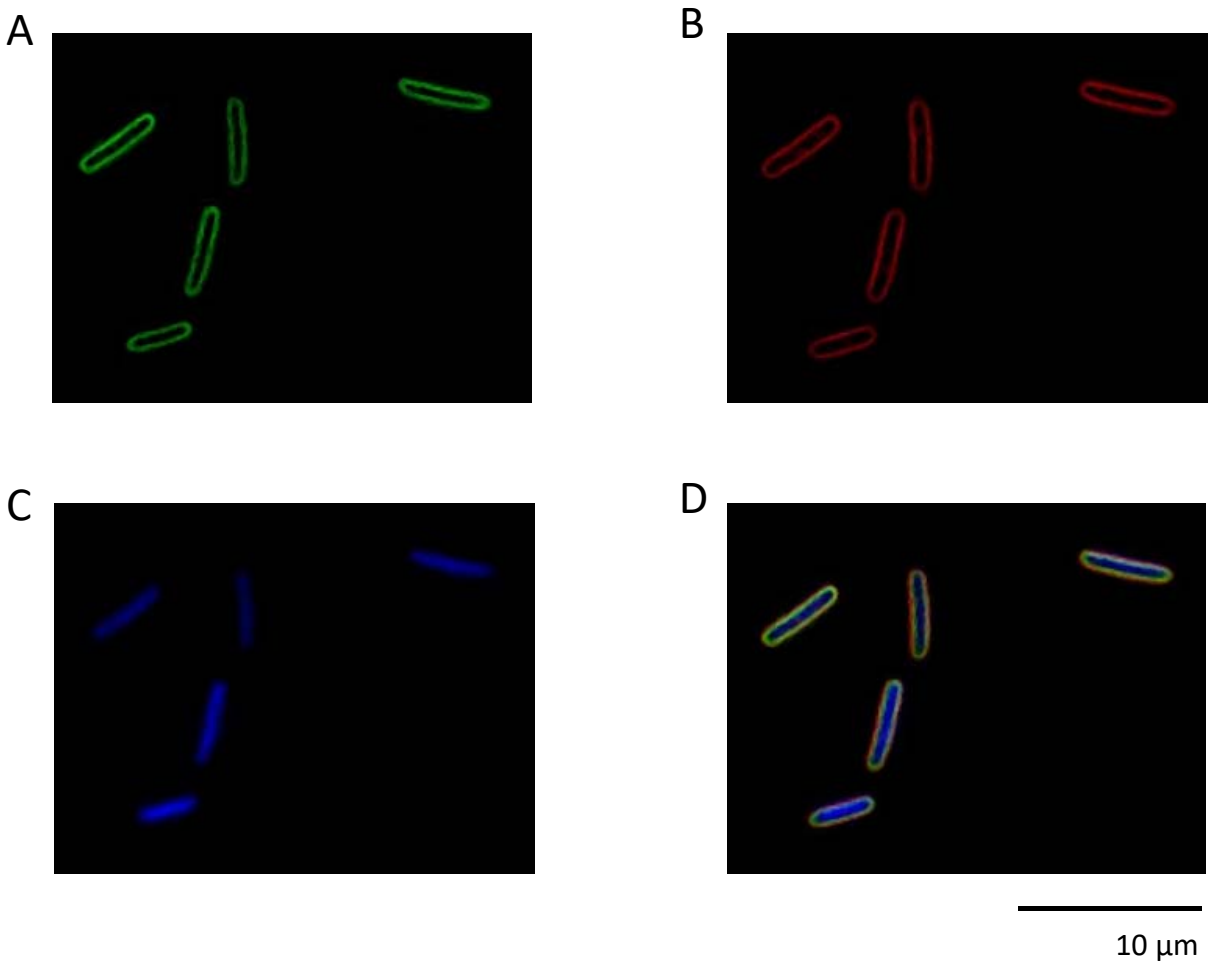


Fig. 6

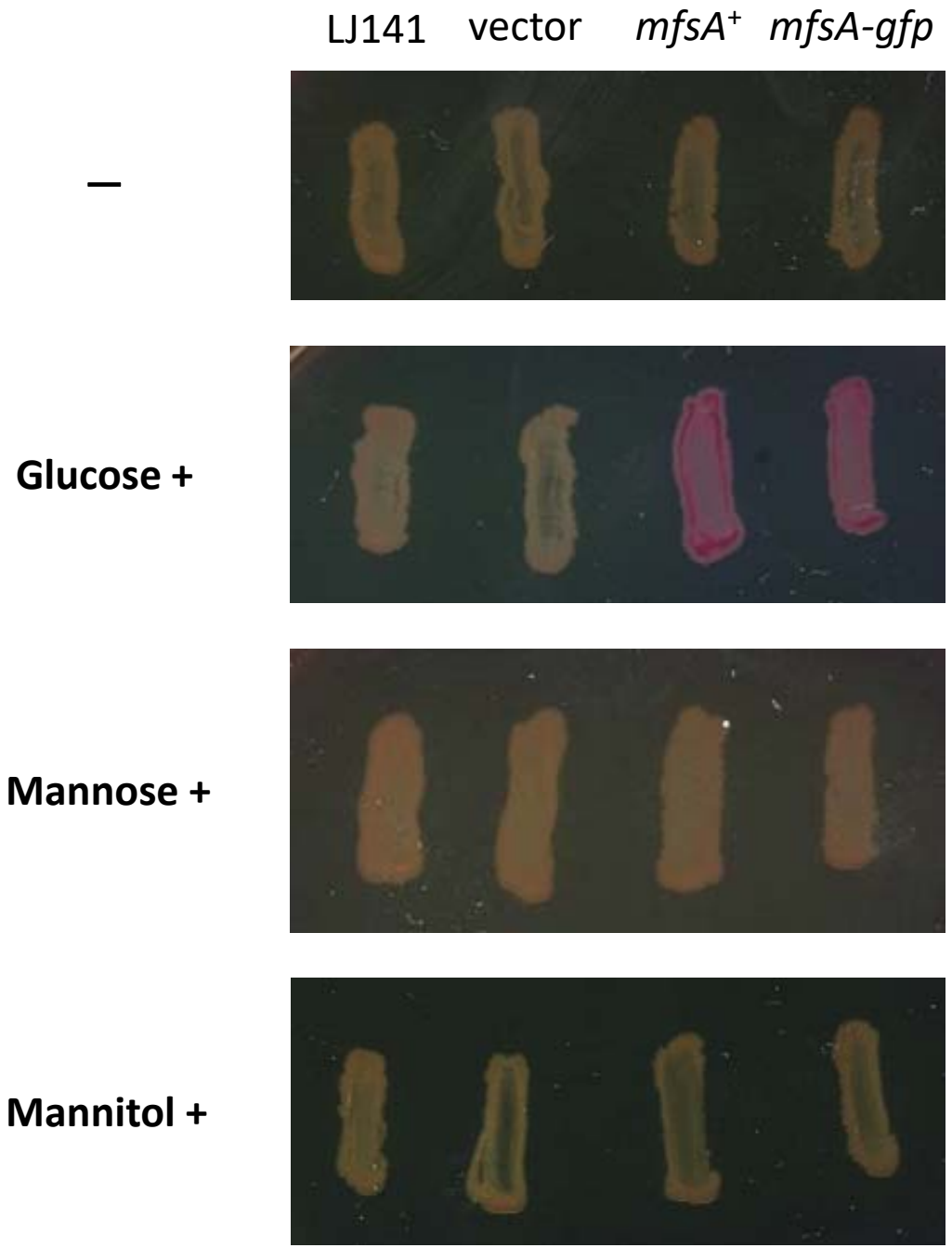


Table S1. Primers.

F4565-UF-BamHI: GGATCCTATACGAAAATGCCAAAACATCCC
F4565-UR-SalI: GTCGACTACTGGAATCAGGATGTCATTGGC
F4565-DF-SalI: GTCGACCTCTTATGTAGTACCACTTATTGG
F4565-DR-SphI: GCATGCTCCTCTTGTGGCTTTAGACGTTCG
F4565-F-BamHI: GGATCCATGAGTTCAGAAAATGTTCAAACC
F4565-stop-R-NotI: GCGGCCGCATTAGTGTCCGCCGCCTTCGCT
F4565-GR-NotI: GCGGCCGCAGTGTCCGCCGCCTTCGCTTTC
pFj29-1st-F: GGATCCGGTACCGATATGGCGGCCGCAGTAAAGGAGAAGAAC
pFj29-2nd-F: AGATCTCTTTAAGAAGGAGATATACATATGGGATCCGGTACCGATATG
Gfpmut3-R-SphI: GCATGCTTATTTGTATAGTTCATCCATGCC
F4565-22bF-NdeI: CATATGAGTTCAGAAAATGTTCAAACCAAA
F4565-22bR-XhoI: CTCGAGGTGTCCGCCGCCTTCGCTTTCAAC
GfpmutS-stopR-XhoI: CTCGAGTTATTTGTATAGTTCATCCATGCC

Table S2. Upregulated genes.

| | Δ*mfsA* with glucose versus WT with glucose | | | | WT without glucose versus WT with glucose | | | |
|---|---|---|---|---|---|---|---|---|
| | Fjoh | definition | ratio | *t*-test | Fjoh | definition | ratio | *t*-test |
| 1 | Fjoh_3856 | Fjoh_3856-hypothetical protein | 6436 | 8.74E-11 | Fjoh_3856 | Fjoh_3856-hypothetical protein | 4616 | 3.91E-08 |
| 2 | Fjoh_2478 | Fjoh_2478-hypothetical protein | 2445 | 1.01E-05 | Fjoh_2478 | Fjoh_2478-hypothetical protein | 1589 | 2.08E-05 |
| 3 | Fjoh_2477 | Fjoh_2477-cytochrome-c peroxidase | 1045 | 6.04E-08 | Fjoh_4848 | Fjoh_4848-hypothetical protein | 889 | 0.002141 |
| 4 | Fjoh_3855 | Fjoh_3855-hypothetical protein | 1014 | 3.37E-07 | Fjoh_2477 | Fjoh_2477-cytochrome-c peroxidase | 855 | 3.06E-07 |
| 5 | Fjoh_3854 | Fjoh_3854-hypothetical protein | 867 | 3.81E-07 | Fjoh_4849 | Fjoh_4849-hypothetical protein | 697 | 1.17E-05 |
| 6 | Fjoh_3857 | Fjoh_3857-TonB-dependent receptor | 650 | 2.17E-05 | Fjoh_4850 | Fjoh_4850-hypothetical protein | 610 | 3.8E-08 |
| 7 | Fjoh_3853 | Fjoh_3853-peptidase S41 | 586 | 3.63E-06 | Fjoh_3855 | Fjoh_3855-hypothetical protein | 606 | 8.54E-05 |
| 8 | Fjoh_0549 | Fjoh_0549-hypothetical protein | 466 | 3.44E-08 | Fjoh_1960 | Fjoh_1960-hypothetical protein | 605 | 5.91E-06 |
| 9 | Fjoh_0852 | Fjoh_0852-hypothetical protein | 432 | 7.19E-09 | Fjoh_4847 | Fjoh_4847-hypothetical protein | 567 | 0.006007 |
| 10 | Fjoh_3858 | Fjoh_3858-FecR anti-FecI sigma factor | 422 | 0.000156 | Fjoh_1959 | Fjoh_1959-hypothetical protein | 425 | 0.000585 |
| 11 | Fjoh_1147 | Fjoh_1147-response regulator receiver protein | 386 | 5.69E-05 | Fjoh_3854 | Fjoh_3854-hypothetical protein | 402 | 1.28E-06 |
| 12 | Fjoh_1148 | Fjoh_1148-meta-pathway phenol degradation-like protein | 307 | 0.00022 | Fjoh_0549 | Fjoh_0549-hypothetical protein | 397 | 1.97E-07 |
| 13 | Fjoh_2894 | Fjoh_2894-TonB-dependent receptor, plug | 290 | 1.22E-07 | Fjoh_1958 | Fjoh_1958-hypothetical protein | 390 | 0.0132 |
| 14 | Fjoh_3852 | Fjoh_3852-hypothetical protein | 287 | 7.09E-09 | Fjoh_1833 | Fjoh_1833-hypothetical protein | 324 | 2.45E-22 |
| 15 | Fjoh_4168 | Fjoh_4168-hypothetical protein | 277 | 6.6E-09 | Fjoh_1147 | Fjoh_1147-response regulator receiver protein | 289 | 0.000191 |
| 16 | Fjoh_1149 | Fjoh_1149-hypothetical protein | 262 | 0.004941 | Fjoh_1957 | Fjoh_1957-ATPase central domain-containing protein | 257 | 1.57E-06 |
| 17 | Fjoh_4169 | Fjoh_4169-TonB-dependent receptor | 223 | 6E-06 | Fjoh_2894 | Fjoh_2894-TonB-dependent receptor, plug | 240 | 9.35E-07 |
| 18 | Fjoh_4889 | Fjoh_4889-hypothetical protein | 185 | 5.7E-07 | Fjoh_2102 | Fjoh_2102-amino acid adenylation protein | 229 | 1.63E-07 |
| 19 | Fjoh_2102 | Fjoh_2102-amino acid adenylation protein | 171 | 1.47E-07 | Fjoh_1148 | Fjoh_1148-meta-pathway phenol degradation-like protein | 218 | 0.000551 |
| 20 | Fjoh_2892 | Fjoh_2892-hypothetical protein | 163 | 4.56E-09 | Fjoh_3857 | Fjoh_3857-TonB-dependent receptor | 208 | 4.87E-05 |
| 21 | Fjoh_2893 | Fjoh_2893-hypothetical protein | 160 | 2.07E-09 | Fjoh_4168 | Fjoh_4168-hypothetical protein | 207 | 4.99E-08 |
| 22 | Fjoh_3414 | Fjoh_3414-hypothetical protein | 141 | 3.21E-06 | Fjoh_2688 | Fjoh_2688-SH3 type 3 domain-containing protein | 205 | 7.88E-06 |
| 23 | Fjoh_2891 | Fjoh_2891-hypothetical protein | 136 | 8.48E-09 | Fjoh_3853 | Fjoh_3853-peptidase S41 | 195 | 9.36E-06 |
| 24 | Fjoh_2103 | Fjoh_2103-alpha/beta hydrolase fold protein | 116 | 1.13E-06 | Fjoh_4855 | Fjoh_4855-hypothetical protein | 194 | 2.96E-08 |
| 25 | Fjoh_4177 | Fjoh_4177-glycoside hydrolase | 101 | 1.97E-06 | Fjoh_3858 | Fjoh_3858-FecR anti-FecI sigma factor | 176 | 0.001114 |
| 26 | Fjoh_2098 | Fjoh_2098-TonB-dependent receptor | 101 | 6.4E-05 | Fjoh_0852 | Fjoh_0852-hypothetical protein | 175 | 4.49E-08 |
| 27 | Fjoh_3477 | Fjoh_3477-hypothetical protein | 99 | 7.76E-07 | Fjoh_1149 | Fjoh_1149-hypothetical protein | 173 | 0.006355 |
| 28 | Fjoh_3171 | Fjoh_3171-pyridoxal-dependent decarboxylase | 98 | 2.38E-07 | Fjoh_3227 | Fjoh_3227-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 152 | 5.49E-05 |
| 29 | Fjoh_4723 | Fjoh_4723-endonuclease I | 95 | 8.98E-09 | Fjoh_4169 | Fjoh_4169-TonB-dependent receptor | 149 | 2.52E-05 |
| 30 | Fjoh_3841 | Fjoh_3841-hypothetical protein | 93 | 4.58E-05 | Fjoh_2103 | Fjoh_2103-alpha/beta hydrolase fold protein | 148 | 5.92E-07 |
| 31 | Fjoh_3478 | Fjoh_3478-hypothetical protein | 93 | 1.95E-08 | Fjoh_2892 | Fjoh_2892-hypothetical protein | 143 | 3.47E-08 |
| 32 | Fjoh_3401 | Fjoh_3401-hypothetical protein | 90 | 3.43E-05 | Fjoh_4889 | Fjoh_4889-hypothetical protein | 140 | 1.48E-06 |
| 33 | Fjoh_3170 | Fjoh_3170-nitroreductase | 88 | 1.44E-07 | Fjoh_1832 | Fjoh_1832-hypothetical protein | 138 | 8.88E-06 |
| 34 | Fjoh_3476 | Fjoh_3476-OmpA/MotB domain-containing protein | 88 | 0.000179 | Fjoh_2098 | Fjoh_2098-TonB-dependent receptor | 137 | 0.000183 |
| 35 | Fjoh_1146 | Fjoh_1146-hypothetical protein | 88 | 2.27E-07 | Fjoh_2893 | Fjoh_2893-hypothetical protein | 137 | 2.76E-08 |
| 36 | Fjoh_4175 | Fjoh_4175-glycoside hydrolase | 85 | 3.66E-06 | Fjoh_1423 | Fjoh_1423-hypothetical protein | 127 | 0.001784 |
| 37 | Fjoh_4174 | Fjoh_4174-carbohydrate-binding family 6 protein | 84 | 2.75E-08 | Fjoh_2084 | Fjoh_2084-hypothetical protein | 125 | 3.36E-21 |
| 38 | Fjoh_1423 | Fjoh_1423-hypothetical protein | 83 | 0.002033 | Fjoh_2891 | Fjoh_2891-hypothetical protein | 122 | 2.79E-08 |
| 39 | Fjoh_3349 | Fjoh_3349-hypothetical protein | 81 | 7.08E-05 | Fjoh_3414 | Fjoh_3414-hypothetical protein | 121 | 7.56E-06 |
| 40 | Fjoh_2185 | Fjoh_2185-hypothetical protein | 80 | 2.08E-06 | Fjoh_3239 | Fjoh_3239-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 120 | 1.23E-05 |
| 41 | Fjoh_2097 | Fjoh_2097-amino acid adenylation protein | 80 | 1.58E-05 | Fjoh_2095 | Fjoh_2095-amino acid adenylation protein | 117 | 4.8E-06 |
| 42 | Fjoh_4176 | Fjoh_4176-carbohydrate-binding family 6 protein | 77 | 4.51E-05 | Fjoh_2094 | Fjoh_2094-class III aminotransferase | 113 | 0.000261 |
| 43 | Fjoh_2095 | Fjoh_2095-amino acid adenylation protein | 72 | 4.36E-06 | Fjoh_2097 | Fjoh_2097-amino acid adenylation protein | 110 | 1.78E-05 |
| 44 | Fjoh_4170 | Fjoh_4170-FecR anti-FecI sigma factor | 71 | 0.010909 | Fjoh_3228 | Fjoh_3228-RND family efflux transporter MFP subunit | 109 | 1.91E-06 |
| 45 | Fjoh_2094 | Fjoh_2094-class III aminotransferase | 70 | 0.000225 | Fjoh_4851 | Fjoh_4851-hypothetical protein | 107 | 0.000228 |
| 46 | Fjoh_2084 | Fjoh_2084-hypothetical protein | 70 | 2.33E-18 | Fjoh_3852 | Fjoh_3852-hypothetical protein | 104 | 2.61E-08 |
| 47 | Fjoh_3227 | Fjoh_3227-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 68 | 3.56E-05 | Fjoh_1738 | Fjoh_1738-malate synthase | 99 | 4.98E-06 |
| 48 | Fjoh_2099 | Fjoh_2099-thioesterase | 67 | 0.001056 | Fjoh_4856 | Fjoh_4856-hypothetical protein | 98 | 1.08E-15 |
| 49 | Fjoh_3851 | Fjoh_3851-polyphosphate kinase | 66 | 2.1E-05 | Fjoh_4854 | Fjoh_4854-hypothetical protein | 96 | 1.56E-13 |
| 50 | Fjoh_3350 | Fjoh_3350-hypothetical protein | 65 | 0.003495 | Fjoh_3232 | Fjoh_3232-UspA domain-containing protein | 93 | 0.000199 |
| 51 | Fjoh_4562 | Fjoh_4562-TonB-dependent receptor, plug | 64 | 2.44E-05 | Fjoh_2099 | Fjoh_2099-thioesterase | 93 | 0.001601 |
| 52 | Fjoh_4171 | Fjoh_4171-ECF subfamily RNA polymerase sigma-24 factor | 63 | 0.019574 | Fjoh_2093 | Fjoh_2093-amino acid adenylation protein | 91 | 1.99E-07 |
| 53 | Fjoh_1518 | Fjoh_1518-hypothetical protein | 62 | 8.44E-05 | Fjoh_3477 | Fjoh_3477-hypothetical protein | 91 | 3.26E-06 |
| 54 | Fjoh_3253 | Fjoh_3253-ECF subfamily RNA polymerase sigma-24 factor | 61 | 5.25E-05 | Fjoh_3225 | Fjoh_3225-hypothetical protein | 89 | 0.000234 |
| 55 | Fjoh_3418 | Fjoh_3418-hypothetical protein | 60 | 1.5E-16 | Fjoh_1739 | Fjoh_1739-isocitrate lyase | 88 | 0.005507 |
| 56 | Fjoh_3154 | Fjoh_3154-hypothetical protein | 60 | 7.02E-07 | Fjoh_2100 | Fjoh_2100-hypothetical protein | 87 | 1.66E-19 |
| 57 | Fjoh_2104 | Fjoh_2104-hypothetical protein | 59 | 0.013886 | Fjoh_3841 | Fjoh_3841-hypothetical protein | 87 | 8.02E-05 |
| 58 | Fjoh_4577 | Fjoh_4577-metallophosphoesterase | 58 | 0.000581 | Fjoh_3476 | Fjoh_3476-OmpA/MotB domain-containing protein | 83 | 9.57E-05 |
| 59 | Fjoh_4325 | Fjoh_4325-hypothetical protein | 57 | 3.71E-06 | Fjoh_3692 | Fjoh_3692-hypothetical protein | 82 | 7.31E-13 |
| 60 | Fjoh_2100 | Fjoh_2100-hypothetical protein | 57 | 5.94E-18 | Fjoh_4174 | Fjoh_4174-carbohydrate-binding family 6 protein | 81 | 1.27E-07 |
| 61 | Fjoh_3251 | Fjoh_3251-TonB-dependent receptor | 57 | 0.000645 | Fjoh_2185 | Fjoh_2185-hypothetical protein | 80 | 4.86E-06 |
| 62 | Fjoh_0821 | Fjoh_0821-TonB-dependent receptor | 56 | 2.4E-05 | Fjoh_2096 | Fjoh_2096-beta-lactamase domain-containing protein | 79 | 9.86E-05 |
| 63 | Fjoh_2101 | Fjoh_2101-cyclic peptide transporter | 55 | 0.000392 | Fjoh_4723 | Fjoh_4723-endonuclease I | 78 | 2.16E-08 |
| 64 | Fjoh_3173 | Fjoh_3173-L-lysine 6-monooxygenase | 55 | 7.01E-10 | Fjoh_3478 | Fjoh_3478-hypothetical protein | 78 | 4.85E-08 |
| 65 | Fjoh_1368 | Fjoh_1368-TonB-dependent receptor | 54 | 5.33E-06 | Fjoh_1146 | Fjoh_1146-hypothetical protein | 77 | 1.04E-06 |
| 66 | Fjoh_2093 | Fjoh_2093-amino acid adenylation protein | 54 | 2.42E-07 | Fjoh_2088 | Fjoh_2088-amino acid adenylation protein | 76 | 0.000701 |
| 67 | Fjoh_2096 | Fjoh_2096-beta-lactamase domain-containing protein | 53 | 4.13E-05 | Fjoh_3226 | Fjoh_3226-RND efflux system outer membrane lipoprotein | 75 | 9.26E-06 |
| 68 | Fjoh_3228 | Fjoh_3228-RND family efflux transporter MFP subunit | 53 | 2.67E-06 | Fjoh_4175 | Fjoh_4175-glycoside hydrolase | 73 | 1.25E-05 |
| 69 | Fjoh_2937 | Fjoh_2937-hypothetical protein | 52 | 1.13E-07 | Fjoh_2104 | Fjoh_2104-hypothetical protein | 73 | 0.012579 |
| 70 | Fjoh_0546 | Fjoh_0546-hypothetical protein | 51 | 1.1E-08 | Fjoh_2087 | Fjoh_2087-NAD-dependent epimerase/dehydratase | 72 | 2.05E-05 |
| 71 | Fjoh_2088 | Fjoh_2088-amino acid adenylation protein | 51 | 0.001119 | Fjoh_4177 | Fjoh_4177-glycoside hydrolase | 72 | 7.23E-06 |
| 72 | Fjoh_3232 | Fjoh_3232-UspA domain-containing protein | 50 | 0.00018 | Fjoh_2101 | Fjoh_2101-cyclic peptide transporter | 71 | 0.000292 |
| 73 | Fjoh_2089 | Fjoh_2089-amino acid adenylation protein | 48 | 5.25E-05 | Fjoh_2089 | Fjoh_2089-amino acid adenylation protein | 71 | 5.81E-05 |
| 74 | Fjoh_4857 | Fjoh_4857-beta-glucosidase | 47 | 1.23E-05 | Fjoh_1518 | Fjoh_1518-hypothetical protein | 68 | 0.000125 |
| 75 | Fjoh_2090 | Fjoh_2090-amino acid adenylation protein | 47 | 3.46E-06 | Fjoh_3401 | Fjoh_3401-hypothetical protein | 68 | 9.29E-05 |
| 76 | Fjoh_3239 | Fjoh_3239-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 46 | 9.61E-06 | Fjoh_0821 | Fjoh_0821-TonB-dependent receptor | 68 | 2.75E-05 |
| 77 | Fjoh_2087 | Fjoh_2087-NAD-dependent epimerase/dehydratase | 46 | 1.12E-05 | Fjoh_3238 | Fjoh_3238-RND family efflux transporter MFP subunit | 68 | 2.68E-07 |
| 78 | Fjoh_3153 | Fjoh_3153-hypothetical protein | 45 | 1.74E-15 | Fjoh_2090 | Fjoh_2090-amino acid adenylation protein | 67 | 4.18E-06 |
| 79 | Fjoh_3225 | Fjoh_3225-hypothetical protein | 45 | 0.000201 | Fjoh_2092 | Fjoh_2092-AMP-dependent synthetase/ligase | 65 | 9.19E-06 |
| 80 | Fjoh_2408 | Fjoh_2408-hypothetical protein | 45 | 3.22E-13 | Fjoh_3251 | Fjoh_3251-TonB-dependent receptor | 65 | 0.001416 |
| 81 | Fjoh_2092 | Fjoh_2092-AMP-dependent synthetase/ligase | 43 | 1.14E-05 | Fjoh_3349 | Fjoh_3349-hypothetical protein | 64 | 0.00015 |
| 82 | Fjoh_3175 | Fjoh_3175-MATE efflux family protein | 42 | 4.48E-06 | Fjoh_4176 | Fjoh_4176-carbohydrate-binding family 6 protein | 63 | 6.16E-05 |
| 83 | Fjoh_4561 | Fjoh_4561-hypothetical protein | 41 | 0.000353 | Fjoh_1368 | Fjoh_1368-TonB-dependent receptor | 61 | 4.73E-06 |
| 84 | Fjoh_3155 | Fjoh_3155-Rhs element Vgr protein | 41 | 2.85E-05 | Fjoh_2091 | Fjoh_2091-short-chain dehydrogenase/reductase SDR | 59 | 0.000276 |
| 85 | Fjoh_2502 | Fjoh_2502-hypothetical protein | 41 | 6.65E-11 | Fjoh_4853 | Fjoh_4853-hypothetical protein | 59 | 4.04E-15 |
| 86 | Fjoh_3152 | Fjoh_3152-phage tail protein | 40 | 6.08E-07 | Fjoh_4577 | Fjoh_4577-metallophosphoesterase | 58 | 0.000728 |
| 87 | Fjoh_0820 | Fjoh_0820-hypothetical protein | 40 | 4.91E-05 | Fjoh_3253 | Fjoh_3253-ECF subfamily RNA polymerase sigma-24 factor | 57 | 0.000126 |
| 88 | Fjoh_1142 | Fjoh_1142-sulfatase | 38 | 9.1E-07 | Fjoh_4852 | Fjoh_4852-hypothetical protein | 56 | 0.017921 |
| 89 | Fjoh_3247 | Fjoh_3247-leucine-rich repeat-containing protein | 37 | 3.82E-05 | Fjoh_0546 | Fjoh_0546-hypothetical protein | 52 | 1.93E-08 |
| 90 | Fjoh_2895 | Fjoh_2895-FecR anti-FecI sigma factor | 37 | 0.026316 | Fjoh_2689 | Fjoh_2689-hypothetical protein | 52 | 9.89E-08 |
| 91 | Fjoh_2091 | Fjoh_2091-short-chain dehydrogenase/reductase SDR | 37 | 0.000487 | Fjoh_2408 | Fjoh_2408-hypothetical protein | 51 | 6.92E-14 |
| 92 | Fjoh_2437 | Fjoh_2437-hypothetical protein | 37 | 1.54E-06 | Fjoh_2437 | Fjoh_2437-hypothetical protein | 51 | 1.06E-06 |
| 93 | Fjoh_3157 | Fjoh_3157-GPW/gp25 family protein | 36 | 2.06E-07 | Fjoh_4857 | Fjoh_4857-beta-glucosidase | 50 | 1.07E-05 |
| 94 | Fjoh_2936 | Fjoh_2936-PAS/PAC sensor protein | 36 | 4.8E-05 | Fjoh_0820 | Fjoh_0820-hypothetical protein | 50 | 4.89E-05 |
| 95 | Fjoh_3174 | Fjoh_3174-IucA/IucC family protein | 36 | 6.79E-06 | Fjoh_4170 | Fjoh_4170-FecR anti-FecI sigma factor | 49 | 0.017712 |
| 96 | Fjoh_1346 | Fjoh_1346-hypothetical protein | 35 | 1.11E-07 | Fjoh_2086 | Fjoh_2086-4'-phosphopantetheinyl transferase | 48 | 0.000427 |
| 97 | Fjoh_1715 | Fjoh_1715-ADP-heptose--LPS heptosyltransferase-like protein | 35 | 1.13E-05 | Fjoh_4562 | Fjoh_4562-TonB-dependent receptor, plug | 48 | 7.25E-06 |
| 98 | Fjoh_3140 | Fjoh_3140-catalase | 35 | 8.29E-06 | Fjoh_4561 | Fjoh_4561-hypothetical protein | 47 | 0.000646 |
| 99 | Fjoh_3859 | Fjoh_3859-ECF subfamily RNA polymerase sigma-24 factor | 34 | 0.000186 | Fjoh_3350 | Fjoh_3350-hypothetical protein | 47 | 0.003961 |
| 100 | Fjoh_3226 | Fjoh_3226-RND efflux system outer membrane lipoprotein | 33 | 9.55E-06 | Fjoh_4171 | Fjoh_4171-ECF subfamily RNA polymerase sigma-24 factor | 46 | 0.027402 |

Table S3. Downregulated genes.

| | *Δ mft* with glucose versus WT with glucose | | | | WT with glucose versus WT with glucose | | | |
|---|---|---|---|---|---|---|---|---|
| | Fjoh | definition | ratio | *t*-test | Fjoh | definition | ratio | t-test |
| 1 | Fjoh_4837 | Fjoh_4837-urease subunit gamma | 0.0011 | 1.33E-06 | Fjoh_1780 | Fjoh_1780-hypothetical protein | 0.0007 | 3.48E-05 |
| 2 | Fjoh_4836 | Fjoh_4836-urease subunit beta | 0.0012 | 5.5E-07 | Fjoh_1781 | Fjoh_1781-hypothetical protein | 0.0008 | 2.37E-06 |
| 3 | Fjoh_4863 | Fjoh_4863-RND family efflux transporter MFP subunit | 0.0021 | 4.41E-06 | Fjoh_4836 | Fjoh_4836-urease subunit beta | 0.0015 | 5.52E-07 |
| 4 | Fjoh_4861 | Fjoh_4861-RND efflux system outer membrane lipoprotein | 0.0025 | 2.35E-06 | Fjoh_4837 | Fjoh_4837-urease subunit gamma | 0.0015 | 1.34E-06 |
| 5 | Fjoh_4862 | Fjoh_4862-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 0.0026 | 5.58E-07 | Fjoh_2417 | Fjoh_2417-hypothetical protein | 0.0015 | 0.000155 |
| 6 | Fjoh_4835 | Fjoh_4835-urease subunit alpha | 0.0033 | 1.81E-05 | Fjoh_2418 | Fjoh_2418-nitrite reductase | 0.0015 | 4.46E-05 |
| 7 | Fjoh_1281 | Fjoh_1281-major facilitator transporter | 0.0036 | 2.39E-05 | Fjoh_1778 | Fjoh_1778-hypothetical protein | 0.0016 | 2.17E-05 |
| 8 | Fjoh_1353 | Fjoh_1353-outer membrane efflux protein | 0.0039 | 0.000166 | Fjoh_1779 | Fjoh_1779-hypothetical protein | 0.0017 | 1.16E-09 |
| 9 | Fjoh_2338 | Fjoh_2338-hypothetical protein | 0.0040 | 0.000443 | Fjoh_2416 | Fjoh_2416-electron transport protein SCO1/SenC | 0.0017 | 1.96E-09 |
| 10 | Fjoh_1355 | Fjoh_1355-major facilitator transporter | 0.0041 | 0.000225 | Fjoh_4056 | Fjoh_4056-L-aspartate oxidase | 0.0018 | 5.93E-09 |
| 11 | Fjoh_4498 | Fjoh_4498-hypothetical protein | 0.0045 | 0.00037 | Fjoh_1068 | Fjoh_1068-hypothetical protein | 0.0020 | 1.7E-08 |
| 12 | Fjoh_2339 | Fjoh_2339-hypothetical protein | 0.0046 | 5.98E-05 | Fjoh_1281 | Fjoh_1281-major facilitator transporter | 0.0023 | 2.35E-05 |
| 13 | Fjoh_2290 | Fjoh_2290-hypothetical protein | 0.0048 | 3.97E-07 | Fjoh_4863 | Fjoh_4863-RND family efflux transporter MFP subunit | 0.0024 | 4.43E-06 |
| 14 | Fjoh_1354 | Fjoh_1354-secretion protein HlyD family protein | 0.0048 | 4.53E-06 | Fjoh_4055 | Fjoh_4055-quinolinate synthetase complex subunit A | 0.0028 | 6.08E-05 |
| 15 | Fjoh_4497 | Fjoh_4497-hypothetical protein | 0.0053 | 2.72E-05 | Fjoh_3910 | Fjoh_3910-(NiFe) hydrogenase maturation protein HypF | 0.0029 | 0.000331 |
| 16 | Fjoh_2004 | Fjoh_2004-beta-lactamase domain-containing protein | 0.0058 | 1.38E-06 | Fjoh_4861 | Fjoh_4861-RND efflux system outer membrane lipoprotein | 0.0029 | 2.36E-06 |
| 17 | Fjoh_3836 | Fjoh_3836-thiamine pyrophosphate binding domain-containing protein | 0.0062 | 2.73E-07 | Fjoh_4862 | Fjoh_4862-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 0.0032 | 5.61E-07 |
| 18 | Fjoh_4834 | Fjoh_4834-urease accessory protein UreE | 0.0062 | 1.49E-07 | Fjoh_4053 | Fjoh_4053-CRP/FNR family transcriptional regulator | 0.0032 | 0.000252 |
| 19 | Fjoh_3835 | Fjoh_3835-amine oxidase | 0.0063 | 6.24E-06 | Fjoh_4059 | Fjoh_4059-hypothetical protein | 0.0034 | 6.24E-20 |
| 20 | Fjoh_4108 | Fjoh_4108-phosphatidylserine decarboxylase | 0.0085 | 8.58E-06 | Fjoh_1582 | Fjoh_1582-hypothetical protein | 0.0035 | 3.69E-17 |
| 21 | Fjoh_4833 | Fjoh_4833-urease accessory protein UreF | 0.0090 | 0.000611 | Fjoh_3907 | Fjoh_3907-nickel-dependent hydrogenase, large subunit | 0.0036 | 1E-08 |
| 22 | Fjoh_2005 | Fjoh_2005-hypothetical protein | 0.0099 | 1.6E-06 | Fjoh_3911 | Fjoh_3911-FKBP-type peptidyl-prolyl cis-trans isomerase | 0.0036 | 6.79E-05 |
| 23 | Fjoh_1068 | Fjoh_1068-hypothetical protein | 0.0103 | 1.89E-08 | Fjoh_3905 | Fjoh_3905-hydrogenase expression/synthesis, HypA | 0.0036 | 1.25E-09 |
| 24 | Fjoh_1781 | Fjoh_1781-hypothetical protein | 0.0106 | 2.64E-06 | Fjoh_4835 | Fjoh_4835-urease subunit alpha | 0.0037 | 1.82E-05 |
| 25 | Fjoh_4838 | Fjoh_4838-hypothetical protein | 0.0111 | 3.15E-17 | Fjoh_4058 | Fjoh_4058-anaerobic ribonucleoside triphosphate reductase | 0.0039 | 0.000648 |
| 26 | Fjoh_1618 | Fjoh_1618-hypothetical protein | 0.0119 | 1.06E-10 | Fjoh_2542 | Fjoh_2542-coproporphyrinogen III oxidase | 0.0040 | 1.82E-06 |
| 27 | Fjoh_1780 | Fjoh_1780-hypothetical protein | 0.0127 | 3.93E-05 | Fjoh_1618 | Fjoh_1618-hypothetical protein | 0.0044 | 9.62E-11 |
| 28 | Fjoh_4053 | Fjoh_4053-CRP/FNR family transcriptional regulator | 0.0127 | 0.000274 | Fjoh_1353 | Fjoh_1353-outer membrane efflux protein | 0.0045 | 0.000167 |
| 29 | Fjoh_1582 | Fjoh_1582-hypothetical protein | 0.0129 | 4.22E-17 | Fjoh_4498 | Fjoh_4498-hypothetical protein | 0.0045 | 0.00037 |
| 30 | Fjoh_0902 | Fjoh_0902-4Fe-4S ferredoxin | 0.0144 | 3.76E-15 | Fjoh_3904 | Fjoh_3904-hydrogenase nickel incorporation protein HypB | 0.0045 | 8.14E-06 |
| 31 | Fjoh_2416 | Fjoh_2416-electron transport protein SCO1/SenC | 0.0148 | 2.34E-09 | Fjoh_3908 | Fjoh_3908-Ni/Fe-hydrogenase, b-type cytochrome subunit | 0.0045 | 0.000176 |
| 32 | Fjoh_2418 | Fjoh_2418-nitrite reductase | 0.0159 | 5.14E-05 | Fjoh_1581 | Fjoh_1581-globin | 0.0045 | 1.33E-05 |
| 33 | Fjoh_2417 | Fjoh_2417-hypothetical protein | 0.0164 | 0.000177 | Fjoh_3442 | Fjoh_3442-hypothetical protein | 0.0046 | 0.000324 |
| 34 | Fjoh_2414 | Fjoh_2414-hemerythrin HHE cation binding domain-containing protein | 0.0167 | 6.49E-06 | Fjoh_3913 | Fjoh_3913-high-affinity nickel-transporter | 0.0046 | 2.27E-07 |
| 35 | Fjoh_4832 | Fjoh_4832-urease accessory protein UreG | 0.0173 | 8.19E-08 | Fjoh_1355 | Fjoh_1355-major facilitator transporter | 0.0048 | 0.000226 |
| 36 | Fjoh_0903 | Fjoh_0903-peptidase U32 | 0.0176 | 7.09E-06 | Fjoh_4060 | Fjoh_4060-anaerobic ribonucleoside-triphosphate reductase activating protein | 0.0049 | 2.43E-08 |
| 37 | Fjoh_1581 | Fjoh_1581-globin | 0.0181 | 1.54E-05 | Fjoh_3906 | Fjoh_3906-hydrogenase (NiFe) small subunit HydA | 0.0049 | 2.04E-06 |
| 38 | Fjoh_0435 | Fjoh_0435-hypothetical protein | 0.0196 | 5.89E-09 | Fjoh_1354 | Fjoh_1354-secretion protein HlyD family protein | 0.0049 | 4.54E-06 |
| 39 | Fjoh_2542 | Fjoh_2542-coproporphyrinogen III oxidase | 0.0199 | 2.19E-06 | Fjoh_4057 | Fjoh_4057-hemerythrin HHE cation binding domain-containing protein | 0.0050 | 2.54E-09 |
| 40 | Fjoh_1440 | Fjoh_1440-hypothetical protein | 0.0202 | 3.37E-13 | Fjoh_4497 | Fjoh_4497-hypothetical protein | 0.0055 | 2.72E-05 |
| 41 | Fjoh_4831 | Fjoh_4831-urease accessory protein UreD | 0.0203 | 2.73E-06 | Fjoh_3909 | Fjoh_3909-hydrogenase maturation protease | 0.0057 | 4.71E-08 |
| 42 | Fjoh_4054 | Fjoh_4054-hypothetical protein | 0.0221 | 4.68E-16 | Fjoh_4061 | Fjoh_4061-hypothetical protein | 0.0061 | 2.06E-06 |
| 43 | Fjoh_1778 | Fjoh_1778-hypothetical protein | 0.0223 | 2.69E-05 | Fjoh_4834 | Fjoh_4834-urease accessory protein UreE | 0.0062 | 1.48E-07 |
| 44 | Fjoh_1619 | Fjoh_1619-hypothetical protein | 0.0229 | 2.42E-12 | Fjoh_2338 | Fjoh_2338-hypothetical protein | 0.0064 | 0.000452 |
| 45 | Fjoh_3905 | Fjoh_3905-hydrogenase expression/synthesis, HypA | 0.0230 | 1.62E-09 | Fjoh_3912 | Fjoh_3912-hydrogenase expression/formation protein HypD | 0.0064 | 2.99E-07 |
| 46 | Fjoh_4828 | Fjoh_4828-hypothetical protein | 0.0233 | 3.64E-08 | Fjoh_0504 | Fjoh_0504-hypothetical protein | 0.0068 | 4.71E-05 |
| 47 | Fjoh_4830 | Fjoh_4830-hypothetical protein | 0.0239 | 8.33E-05 | Fjoh_3903 | Fjoh_3903-hydrogenase assembly chaperone HypC/HupF | 0.0070 | 4.98E-15 |
| 48 | Fjoh_3910 | Fjoh_3910-(NiFe) hydrogenase maturation protein HypF | 0.0245 | 0.000398 | Fjoh_3835 | Fjoh_3835-amine oxidase | 0.0071 | 6.29E-06 |
| 49 | Fjoh_0434 | Fjoh_0434-hypothetical protein | 0.0253 | 5.12E-07 | Fjoh_3836 | Fjoh_3836-thiamine pyrophosphate binding domain-containing protein | 0.0074 | 2.77E-07 |
| 50 | Fjoh_3904 | Fjoh_3904-hydrogenase nickel incorporation protein HypB | 0.0255 | 1.03E-05 | Fjoh_2004 | Fjoh_2004-beta-lactamase domain-containing protein | 0.0075 | 1.4E-06 |
| 51 | Fjoh_1309 | Fjoh_1309-short-chain dehydrogenase/reductase SDR | 0.0260 | 1.29E-06 | Fjoh_0902 | Fjoh_0902-4Fe-4S ferredoxin | 0.0078 | 3.42E-15 |
| 52 | Fjoh_4829 | Fjoh_4829-Urea transporter | 0.0262 | 0.000611 | Fjoh_4838 | Fjoh_4838-hypothetical protein | 0.0078 | 2.64E-17 |
| 53 | Fjoh_3217 | Fjoh_3217-cyclic nucleotide-binding protein | 0.0276 | 6.28E-08 | Fjoh_0903 | Fjoh_0903-peptidase U32 | 0.0082 | 6.39E-06 |
| 54 | Fjoh_1915 | Fjoh_1915-hypothetical protein | 0.0284 | 7.91E-06 | Fjoh_4108 | Fjoh_4108-phosphatidylserine decarboxylase | 0.0083 | 8.56E-06 |
| 55 | Fjoh_3911 | Fjoh_3911-FKBP-type peptidyl-prolyl cis-trans isomerase | 0.0293 | 8.74E-05 | Fjoh_2339 | Fjoh_2339-hypothetical protein | 0.0086 | 6.22E-05 |
| 56 | Fjoh_4407 | Fjoh_4407-RND family efflux transporter MFP subunit | 0.0311 | 1.52E-08 | Fjoh_3902 | Fjoh_3902-hydrogenase expression/formation protein HypE | 0.0096 | 0.000322 |
| 57 | Fjoh_1622 | Fjoh_1622-hypothetical protein | 0.0322 | 4.54E-13 | Fjoh_2290 | Fjoh_2290-hypothetical protein | 0.0104 | 4.28E-07 |
| 58 | Fjoh_3913 | Fjoh_3913-high-affinity nickel-transporter | 0.0327 | 3.24E-07 | Fjoh_2005 | Fjoh_2005-hypothetical protein | 0.0116 | 1.63E-06 |
| 59 | Fjoh_4408 | Fjoh_4408-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 0.0332 | 0.00035 | Fjoh_4054 | Fjoh_4054-hypothetical protein | 0.0118 | 4.04E-16 |
| 60 | Fjoh_3903 | Fjoh_3903-hydrogenase assembly chaperone HypC/HupF | 0.0336 | 7.36E-15 | Fjoh_1619 | Fjoh_1619-hypothetical protein | 0.0120 | 2.06E-12 |
| 61 | Fjoh_4442 | Fjoh_4442-short-chain dehydrogenase/reductase SDR | 0.0337 | 6.28E-06 | Fjoh_4833 | Fjoh_4833-urease accessory protein UreF | 0.0121 | 0.000627 |
| 62 | Fjoh_2826 | Fjoh_2826-FAD-binding monooxygenase | 0.0345 | 0.000495 | Fjoh_2414 | Fjoh_2414-hemerythrin HHE cation binding domain-containing protein | 0.0128 | 6.22E-06 |
| 63 | Fjoh_3218 | Fjoh_3218-dihydroxy-acid dehydratase | 0.0355 | 1.91E-05 | Fjoh_4832 | Fjoh_4832-urease accessory protein UreG | 0.0165 | 8.08E-08 |
| 64 | Fjoh_1779 | Fjoh_1779-hypothetical protein | 0.0366 | 1.92E-09 | Fjoh_1440 | Fjoh_1440-hypothetical protein | 0.0197 | 3.34E-13 |
| 65 | Fjoh_4056 | Fjoh_4056-L-aspartate oxidase | 0.0369 | 9.49E-09 | Fjoh_4831 | Fjoh_4831-urease accessory protein UreD | 0.0221 | 2.78E-06 |
| 66 | Fjoh_4403 | Fjoh_4403-succinylglutamate desuccinylase/aspartoacylase | 0.0376 | 3.82E-07 | Fjoh_0435 | Fjoh_0435-hypothetical protein | 0.0221 | 6.09E-09 |
| 67 | Fjoh_3305 | Fjoh_3305-hypothetical protein | 0.0383 | 1.21E-05 | Fjoh_4828 | Fjoh_4828-hypothetical protein | 0.0264 | 3.79E-08 |
| 68 | Fjoh_4443 | Fjoh_4443-hypothetical protein | 0.0393 | 3.25E-18 | Fjoh_3217 | Fjoh_3217-cyclic nucleotide-binding protein | 0.0276 | 6.31E-08 |
| 69 | Fjoh_4055 | Fjoh_4055-quinolinate synthetase complex subunit A | 0.0394 | 8.88E-05 | Fjoh_1622 | Fjoh_1622-hypothetical protein | 0.0283 | 4.29E-13 |
| 70 | Fjoh_1134 | Fjoh_1134-hypothetical protein | 0.0397 | 3.4E-14 | Fjoh_4830 | Fjoh_4830-hypothetical protein | 0.0287 | 8.73E-05 |
| 71 | Fjoh_4840 | Fjoh_4840-hyaluronan synthase | 0.0421 | 0.000724 | Fjoh_2525 | Fjoh_2525-PAS/PAC sensor protein | 0.0290 | 6.56E-07 |
| 72 | Fjoh_3902 | Fjoh_3902-hydrogenase expression/formation protein HypE | 0.0425 | 0.00043 | Fjoh_4829 | Fjoh_4829-Urea transporter | 0.0307 | 0.000635 |
| 73 | Fjoh_4409 | Fjoh_4409-RND efflux system outer membrane lipoprotein | 0.0436 | 0.001516 | Fjoh_2536 | Fjoh_2536-copper-exporting ATPase | 0.0309 | 4.63E-07 |
| 74 | Fjoh_2841 | Fjoh_2841-alcohol dehydrogenase | 0.0437 | 9.35E-05 | Fjoh_2826 | Fjoh_2826-FAD-binding monooxygenase | 0.0312 | 0.000482 |
| 75 | Fjoh_3912 | Fjoh_3912-hydrogenase expression/formation protein HypD | 0.0456 | 4.92E-07 | Fjoh_1309 | Fjoh_1309-short-chain dehydrogenase/reductase SDR | 0.0328 | 1.4E-06 |
| 76 | Fjoh_3442 | Fjoh_3442-hypothetical protein | 0.0460 | 0.000466 | Fjoh_0434 | Fjoh_0434-hypothetical protein | 0.0350 | 5.85E-07 |
| 77 | Fjoh_3907 | Fjoh_3907-nickel-dependent hydrogenase, large subunit | 0.0474 | 1.79E-08 | Fjoh_2413 | Fjoh_2413-nitric oxide reductase large subunit-like protein | 0.0376 | 0.000341 |
| 78 | Fjoh_1904 | Fjoh_1904-hypothetical protein | 0.0502 | 7.03E-08 | Fjoh_2841 | Fjoh_2841-alcohol dehydrogenase | 0.0386 | 8.89E-05 |
| 79 | Fjoh_4402 | Fjoh_4402-Cl- channel, voltage-gated family protein | 0.0504 | 8.19E-06 | Fjoh_4442 | Fjoh_4442-short-chain dehydrogenase/reductase SDR | 0.0388 | 6.68E-06 |
| 80 | Fjoh_4059 | Fjoh_4059-hypothetical protein | 0.0505 | 1.29E-19 | Fjoh_4407 | Fjoh_4407-RND family efflux transporter MFP subunit | 0.0411 | 1.73E-08 |
| 81 | Fjoh_0641 | Fjoh_0641-hypothetical protein | 0.0578 | 2.28E-18 | Fjoh_1134 | Fjoh_1134-hypothetical protein | 0.0434 | 3.6E-14 |
| 82 | Fjoh_4029 | Fjoh_4029-oxidoreductase FAD-binding subunit | 0.0593 | 4.72E-07 | Fjoh_3218 | Fjoh_3218-dihydroxy-acid dehydratase | 0.0435 | 2.09E-05 |
| 83 | Fjoh_2751 | Fjoh_2751-hypothetical protein | 0.0593 | 1.98E-09 | Fjoh_4443 | Fjoh_4443-hypothetical protein | 0.0435 | 3.53E-18 |
| 84 | Fjoh_0997 | Fjoh_0997-hypothetical protein | 0.0593 | 3.31E-20 | Fjoh_3305 | Fjoh_3305-hypothetical protein | 0.0438 | 1.29E-05 |
| 85 | Fjoh_2752 | Fjoh_2752-ECF subfamily RNA polymerase sigma-24 factor | 0.0606 | 3.67E-14 | Fjoh_3245 | Fjoh_3245-patatin | 0.0451 | 6.52E-08 |
| 86 | Fjoh_2966 | Fjoh_2966-hypothetical protein | 0.0609 | 7.27E-16 | Fjoh_4408 | Fjoh_4408-hydrophobe/amphiphile efflux-1 (HAE1) family protein | 0.0457 | 0.000392 |
| 87 | Fjoh_4864 | Fjoh_4864-hypothetical protein | 0.0612 | 3.27E-06 | Fjoh_4409 | Fjoh_4409-RND efflux system outer membrane lipoprotein | 0.0468 | 0.001555 |
| 88 | Fjoh_2413 | Fjoh_2413-nitric oxide reductase large subunit-like protein | 0.0613 | 0.000422 | Fjoh_4827 | Fjoh_4827-TonB-dependent receptor, plug | 0.0479 | 4.22E-08 |
| 89 | Fjoh_4519 | Fjoh_4519-hypothetical protein | 0.0618 | 7.99E-20 | Fjoh_0997 | Fjoh_0997-hypothetical protein | 0.0482 | 2.62E-20 |
| 90 | Fjoh_3908 | Fjoh_3908-Ni/Fe-hydrogenase, b-type cytochrome subunit | 0.0646 | 0.000308 | Fjoh_1519 | Fjoh_1519-5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase | 0.0497 | 7.06E-05 |
| 91 | Fjoh_2750 | Fjoh_2750-hypothetical protein | 0.0649 | 0.000106 | Fjoh_4864 | Fjoh_4864-hypothetical protein | 0.0526 | 2.95E-06 |
| 92 | Fjoh_1133 | Fjoh_1133-ECF subfamily RNA polymerase sigma-24 factor | 0.0654 | 9.12E-13 | Fjoh_0438 | Fjoh_0438-hypothetical protein | 0.0534 | 4.14E-07 |
| 93 | Fjoh_3906 | Fjoh_3906-hydrogenase (NiFe) small subunit HydA | 0.0655 | 4.22E-06 | Fjoh_1904 | Fjoh_1904-hypothetical protein | 0.0535 | 7.39E-08 |
| 94 | Fjoh_4827 | Fjoh_4827-TonB-dependent receptor, plug | 0.0657 | 6.08E-08 | Fjoh_2337 | Fjoh_2337-hypothetical protein | 0.0543 | 8.72E-06 |
| 95 | Fjoh_0643 | Fjoh_0643-hypothetical protein | 0.0664 | 1.43E-20 | Fjoh_4840 | Fjoh_4840-hyaluronan synthase | 0.0549 | 0.000808 |
| 96 | Fjoh_3909 | Fjoh_3909-hydrogenase maturation protease | 0.0682 | 1.09E-07 | Fjoh_3840 | Fjoh_3840-hypothetical protein | 0.0550 | 3.22E-05 |
| 97 | Fjoh_0519 | Fjoh_0519-hypothetical protein | 0.0686 | 0.000389 | Fjoh_2340 | Fjoh_2340-helix-turn-helix domain-containing protein | 0.0572 | 0.001234 |
| 98 | Fjoh_4058 | Fjoh_4058-anaerobic ribonucleoside triphosphate reductase | 0.0689 | 0.001107 | Fjoh_3901 | Fjoh_3901-TonB-dependent receptor | 0.0636 | 4.79E-06 |
| 99 | Fjoh_0639 | Fjoh_0639-hypothetical protein | 0.0689 | 1.23E-19 | Fjoh_4029 | Fjoh_4029-oxidoreductase FAD-binding subunit | 0.0641 | 5.02E-07 |
| 100 | Fjoh_3956 | Fjoh_3956-endoribonuclease L-PSP | 0.0701 | 7.68E-07 | Fjoh_3956 | Fjoh_3956-endoribonuclease L-PSP | 0.0649 | 7.23E-07 |

Fig. S1

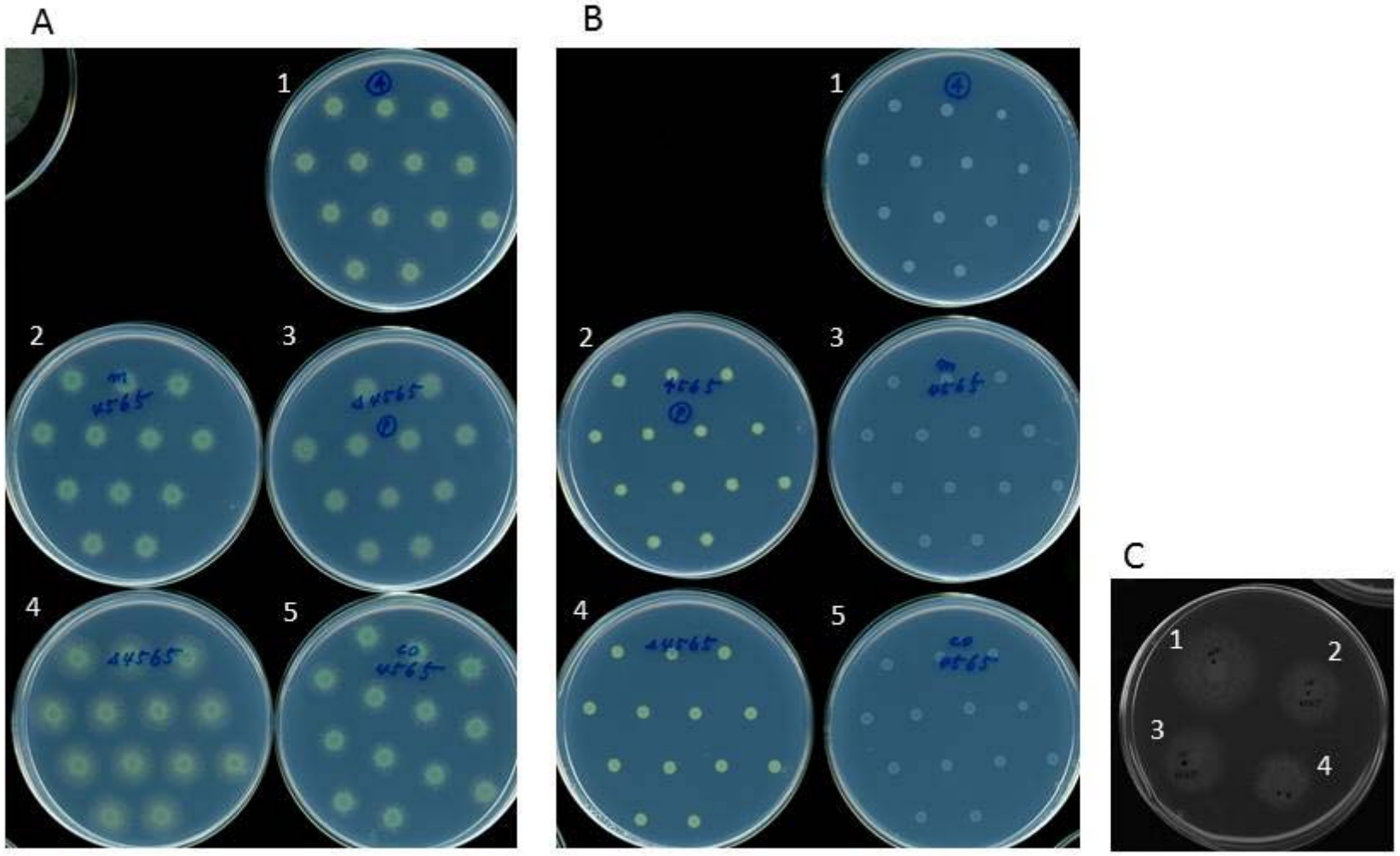
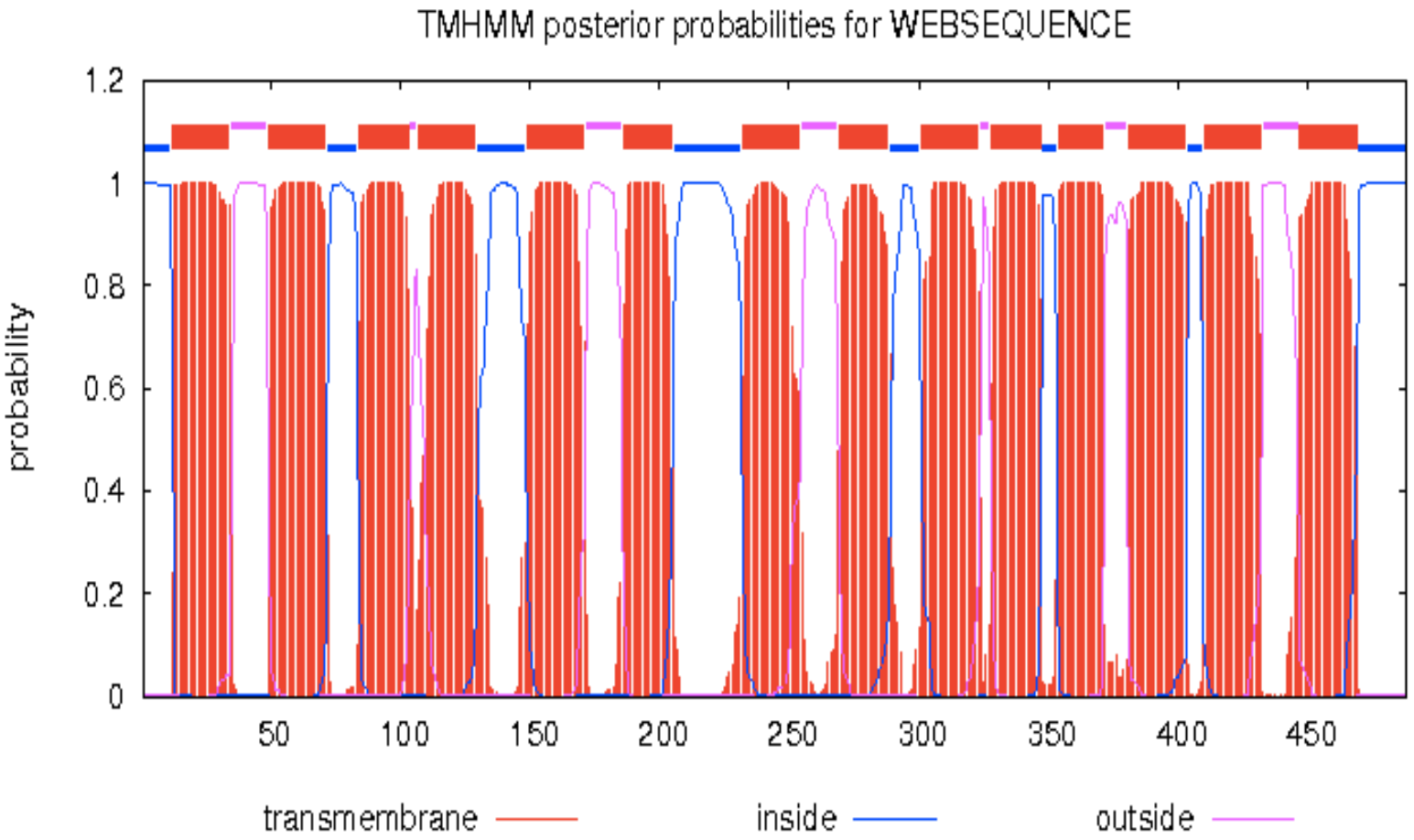


Fig. S2


TMHMM posterior probabilities for WEBSEQUENCE
probability
1.2
1
0.8
0.6
0.4
0.2
0
50
100
150
200
250
300
350
400
450
transmembrane
inside
outside