

An l-glucose Catabolic Pathway in Paracoccus Species 43P

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supplemental Table 1. Primers used in this study

Primer	Sequence (5'-3') ^a
Primers used for amplification and sequencing of 16S rRNA gene	
27F	AGAGTTTGATCCTGGCTCAG
1492R	CGTTACCTTGTACGACTT
43P16S_400F	GGGGAATCTTAGACAATGGGGG
43P16S_1000R	GAACGTCTCACGACACGAGCTG
Degenerate primers used for identification of <i>lgdA</i> and <i>lgnH</i>	
LGDH_NterF	ATHGGNACNGGNTTYATGGG
LGDH_midR	GGRTCNGCCATRTARTCYTC
LGnDH_NterFII	ATHGARAARGARGGNCAAYAC
LGnDH_midRIII	CCCATNCCDATCATNCCRCA
Primers used for inverse PCR and primer walking	
LGDH_invF	GCCGAATGCGGTGCGACGTTGC
LGDH_invFII	GTCAATTTCTGAGGAATGATAT
LGDH_invR	GCGGTTCGATCTGATCGCCGAAGG
LGDH_invRII	AAAGACTGGATCGCGCCGATCG
43P1679_F	ATTGGTGCGAAACGCTGCAACC
43P1678_invF	GGTCAAGGATGTGACCGGCGCG
43P1678_invFII	CGGCTATGTCGCGCTGAAAAAGG
43P1678_invFIII	GTTCTGGGCCAGCAGATCCTGA
43P1678_invFIV	CGCTTTATCCCTTTAACCTGTG
43P1678_invR	GGCGCGGTTACGTCGTTTCAGC
43P1678_invRII	CGGGCATATCCTCAAGCTGGAC
43P1678_invRIII	CCTCGATTTGTCCGGTAAAGGC
43P1677_R	GCTTCGATCATGCTGCCGGTGT
43P1676_invF	GACGACCGGCTGGAGAATGC
43P1676_invFII	ACGATCTGGTGCGCGGCTTT
43P1676_invFIII	CTATGCCAAGACCAAGAAGC
43P1676_invFIV	GCGGTTTCAGGACTTTACCA
43P1676_invFV	GCTATAGCTGCATGGGGTACGA
43P1676_invR	CGGTAGAAGATCAGCGGAAAGC
43P1676_invRII	CGCACAGGCCGCGTTTCCCG
43P1674_invF	AATGACTGCAAGGTCTGCGT
43P1674_invFII	GGTGCAGGCATGAAGCTGTT
43P1675_invR	AAAGTCGATCTGCGGCTGCT
43P1675_invRII	CCCCATTTGATTTCTGCTG
43P1675_invRIII	TATCTGCCCGATGGCAGCGA
LGNDH_invF	AATGGAAAAGGTCATTCTGAA
LGNDH_invFII	GATGTCGTGTTTCAAGCTGT
LGNDH_invFIII	CAATGCGACTATGACGGACT
LGNDH_invR	AGGGTGACCAAAGGATTAAC
LGNDH_invRII	ACATCACATCTTCGTCGATG
LGNDH_invRIII	ACAGGCTGTTGTTGCGATGC
LGNDH_invRIV	CAGTCGATATAACCGGCCTC
43P4929_invF	TGTAGTCGTTTTCGCGCATGG
43P4929_invFII	TCGAGAGATTTTCGAAACGC
43P4929_invFIII	GTGCATGTCCAGAACGTTAT
43P4929_invR	AGAGCCAGATCCTGAAGCTG

43P4929_invRII	CAACTCGCTTTTTGAGGCTT
43P4929_midF	CAATGGCTTCGGCAATATCC
43P4928_invF	CAAGCCTCAAAAAGCGAGTT
43P4928_invR	GCTTTCATCGCGATATTTCC
43P4926_midR	GACTTCGTCAAACAGCATGAGT
43P4926_midFII	GTGATTGCACACCGTGATCT
43P4926_NterR	ATTTCCCCGAAGTAAGCGCTT
43P4924_NterR	GAGATCTGCGACACAAGGCT
43P4923_CterF	CGGAACATCATGATCCTGGCA
43P4923_NterR	GCCAACAAATTCCCCAATCGC

Primers used for construction of expression plasmids

LgdA_NdeF	CCGGGCATATGAGCAATGCTGAAAAAGCCCTC
LgdA_XhoR	CCGGGCTCGAGGAAATTGACGGGCTGGCCGGTCT
LgnH_NdeF	GAGAGACATATGAAGGCGCTTATCATCGAGA
LgnH_XhoR	GGCCCTCGAGTCATAGTGCCACCATCACTT
LgnI_NdeF	GGGAGGCATATGAGCGACAGTTTCAAAAAA
LgnI_XhoR	GAGACTCGAGTTAGAACCCTGCGAGTGCCC
LgnF_NheF	GAGAGAGCTAGCACGAATGGAATAGCGCCACG
LgnF_XhoR	TAACTCGAGTCAGGGCCAGATACGGGTGG
LgnG_NdeF	GAGAGACATATGAATTTTCCAACCTCTCCCC
LgnG_EcoR	TTTGAATTCTCATGCGAGGCCTCCTGATGTCT
LgnE_NdeF	GAGAGACATATGAAAGCTAAAACAATCCGGC
LgnE_EcoR	GAAGAATTCTCACGTCACCACCGCAAGCTGCCAA
DgoD10B_NdeF	GAGAGACATATGAAAATCACCAAAATTACCAC
DgoD10B_XhoR	GGGCTCGAGTTACC ACTCTGCTACGCTGTTAT

Primers used for construction of suicide plasmids

LgdA1700_EcoF	GGGGAATTCTTCCGGCATGAAHATCGTTCG
LgdA1700_BamR	GGGGGATCCCTTCAGCGCCTTTTTTCAGCG
LgnE2000_HinF	CAGAGAAGCTTATCAACGAAGCAAATCCGTC
LgnE2000_XbaR	GATGCTCTAGAGTCGATATAACCGGCCTCCA
LgnH1500_EcoF	GGGGAATTTCGATCATCTCGGCTTATCAGG
LgnH1500_BamR	GGGGGATCCATAAGAAGCCATCGATGCGG
LgnI1500_XbaF	CAGTCATCTAGACTGCCGCTACAACAAAACGC
LgnI1500_SacIR	GACTGAGAGCTCGGTGTCAGAACGCGGATAGC
Pkan_SacIIF	CACAACCGCGGATGTCAGCTACTGGGCTATCTGGA
Kan_SacIIR	CACAACCGCGGTTGGTTCGGTCATTTCGAACC
Pkan_F	ATGTCAGCTACTGGGCTATCTGGA
Kan_R	TTGGTTCGGTCATTTCGAACC
Mob_F	ACTCGCATAGGCTTGGGTCG
Mob_R	CTGGCAATTCCGGTTCGCTT

Primers used for confirmation of gene disruption

lgdA_conF	AAGGACATCGAGGACGTGAT
lgdA_conR	ACGCTCGATTTTCGTCATTGC
lgnE_conF	AATGCCATGACGACAAACGC
lgnE_conR	TTTCCTCACCACGAATGACG
lgnH_conF	ATTCGGCATCGGAACCAACC
lgnI_conF	GCATGAAGGCGCTTATCATC
lgnI_conR	CGTGCTGACGGTACACCTCC

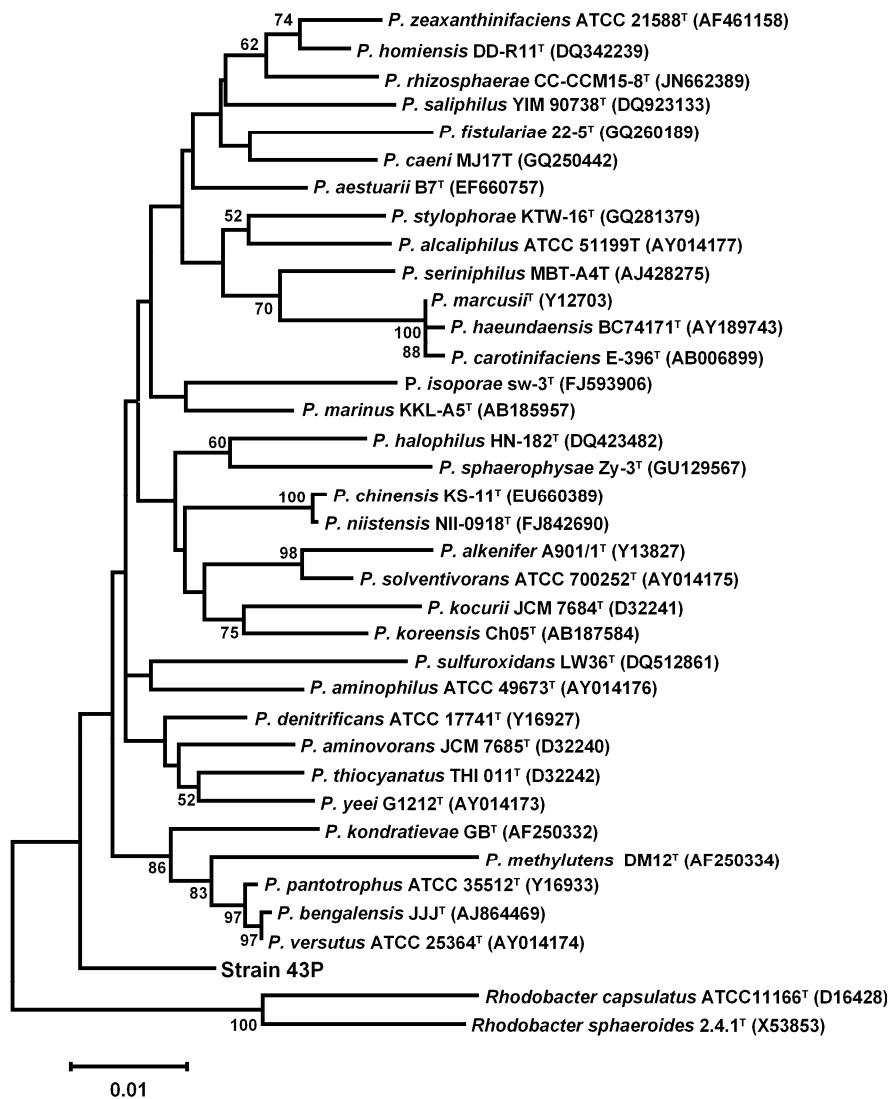
^a Restriction sites introduced are shown in italics, and the initiation and termination codons are in bold.

supplemental Table 2. Purification of L-GDH

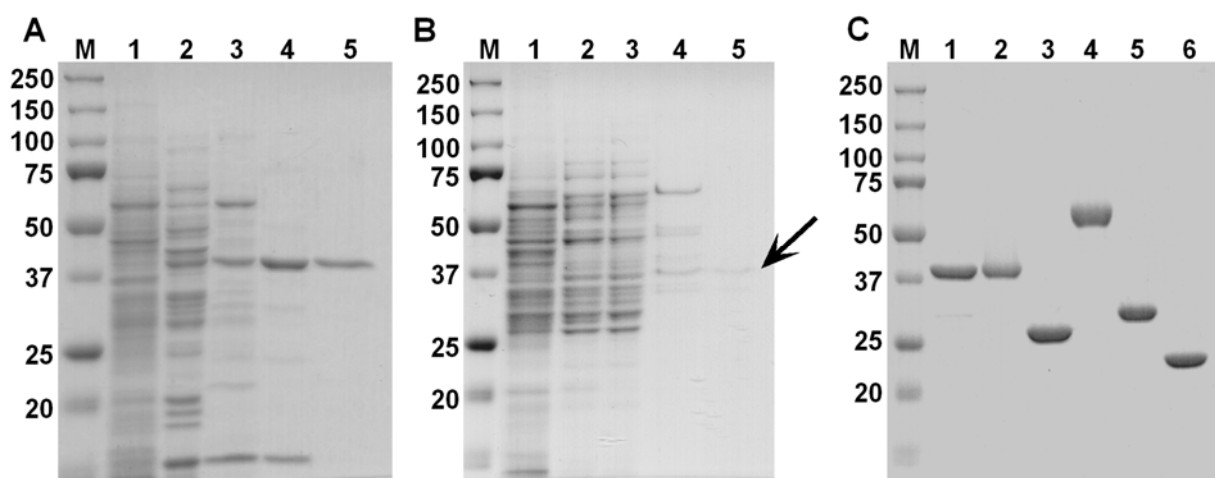
Purification step	Total protein (mg)	Total activity ($\mu\text{mol min}^{-1}$)	Specific activity ($\mu\text{mol min}^{-1}\text{mg}^{-1}$)	Yield (%)
Cell-free extract	734	33.1	0.05	100
DEAE-cellulose	117	22.6	0.19	68.2
Butyl Toyopearl	8.53	20.6	2.42	62.8
Mono Q 5/50	1.51	4.86	3.21	14.7
Superdex 200 10/300	0.21	0.982	4.71	3.00

supplemental Table 3. Purification of L-GnDH

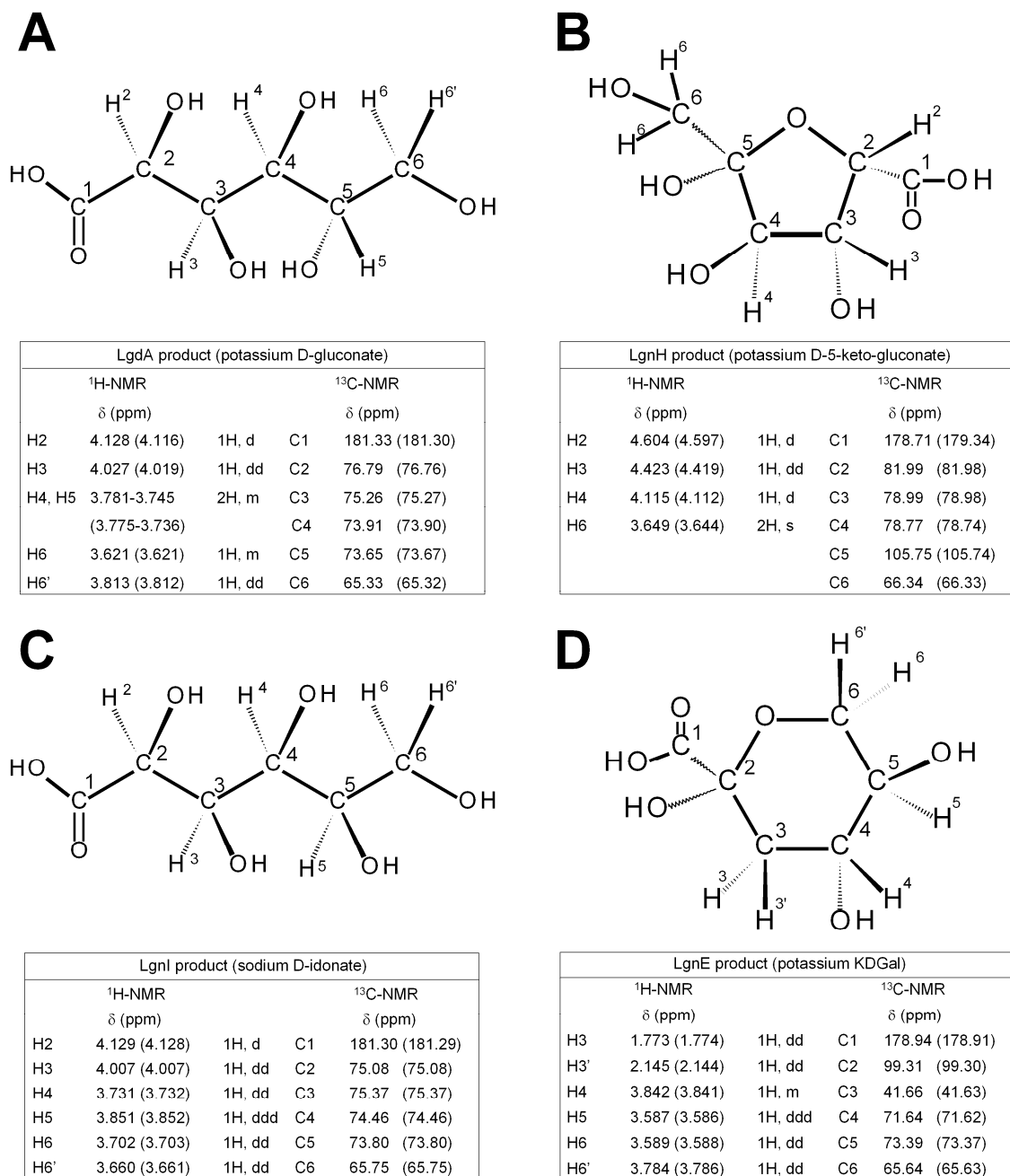
Purification step	Total protein (mg)	Total activity ($\mu\text{mol min}^{-1}$)	Specific activity ($\mu\text{mol min}^{-1}\text{mg}^{-1}$)	Yield (%)
Cell-free extract	149	33.9	0.23	100
1st DEAE-cellulose	45.7	27.1	0.59	80.0
2nd DEAE-cellulose	16.4	17.7	1.07	52.1
Mono Q 5/50	0.36	4.61	12.7	13.6
Superdex 200 10/300	0.04	0.851	22.0	2.51



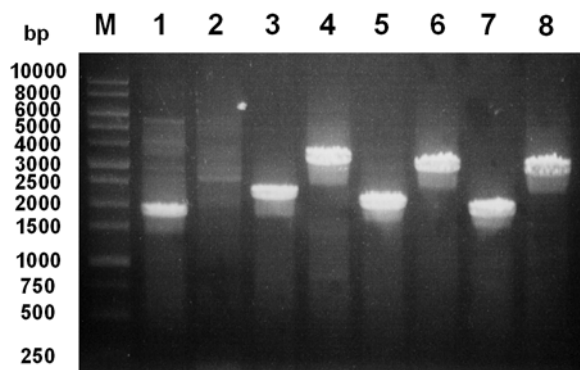
supplemental Figure 1. Phylogenetic tree based on 16S rRNA gene sequences of strain 43P and *Paracoccus* and *Rhodobacter* strains. The tree was drawn using the NJ method with the MEGA5 package. Bootstrap values were calculated from 1000 repeats and those greater than 50% are shown at branch points. The bar represents 0.01 substitution.



supplemental Figure 2. SDS-PAGE of purification steps of L-GDH (A) and L-GnDH (B) and the purified recombinant enzymes (C). Lanes M denote molecular markers (kDa). (A) Lane 1, cell-free extracts; 2, after DEAE-cellulose; 3, after Butyl-Toyopearl; 4, after Mono Q 5/50 GL; and 5, after Superdex 200 10/300 GL chromatography. (B) Lane 1, cell-free extracts; 2, after first DEAE-cellulose; 3, after second DEAE-cellulose; 4, after Mono Q 5/50 GL; and 5, after Superdex 200 10/300 GL chromatography. The arrow indicates the position of L-GnDH. (C) Lane 1, LgdA; 2, LgnH; 3, LgnI; 4, LgnE; 5, LgnF; and 6, LgnG.



supplemental Figure 3. NMR analysis of LgdA (A), LgnH (B), LgnI (C), and LgnE (D) reaction products. Chemical shifts of the reaction products are shown in tables below, where those of the authentic standards, potassium D-gluconate (A), potassium D-5-keto-gluconate (B), sodium D-idonate (C) and potassium KDGal (D), are shown in brackets. The deduced structures are also shown. All the chemical shifts were referenced to a 4,4-dimethyl-4-silapentane-1-sulfonic acid internal standard (0 ppm).



supplemental Figure 4. PCR analysis of gene disruption mutants. PCR reactions for amplifying *lgdA* (lanes 1 and 2), *lgnE* (3 and 4), *lgnH* (5 and 6), and *lgnI* (7 and 8) were conducted with primer pairs described in supplemental Table 1 using strain 43P chromosomal DNA (lanes 1, 3, 5, and 7), Δ *lgdA* strain (2), Δ *lgnE* strain (4), Δ *lgnH* strain (6), and Δ *lgnI* strain (8) as templates. Lane M denotes molecular mass markers.

supplemental Figure 5. Phylogenetic trees based on the amino acid sequences of LgdA (A), LgnH (B), LgnI (C), LgnE (D), LgnF (E), and LgnG (F) and their related sequences from the KEGG genome and Uniprot KB databases. The sequences of enzymes with known function from respective protein families in the Uniprot KB database are shown in green with their accession numbers, and those of "potential" L-glucose and L-gluconate utilizing organisms from the KEGG genome database are shown in red and blue, respectively, with KEGG organisms and accession numbers. The other sequences from the KEGG genome database showing high similarities are indicated only with lines. Line colors denote affiliations of organisms with the KEGG organisms; red, orange, yellow and magenta, *Alpha-, Beta-, Gamma- and Deltaproteobacteria*; green, *Firmicutes*; cyan, *Actinobacteria*; denim, *Chlamydiae*; cream, *Spirochetes*; purple, *Acidobacteria*; blue, *Bacteroidetes*; mustard, *Fusobacteria*; camel, *Verrucomicrobia*; and lilac, Hyperthermophilic bacteria. The bars represent 0.1 substitutions.