Supplementary materials

Table S1: Total ABE concentration, yield, and productivity obtained during batch ABE fermentation by *C. beijerinckii* NCIMB 8052

Parameters (Max. values)	Control	$CaCO_3$ (4 g/L) treatment
Acetone (g/L)	3.1 ± 0.7	8.0 ± 0.3
Ethanol (g/L)	0.9 ± 0.2	1.1 ± 0.1
Butanol (g/L)	11.4 ± 0.8	16.3 ± 0.3
*Total ABE (g/L)	15.4 ± 1.8	25.5 ± 0.5
Initial glucose (g/L)	59.8 ± 1.0	65 ± 1.0
*Final glucose (g/L)	17.8 ± 1.0	0.0
Cell density (540 nm)	6.1 ± 0.4	9.9 ± 0.4
*Cell Dry Weight (g/L)	3.5 ± 0.5	6.2 ±0.5
Fermentation time (h)	72	60
Total glucose utilized (g)	42.0 ± 0.0	65 ± 1.0
ABE Yield (g/g)	0.37 ± 0.04	0.39 ± 0.00
ABE Productivity (g/L/h)	0.21 ± 0.03	0.42 ± 0.01

*The marked effects of $CaCO_3$ on *C. beijerinckii* during ABE fermentation

Table S2A: Isoelectric points of solventogenic enzymes of *C. beijerinckii* NCIMB 8052

Enzyme	**Isoelectric point**
Pflb pyruvate-ferredoxin oxidoreductase	5.8
ak acetate kinase	5.1
pta phosphotrans acetylase	4.7
adhE aldhyde dehydrogenase	5.8
bdhAB alcohol dehydrogenase	5.5
thl thiolase	5.8
adc acetoacetate decarboxylase	6.2
ctfAB CoA-transferase	subunit A 4.9 subunit B 6.9
hbd 3-hydroxybutyryl-CoA dehydrogenase	5.3
crt crotonase	5.5
Bcd butyryl-CoA dehydrogenase	5.3
buk butyrate kinase	4.9
ptb phosphotransbutyrylase	9.2

Table S2B: Isoelectric points of annotated pathways in *C. beijerinckii* NCIMB 8052

Carbohydrate utilization genes: Glycolysis/Gluconeogenesis

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
Putative glucokinase, ROK family	4.8	[EC:2.7.1.2]
Glucose-6-phosphate isomerase	4.9	[EC:5.3.1.9]
6-Phosphofructokinase	5.4	[EC:2.7.1.11]
Fructose-1,6-bisphosphatase	6.1	fructose-1,6-bisphosphataseIII [EC:3.1.3.11]
Fructose-1,6-bisphosphate aldolase	5.6	fructose-bisphosphate aldolase, class I [EC:4.1.2.13]
Fructose-bisphosphate aldolase	6.2	fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
Triosephosphate isomerase	4.8	[EC:5.3.1.1]
Glyceraldehyde-3-phosphate Dehydrogenase, type I	5.3	[EC:1.2.1.12]
Phosphoglycerate kinase	4.9	[EC:2.7.2.3]
Phosphoglyceromutase	5.0	phosphoglycerate mutase [EC:5.4.2.1]
Phosphoglycerate mutase	6.3	phosphoglycerate mutase [EC:5.4.2.1]
Phosphoglycerate mutase 1 family	9.2	phosphoglycerate mutase [EC:5.4.2.1]
Phosphopyruvate hydratase	4.4	enolase [EC:4.2.1.11]

Pyruvate kinase	5.1	[EC:2.7.1.40]
L-Lactate dehydrogenase	4.9	[EC:1.1.1.27]
Iron-containing alcohol dehydrogenase (bifunctional acetaldehyde-CoA/alcohol dehydrogenase)	5.5	alcohol dehydrogenase [EC:1.1.1.1] acetaldehyde dehydrogenase [EC:1.2.1.10]
Aldose 1-epimerase	4.6	[EC:5.1.3.3]
Glycoside hydrolase, family protein	4.7	6-phospho-beta-glucosidase [EC:3.2.1.86]
6-Phospho-beta-glucosidase	5.1	[EC:3.2.1.86]
PTS system, glucose subfamily, IIA subunit	6.2	PTS system, glucose-specific IIA component [EC:2.7.1.69]
PTS system, alpha-glucoside-specific IIBC subunit	8.2	PTS system, arbutin-like IIB component [EC:2.7.1.69] PTS system, arbutin-like IIC component
Cellobiose/arbutin/salicin-specific PTS system components IIBC	9.2	PTS system, arbutin-, cellobiose-, and salicin-specific IIB component [EC:2.7.1.69] PTS system, arbutin-, cellobiose-, and salicin-specific IIC component

Pentose phosphate pathway

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
Glucose-6-phosphate isomerase	4.9	[EC:5.3.1.9]
Hypothetical protein	5.6	6-phosphogluconolactonase [EC:3.1.1.31]
6-Phosphogluconate dehydrogenase-like protein	5.1	6-phosphogluconate dehydrogenase [EC:1.1.1.44]
Ribulose-phosphate 3-epimerase	5.0	[EC:5.1.3.1]
Transketolase, central region	5.0	transketolase [EC:2.2.1.1]
Transketolase domain protein	6.0	transketolase [EC:2.2.1.1]
Transketolase	5.2	transketolase [EC:2.2.1.1]
Putative transaldolase	4.8	transketolase [EC:2.2.1.1]
Ribose 5-phosphate isomerase	5.0	ribose 5-phosphate isomerase A [EC:5.3.1.6]
Sugar-phosphate isomerase, RpiB/LacA/LacB family	8.5	ribose 5-phosphate isomerase B [EC:5.3.1.6]
Deoxyribose-phosphate aldolase	6.8	[EC:4.1.2.4]

Ribokinase	5.0	[EC:2.7.1.15]
Phosphopentomutase	5.0	[EC:5.4.2.7]
Ribose-phosphate pyrophosphokinase	5.9	[EC:2.7.6.1]
Phosphoribosylpyrophosphate synthetase	5.9	ribose-phosphate pyrophosphokinase [EC:2.7.6.1]
2-Dehydro-3-deoxyphosphogluconate aldolase /4-hydroxy-2-oxoglutarate aldolase	6.5	[EC:4.1.2.14 4.1.3.16]
PfkB domain protein	5.7	2-dehydro-3-deoxygluconokinase [EC:2.7.1.45]
Fructose-1,6-bisphosphate aldolase	5.1	fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
Fructose-bisphosphate aldolase	6.2	fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
Fructose-1,6-bisphosphate aldolase	5.6	fructose-bisphosphate aldolase, class I [EC:4.1.2.13]
6-Phosphofructokinase	5.4	[EC:2.7.1.11]
Fructose-1,6-bisphosphatase	6.1	fructose-1,6-bisphosphatase III [EC:3.1.3.11]

Pentose and glucuronate interconversions

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
5-Keto-4-deoxyuronate isomerase	5.5	4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase [EC:5.3.1.17]
2-Deoxy-D-gluconate 3-dehydrogenase	5.9	[EC:1.1.1.125]
PfkB domain protein	5.7	2-dehydro-3-deoxygluconokinase [EC:2.7.1.45]
2-Dehydro-3-deoxyphosphogluconate aldolase /4-hydroxy-2-oxoglutarate aldolase	6.5	[EC:4.1.2.14 4.1.3.16]
Glucuronate isomerase	5.2	[EC:5.3.1.12]
Altronate oxidoreductase	4.8	tagaturonate reductase [EC:1.1.1.58]
Altronate hydrolase	5.5	[EC:4.2.1.7]
Mannonate dehydratase	5.3	[EC:4.2.1.8]
Mannitol dehydrogenase, C-terminal domain	4.9	fructuronate reductase [EC:1.1.1.57]
UDP glucose 6-dehydrogenase	5.9	[EC:1.1.1.22]
UTP--glucose-1-phosphate	9.5	[EC:2.7.7.9]

uridylyltransferase		
L-Ribulose-5-phosphate 4-epimerase	5.2	[EC:5.1.3.4]
Ribulose-phosphate 3-epimerase	5.3	[EC:5.1.3.1]
Orotidine 5'-phosphate decarboxylase	4.8	3-hexulose-6-phosphate synthase [EC:4.1.2.-]
L-Arabinose isomerase	5.1	[EC:5.3.1.4]
Xylose isomerase	5.0	[EC:5.3.1.5]
Rhamnulokinase	5.1	[EC:2.7.1.5]
Rhamnulose-1-phosphate aldolase	5.5	[EC:4.1.2.19]

Fructose and mannose metabolism

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
ROK family protein	5.4	fructokinase [EC:2.7.1.4]
Ribokinase-like domain-containing protein	4.6	fructokinase [EC:2.7.1.4]
Mannose-6-phosphate isomerase, class I	4.9	mannose-6-phosphate isomerase [EC:5.3.1.8]
Rhamnulokinase	4.9	[EC:2.7.1.5]
Rhamnulose-1-phosphate aldolase	5.5	[EC:4.1.2.19]
6-Phosphofructokinase	5.4	[EC:2.7.1.11]
Fructose-1,6-bisphosphatase	6.1	fructose-1,6-bisphosphatase III [EC:3.1.3.11]
Mannitol dehydrogenase, C-terminal domain	4.9	mannitol-1-phosphate 5-dehydrogenase [EC:1.1.1.17]
Sorbitol-6-phosphate 2-dehydrogenase	6.4	[EC:1.1.1.140]
1-Phosphofructokinase	5.3	[EC:2.7.1.56]
Xylose isomerase	5.0	[EC:5.3.1.5]
Fructose-1,6-bisphosphate aldolase, class II	5.6	fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
Fructose-bisphosphate aldolase, class II	6.2	[EC:4.1.2.13]
Fructose-bisphosphate aldolase, class I	6.2	[EC:4.1.2.13]
Triosephosphate isomerase	4.8	(EC:5.3.1.1)
PTS system, fructose subfamily, IIC subunit	6.6	
PTS system, mannose/fructose/sorbose family, IIA subunit	6.1	
Phosphotransferase system PTS,	4.8	

sorbose-Specific IIC subunit		
PTS system, mannose/fructose/sorbose family, IIC subunit	5.7	PTS system, mannose-specific IIC component
PTS system, mannose/fructose/sorbose family, IID subunit	9.9	PTS system, mannose-specific IID component
Phosphoenolpyruvate-dependent sugar Phosphotransferase system, EIIA 2	6.3	PTS system, mannitol-specific IIA component [EC:2.7.1.69]
PTS system, mannitol-specific IIC subunit	8.6	
PTS system, glucitol/sorbitol-specific IIA subunit	4.7	PTS system, glucitol/sorbitol-specific IIA component
PTS system, glucitol/sorbitol-specific, IIBC subunit	8	
PTS system, glucitol/sorbitol-specific, IIC subunit	9.4	PTS system, glucitol/sorbitol-specific IIC component

Galactose metabolism

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Galactokinase	5.0	[EC:2.7.1.6]
UTP--glucose-1-phosphate uridylyltransferase	9.5	[EC:2.7.7.9]
Putative glucokinase, ROK family	4.8	glucokinase [EC:2.7.1.2]
Glycoside hydrolase family 2, TIM barrel	5.2	beta-galactosidase [EC:3.2.1.23]
Glycoside hydrolase, family 4	7.0	alpha-galactosidase [EC:3.2.1.22]
Putative PTS IIA-like nitrogen-regulatory protein, PtsN	5.1	PTS system, galactitol-specific IIA component
Phosphotransferase system, lactose/cellobiose-specific IIB subunit	6.2	PTS system, galactitol-specific IIB component [EC:2.7.1.69]
PTS system, galactitol-specific IIB component, putative	5.4	PTS system, galactitol-specific IIB component [EC:2.7.1.69]
PTS system galactitol-specific IIC component	9.1	PTS system galactitol-specific IIC component
6-Phosphofructokinase	5.4	[EC:2.7.1.11]
PTS system fructose subfamily IIA component	6.1	PTS system, N-acetylgalactosamine-specific IIA component [EC:2.7.1.69]

N-acetylgalactosamine-specific PTS system transporter subunit IIB	6.8	PTS system, galactosamine-specific IIB component [EC:2.7.1.69]
N-acetylgalactosamine-specific PTS system transporter subunit IIC	4.6	PTS system, galactosamine-specific IIC component
N-acetylgalactosamine-specific PTS system transporter subunit IID	9.4	PTS system, galactosamine-specific IID component

Starch and sucrose metabolism

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Phosphotransferase system, EIIC	9.5	
Beta-glucosidase	5.0	[EC:3.2.1.21]
Alpha-phosphotrehalase	4.8	trehalose-6-phosphate hydrolase [EC:3.2.1.93]
PTS system, glucose subfamily, IIA subunit	6.2	PTS system, glucose-specific IIA component [EC:2.7.1.69]
PTS system, trehalose-specific IIBC subunit	9.6	
UDP-glucose 6-dehydrogenase	5.3	[EC:1.1.1.22]
UTP--glucose-1-phosphate uridylyltransferase	9.5	[EC:2.7.7.9]
Putative glucokinase, ROK family	4.8	glucokinase [EC:2.7.1.2]
Glucose-6-phosphate isomerase	4.9	[EC:5.3.1.9]
ROK family protein	5.4	fructokinase [EC:2.7.1.4]
PfkB domain protein (ribokinase-like domain-containing protein)	5.7	fructokinase [EC:2.7.1.4]
Glucose-1-phosphate adenylyltransferase, GlgD subunit	7.1	glucose-1-phosphate adenylyltransferase [EC:2.7.7.27]
Glucose-1-phosphate adenylyltransferase	5.0	glucose-1-phosphate adenylyltransferase [EC:2.7.7.27]
Glycogen/starch synthase, ADP-glucose type	5.0	starch synthase [EC:2.4.1.21]
1,4-alpha-glucan branching enzyme	5.2	[EC:2.4.1.18]
Glycogen/starch/alpha-glucan phosphorylase	6.5	starch phosphorylase [EC:2.4.1.1]
4-Alpha-glucanotransferase	4.9	[EC:2.4.1.25]
Glycoside hydrolase, family 4	7.0	maltose-6'-phosphate glucosidase [EC:3.2.1.122]

Aminosugars metabolism

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Glucosamine-6-phosphate isomerase	5.5	glucosamine-6-phosphate deaminase [EC:3.5.99.6]
Glucosamine--fructose-6-phosphate aminotransferase (isomerizing)	4.8	[EC:2.6.1.16]
N-acetylglucosamine-6-phosphate deacetylase	4.9	[EC:3.5.1.25]
UDP-N-acetylglucosamine pyrophosphorylase	6.0	bifunctional protein GlmU [EC:2.7.7.23 2.3.1.157]
UDP-N-acetylglucosamine 2-epimerase	5.7	[EC:5.1.3.14]
PTS system, N-acetylglucosamine-specific IIBC subunit	9.0	
PTS system, glucose subfamily, IIA subunit	6.2	
Glucokinase regulatory-like protein	5.6	N-acetylmuramic acid 6-phosphate etherase [EC:4.2.-.-]
Phosphoglucosamine mutase	4.6	[EC:5.4.2.10]
Glycoside hydrolase, family 18	5.4	chitinase [EC:3.2.1.14]
UDP-N-acetylglucosamine 1-carboxyvinyltransferase	6.4	[EC:2.5.1.7]
MurB, UDP-N-acetylenolpyruvoylglucosamine reductase	5.6	UDP-N-acetylmuramate dehydrogenase [EC:1.1.1.158]

Pyruvate metabolism

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Pyruvate formate-lyase	5.5	formate C-acetyltransferase [EC:2.3.1.54]
Formate acetyltransferase	5.5	formate C-acetyltransferase [EC:2.3.1.54]
Iron-containing alcohol dehydrogenase (Bifunctional acetaldehyde-CoA/alcohol dehydrogenase)	5.6	alcohol dehydrogenase [EC:1.1.1.1] acetaldehyde dehydrogenase [EC:1.2.1.10]
Aldehyde dehydrogenase	5.5	
Acetate kinase	5.1	[EC:2.7.2.1]
Pyruvate kinase	5.1	[EC:2.7.1.40]

Acetyl-CoA carboxylase, carboxyl transferase, alpha subunit	7.3	acetyl-CoA carboxylase carboxyl transferase subunitalpha [EC:6.4.1.2]
Acetyl-CoA carboxylase biotin carboxyl carrier protein	4.5	
Biotin carboxylase	5.5	biotin carboxylase [EC:6.3.4.14] acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2]
Acetyl-CoA carboxylase, carboxyl transferase, beta subunit	5.6	acetyl-CoA carboxylase carboxyl transferase subunit beta [EC:6.4.1.2]
Acylphosphatase	8.7	[EC:3.6.1.7]
L-lactate dehydrogenase	4.8	[EC:1.1.1.27]
D-isomer specific 2-hydroxyacid dehydrogenase, NAD-binding	5.0	D-lactate dehydrogenase [EC:1.1.1.28]
L-1,2-propanediol oxidoreductase	4.7	lactaldehyde reductase [EC:1.1.1.77]
Methylglyoxal synthase	7.8	[EC:4.2.3.3]
Carboxynorspermidine decarboxylase	5.1	
Malate dehydrogenase (oxaloacetate-decarboxylating)	4.7	[EC:1.1.1.38]
Pyruvate carboxylase	5.5	pyruvate carboxylase subunit A [EC:6.4.1.1] pyruvate carboxylase subunit B [EC:6.4.1.1]
Pyruvate phosphate dikinase	4.8	pyruvate,orthophosphate dikinase [EC:2.7.9.1]
Phosphoenolpyruvate synthase	5.9	pyruvate,water dikinase [EC:2.7.9.2]
Pyruvate phosphate dikinase, PEP/pyruvate-binding	8.2	pyruvate,water dikinase [EC:2.7.9.2]
Acetyl-CoA acetyltransferase	6.1	[EC:2.3.1.9]
2-Isopropylmalate synthase	6.0	[EC:2.3.3.13]
aksA Trans-homoaconitate synthase	5.8	homocitrate synthase [EC:2.3.3.14] homocitrate synthase NifV
Homocitrate synthase	5.4	homocitrate synthase [EC:2.3.3.14] homocitrate synthase NifV

Motility and chemotaxis genes/proteins: Bacterial chemotaxis

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
Methyl-accepting chemotaxis sensory transducer	4.8	methyl-accepting chemotaxis protein
CheA signal transduction histidine	5.0	two-component system, chemotaxis

kinase		family, sensor kinase, CheA, [EC:2.7.13.3]
Putative CheW protein	5.0	purine-binding chemotaxis protein CheW
CheC domain protein	4.2	chemotaxis protein CheX
CheD	9.2	chemotaxis protein CheD, [EC:3.5.1.44]
Protein-glutamate O-methyltransferase	5.2	chemotaxis protein methyltransferase CheR [EC:2.1.1.80]
Response regulator receiver modulated CheB methylesterase	8.8	protein-glutamate methylesterase, two-component system,chemotaxis family, response regulator CheB, [EC:3.1.1.61]
Response regulator receiver protein	6.9	two-component system, chemotaxis family, response, regulator CheY
Putative CheW protein	5.0	two-component system, chemotaxis family, response, regulator CheV
FliG, flagellar motor switch protein G	4.8	
Flagellar motor switch protein FliM	4.6	
Flagellar motor switch protein	4.1	flagellar motor switch protein FliN/FliY
MotA/TolQ/ExbB proton channel	4.8	chemotaxis protein MotA
Flagellar motor protein MotA	5.0	chemotaxis protein MotA
OmpA/MotB domain protein	5.0	chemotaxis protein MotB

Flagellar genes/proteins

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
FlhA , flagellar biosynthesis protein A	4.7	
FliR/FlhB, bifunctional flagellar Biosynthesis protein	9.2	
Export protein FliQ, family 3	8.2	flagellar biosynthetic protein FliQ
FliP, flagellar biosynthetic protein	5.0	EC:3.1.1.3
Putative flagellar formation protein	9.4	flagellar protein FliO/FliZ
Flagellar motor switch protein	4.1	flagellar motor switch protein FliN/FliY
FliM, flagellar motor switch protein	4.6	
OmpA/MotB domain protein	5.4	chemotaxis protein MotB
MotA/TolQ/ExbB proton channel	4.8	chemotaxis protein MotA
MotA, flagellar motor protein	5.1	

Protein of unknown function DUF1078 domain protein	4.9	flagellar hook protein FlgE
Flagellar hook capping protein	4.6	flagellar basal-body rod modification protein FlgD
FliJ, flagellar export protein	9.3	
Flagellar protein export ATPase FliI	5.4	[EC:3.6.3.14] flagellum-specific ATP synthase
FliH, flagellar biosynthesis/type III secretory pathway -like protein	5.3	flagellar assembly protein FliH
FliG, flagellar motor switch protein G	4.8	
FliF, flagellar MS-ring protein	5.4	
FliE ,flagellar hook-basal body complex subunit	4.8	
FlgC, flagellar basal body rod protein	6.6	
FlgB, flagellar basal body rod protein	8.8	
FlgM, anti-sigma-28 factor	9.2	negative regulator of flagellin synthesis FlgM
FlgG, flagellar basal body rod protein	4.7	
FlgK, flagellar hook-associated protein	5.0	
FlgL, flagellar hook-associated protein 3	4.8	flagellar hook-associated protein 3 FlgL
Flagellin domain protein	5.1	
Flagellar hook-associated 2 domain protein	5.3	
Flagellar protein FliS	6.6	

Stress response genes/proteins

Stress response genes (Definition)	**Isoelectric point**
Ferric uptake regulator family protein (Peroxide stress response regulator)	8.5
Stress responsive alpha-beta barrel domain protein	5.6
Heat shock protein DnaJ domain-containing protein	9.3
Heat shock protein Hsp20	5.3
Heat shock protein 90	5.0

Fatty acids metabolism genes/proteins: Fatty acid biosynthesis

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**

Biotin carboxylase	5.7	[EC:6.3.4.14 6.4.1.2]
Acetyl-CoA carboxylase, biotin carboxylase	5.7	[EC:6.3.4.14 6.4.1.2]
Acetyl-CoA carboxylase, carboxyl transferase, alpha subunit	7.3	[EC:6.4.1.2]
Acetyl-CoA carboxylase, carboxyl transferase, beta subunit	5.6	[EC:6.4.1.2]
Acetyl-CoA carboxylase, biotin carboxyl carrier protein	4.5	
Malonyl CoA-acyl carrier protein transacylase	5.4	[EC:2.3.1.39]
Beta-ketoacyl synthase-like protein	5.4	[EC:2.3.1.179]
3-Oxoacyl-(acyl-carrier-protein) synthase III	5.3	[EC:2.3.1.180]
Beta-ketoacyl-acyl-carrier-protein synthase I	5.1	[EC:2.3.1.180]
FabG, 3-ketoacyl-(acyl-carrier-protein) reductase	8.3	[EC:1.1.1.100]
Short-chain dehydrogenase/reductase SDR	5.8	[EC:1.1.1.100]
Beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ	8.5	[EC:4.2.1.-]
2-Nitropropane dioxygenase, NPD	6.6	[EC:1.3.1.-]

Fatty acid metabolism

Gene/protein (Definition)	**Isoelectric point**	**Note (Orthology)**
Acetyl-CoA acetyltransferase	6.1	[EC:2.3.1.9]
Enoyl-CoA hydratase/isomerase	5.5	[EC:4.2.1.17]
Iron-containing alcohol dehydrogenase	5.5	[EC:1.1.1.1 1.2.1.10]

Sporulation genes/proteins

Gene/ protein (Definition)	**Isoelectric point**	**Note (Orthology)**
SpoVG family protein	5.1	stage V sporulation protein G
AbrB family transcriptional regulator (SpoVT/AbrB domain protein)	7.6	stage V sporulation protein T
Sporulation stage II, protein E	**6.4**	[EC:3.1.3.16]
RNA polymerase factor sigma-70	8.8	RNA polymerase sporulation-specific sigma factor
Sporulation stage II, protein R	6.6	
Sporulation stage II protein D	6.2	

Sporulation stage III, transcriptional regulator SpoIIID	9.6	
Anti-sigma F factor	8.6	(EC:2.7.11.1)
Sporulation sigma factor SigF	7.7	stage II sporulation protein AB
Sporulation stage V, protein AC	6.2	
Stage V sporulation protein AD	5.4	
Sporulation stage V, protein AE	9.4	
Stage II sporulation P family protein	6.0	
Putative stage IV sporulation YqfD	9.4	
Sporulation sigma factor SigK	9.2	RNA polymerase sporulation-specific sigma factor
Peptidase U4, sporulation factor SpoIIGA	8.8	
Sporulation sigma factor SigE	8.8	RNA polymerase sporulation-specific sigma factor
Sporulation sigma factor SigG	8.7	RNA polymerase sporulation-specific sigma factor
Sporulation protein YlmC/YmxH	5.0	
Sporulation stage IV, protein A	4.9	
Sporulation integral membrane protein YlbJ	9.3	
Stage V sporulation protein S	8.9	
Sporulation protein YtaF	8.4	
Sporulation protease LonB	6.1	EC:3.4.21.53
Sporulation stage V, protein B	9.2	
Sporulation protein YtxC	4.9	
Stage V sporulation protein D	5.5	EC:2.4.1.129 sporulation-specific penicillin binding protein
Stage V sporulation protein E	9.4	cell division protein FtsW
Sporulation stage III, protein AA	8.3	
Stage III sporulation protein SpoAB	5.2	
Stage III sporulation AC family protein	6.5	
Sporulation stage III, protein AD	6.1	
Sporulation stage III, protein AE	8.2	
Sporulation stage III, protein AF	6.4	
Sporulation stage III, protein AG	7.6	
Stage III sporulation protein AH	4.8	
Peptidase S55	6.9	[EC:3.4.21.116] stage IV sporulation protein B
Sporulation protein YtfJ	4.8	
Sporulation stage II, protein M	9.5	
ECF subfamily RNA polymerase Sigma-24 factor	6.1	RNA polymerase sporulation-specific sigma factor

Sporulation protein YunB	8.8	
Sporulation protease LonC	6.7	[EC:3.4.21.53]

Membrane Transport: ABC transporters

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Sulfate ABC transporter, periplasmic Sulfate-binding protein	4.8	sulfate transport system substrate-binding protein
Sulfate ABC transporter, inner Membrane subunit CysT	9.7	sulfate transport system permease protein
Sulfate ABC transporter, inner Membrane subunit CysW	10	sulfate transport system permease protein
Sulfate ABC transporter, ATPase subunit	6.7	sulfate transport system ATP-binding protein [EC:3.6.3.25]
NMT1/THI5-like protein	4.5	sulfonate/nitrate/taurine transport system substrate-binding protein
ABC transporter, periplasmic-binding, putative	9.5	sulfonate/nitrate/taurine transport system substrate-binding protein
Aliphatic sulfonates family ABC transporter, periplsmic ligand-binding protein	5.4	sulfonate/nitrate/taurine transport system substrate-binding protein
Binding-protein-dependent transport systems inner membrane component	8.7	sulfonate/nitrate/taurine transport system permease protein osmoprotectant transport system permease protein lactose/L-arabinose transport system permease protein D-methionine transport system permease protein peptide/nickel transport system permease protein
ABC transporter related	4.7-9.8	sulfonate/nitrate/taurine transport system ATP-binding protein

		maltose/maltodextrin transport system ATP-binding protein putative multiple sugar transport system ATP-binding protein ribose transport system ATP-binding protein methyl-galactoside transport system ATP-binding protein D-xylose transport system ATP-binding protein putative glutamine transport system ATP-binding protein branched-chain amino acid transport system ATP-binding protein branched-chain amino acid transport system ATP-binding protein D-methionine transport system ATP-binding protein iron complex transport system ATP-binding protein zinc transport system ATP-binding protein cobalt/nickel transport system ATP-binding protein antibiotic transport system ATP-binding protein lipopolysaccharide transport system ATP-binding protein

		ATP-binding cassette, subfamily B, bacterial MsbA macrolide transport system permease protein macrolide transport system ATP-binding protein
Molybdenum ABC transporter, periplasmic molybdate-binding protein	4.8	molybdate transport system substrate-binding protein
Molybdate ABC transporter, inner Membrane subunit	9.7	molybdate transport system permease protein
Extracellular solute-binding protein, family 1	4.6	spermidine/putrescine transport system substrate-binding protein lactose/L-arabinose transport system substrate-binding protein sorbitol/mannitol transport system substrate-binding protein
Ornithine carbamoyltransferase	5.2	spermidine/putrescine transport system permease protein
Spermidine/putrescine ABC transporter ATPase subunit	5.4	spermidine/putrescine transport system ATP-binding protein [EC:3.6.3.31]
Substrate-binding region of ABC-type glycine betaine transport system	8.8	osmoprotectant transport system substrate-binding protein osmoprotectant transport system permease protein
Monosaccharide-transporting ATPase	9.5	putative multiple sugar transport system substrate-binding protein putative multiple sugar transport system permease protein ribose transport system permease protein methyl-galactoside transport system permease protein D-xylose transport system permease

		protein
Periplasmic binding protein/LacI transcriptional regulator	4.8	ribose transport system substrate-binding protein methyl-galactoside transport system substrate-binding protein
ABC-type sugar transport system periplasmic component-like protein	5.1	ribose transport system substrate-binding protein
Periplasmic binding protein/LacI Transcriptional regulator	4.8	L-arabinose transport system substrate-binding protein
D-xylose ABC transporter, periplasmic substrate-binding protein	4.9	
Phosphate binding protein	4.8	phosphate transport system substrate-binding protein
Phosphate ABC transporter, inner Membrane subunit PstC	9.7	phosphate transport system permease protein
Phosphate ABC transporter, inner Membrane subunit PstA	9.9	phosphate transport system permease protein
Phosphate ABC transporter, ATPase subunit	5.4	phosphate transport system ATP-binding protein
Extracellular ligand-binding receptor	4.9	branched-chain amino acid transport system substrate-binding protein
Inner-membrane translocator	9.8	branched-chain amino acid transport system permease protein
NlpA lipoprotein	4.9	D-methionine transport system substrate-binding protein
Lipoprotein, YaeC family	4.9	D-methionine transport system substrate-binding protein
Extracellular solute-binding protein, family 5	4.6	peptide/nickel transport system substrate-binding protein
Oligopeptide/dipeptide ABC transporter, ATPase subunit	5.7	peptide/nickel transport system ATP-binding protein
Periplasmic binding protein	5.0	iron complex transport system substrate-binding protein
Transport system permease protein	9.1	iron complex transport system permease protein
Periplasmic solute binding protein	4.6	zinc transport system substrate-binding protein
ABC-3 protein	8.3	zinc transport system permease protein
Cobalt transport protein CbiM	6.8	cobalt/nickel transport system permease protein
Cobalamin biosynthesis protein CbiM	8.6	cobalt/nickel transport system permease protein

Cobalt transport protein	6.8	cobalt/nickel transport system permease protein
Cobalt ABC transporter, inner membrane subunit CbiQ	9.1	cobalt/nickel transport system permease protein
Antibiotic transport-associated permease SpaG/MutG	8.3	antibiotic transport system permease protein
Lantibiotic permease protein SpaE/MutE	9.6	antibiotic transport system permease protein
Protein of unknown function DUF214	8.8	cell division transport system permease protein
Cell division ATP-binding protein FtsE	9.0	cell division transport system ATP-binding protein

Phosphotransferase system (PTS)

Gene/protein (Definition)	Isoelectric point	Note (Orthology)
Phosphoenolpyruvate-protein phosphotransferase	4.6	phosphotransferase system, enzyme I, PtsI [EC:2.7.3.9]
PTS system, glucose subfamily, IIA subunit	6.2	PTS system, glucose-specific IIA component [EC:2.7.1.69] PTS system, D-glucosamine-specific IIB component PTS system, D-glucosamine-specific IIC component PTS system, beta-glucosides-specific IIA component
PTS system, N-acetylglucosamine-specific IIBC subunit	9.0	PTS system, N-acetylglucosamine-specific IIB component[EC:2.7.1.69] PTS system, N-acetylglucosamine-specific IIC component
Phosphotransferase system, EIIC	9.5	PTS system, sucrose-specific IIB component [EC:2.7.1.69] PTS system, sucrose-specific IIC component
PTS system, beta-glucoside-specific IIABC subunit	7.7	PTS system, beta-glucosides-specific IIA component [EC:2.7.1.69] PTS system, beta-glucosides-specific IIB component PTS system, beta-glucosides-specific IIC component
Cellobiose/arbutin/salicin-specific PTS	9.2	PTS system, arbutin-, cellobiose-, and

system components IIBC		salicin-specific IIB component [EC:2.7.1.69] PTS system, arbutin-, cellobiose-, and salicin-specific IIC component
PTS system, trehalose-specific IIBC subunit	9.6	PTS system, trehalose-specific IIB component [EC:2.7.1.69] PTS system, trehalose-specific IIC component
PTS system, alpha-glucoside-specific IIBC subunit	8.2	PTS system, arbutin-like IIB component [EC:2.7.1.69] PTS system, arbutin-like IIC component
Phosphotransferase system PTS, lactose/cellobiose-specific IIA subunit	5.4	PTS system, cellobiose-specific IIA component [EC:2.7.1.69]
Phosphotransferase system, lactose/cellobiose-specific IIB subunit	6.2	PTS system, cellobiose-specific IIB component [EC:2.7.1.69]
PTS system, lactose/cellobiose family IIC subunit	9.2	PTS system, cellobiose-specific IIC component
Phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA 2	6.3	PTS system, mannitol-specific IIA component[EC:2.7.1.69]
PTS system, mannitol-specific IIC subunit	8.6	PTS system, mannitol-specific IIB component [EC:2.7.1.69] PTS system, mannitol-specific IIC component
PTS system, mannose/fructose/sorbose family, IIA subunit	6.1	PTS system, mannose-specific IIA component [EC:2.7.1.69] PTS system, mannose-specific IIB component [EC:2.7.1.69]
Phosphotransferase system PTS, sorbose-specific IIC subunit	4.8	PTS system, mannose-specific IIC component
PTS system, mannose/fructose/sorbose family, IIC subunit	5.7	PTS system, mannose-specific IIC component
PTS system, mannose/fructose/sorbose family, IID subunit	9.9	PTS system, mannose-specific IID component
PTS system fructose subfamily IIA component	6.1	PTS system, N acetylgalactosamine-specific IIA component [EC:2.7.1.69]
N-acetylgalactosamine-specific PTS system transporter subunit IIB	6.8	PTS system, galactosamine-specific IIB component [EC:2.7.1.69]
N-acetylgalactosamine-specific PTS	4.6	PTS system, galactosamine-specific

system transporter subunit IIC		IIC component
N-acetylgalactosamine-specific PTS system transporter subunit IID	9.4	PTS system, galactosamine-specific IID component
PTS system, glucitol/sorbitol-specific IIA subunit	4.7	PTS system, glucitol/sorbitol-specific IIA component [EC:2.7.1.69]
PTS system, glucitol/sorbitol-specific, IIBC subunit	8.0	PTS system, glucitol/sorbitol-specific IIB component [EC:2.7.1.69] PTS system, glucitol/sorbitol-specific IIC component
PTS system, glucitol/sorbitol-specific, IIC subunit	9.4	PTS system, glucitol/sorbitol-specific IIC component
Putative PTS IIA-like nitrogen-regulatory protein PtsN	5.1	PTS system, galactitol-specific IIA component [EC:2.7.1.69]
PTS system, galactitol-specific IIB component, putative	5.4	PTS system, galactitol-specific IIB component [EC:2.7.1.69]
PTS system galactitol-specific IIC component	9.1	PTS system, galactitol-specific IIC component
Phosphotransferase system, lactose/cellobiose-specific IIB subunit	6.2	PTS system, ascorbate-specific IIB component [EC:2.7.1.69]
UlaA, sgaT, Ascorbate-specific PTS system enzyme IIC	8.6	PTS system, ascorbate-specific IIC component
PTS system, fructose subfamily, IIC subunit	6.6	PTS system, fructose-specific IIA component [EC:2.7.1.69] PTS system, fructose-specific IIB component [EC:2.7.1.69] PTS system, fructose-specific IIC component

Table S3: Full list of proteins which were significantly down-regulated in Ca^{2+}-supplemented *C. beijerinckii* 8052 ABE fermentation relative to the untreated cells.

Spot #	**Protein ID**	**PI**	**Description/cellular function**	**Fold change**	**P value (T-test)**
441	YP_001310387.1	5.7	Hypothetical protein	-11.5	0.034
243	YP_001307566.1	5.3	Sporulation stage II protein D	-6.7	0.001
76	YP_001311201.1	5.3	Pullulanase/glycoside hydrolase family	-6.1	0.003
450	YP_001309441.1	5.1	Rubrerythrin/ruberydoxin	-4.9	0.007
244	YP_001311728.1	5.4	Polysaccharide deacetylase	-4.7	0.003
51	YP_001307620.1	5.2	Hypothetical protein with a nucleoside triphosphate hydrolase conserved domain	-4.3	0.032
22	YP_001309233.1	5.5	Arsenite-activated ATPase	-4.2	0.004
375	YP_001310303.1	5.9	Chloramphenicol O-acetyltransferase	-4.1	0.005
498	YP_001310261.1	6.2	TetR family transcriptional regulator	-4.1	0.000*
25	YP_001309141.1	6.0	Exonuclease/nucleoside triphosphate/DEAD-like helicase/DNA polymeras	-3.2	0.045
48	YP_001310746.1	5.3	Dual specificity protein-tyrosine phosphatase	-2.7	0.003

53	YP_001307365.1	6.3	Sigma-54 factor interaction domain-containing protein/ transcriptional regulator	-2.4	0.016
27	YP_001310172.1	5.7	PAS/PAC sensor hybrid histidine kinase	-2.3	0.013
57	YP_001310872.1	6.0	Molybdopterin-guanine dinucleotide biosynthesis protein B	-1.8	0.022
67	YP_001307436.1	6.4	Hypothetical protein	-4.2	0.027
94	YP_001307495.1	6.5	UvrD/REP helicase	-1.9	0.004
128	YP_001308846.1	5.3	Putative prophage ps3 protein 01	-2.3	0.026
131	YP_001310397.1	6.2	Diguanylate cyclase	-1.7	0.008
133	YP_001309899.1	6.4	ABC transporter related	-1.9	0.037
142	YP_001308978.1	6.4	SufS subfamily cysteine desulfurase	-2.1	0.017
148	YP_001308078.1	6.5	Hypothetical protein Cbei_0938	-1.8	0.031
161	YP_001312096.1	6.2	Ammonium transporter	-2.2	0.001
163	YP_001309830.1	6.0	XRE family transcriptional regulator	-3.4	0.013
169	YP_001311681.1	6.3	Diguanylate cyclase	-1.8	0.001
177	YP_001310166.1	5.7	D-Galactose-binding periplasmic protein precursor	-1.7	0.002
182	YP_001309690.1	7.0	dTDP-Glucose 4,6 dehydratase	-1.7	0.018
199	YP_001308366.1	5.1	Regulatory protein, LacI	-2.6	0.034

203	YP_001307470.1	5.3	8-Oxoguanine DNA glycosylase domain-containing protein	-3.2	0.020
211	YP_001307183.1	6.3	Radical SAM domain-containing protein	-2.5	0.012
228	YP_001309511.1	5.3	Membrane dipeptidase	-2.2	0.004
238	YP_001311919.1	6.5	Hypothetical protein	-2.0	0.016
247	YP_001312099.1	6.0	Dihydrodipicolinate synthase	-2.4	0.027
272	YP_001310534.1	5.5	Transcription activator, effector binding	-2.8	0.040
278	YP_001311727.1	6.4	Hypothetical protein	-1.7	0.028
280	YP_001307749.1	5.8	ABC transporter related	-2.3	0.015
294	YP_001309572.1	5.5	5-Keto-4-deoxyuronate isomerase	-2.0	0.004
296	YP_001309599.1	6.4	Ribonuclease H	-1.7	0.023
299	YP_001308172.1	6.3	RNA-binding S1 domain-containing protein	-1.9	0.010
331	YP_001311405.1	6.4	Nucleotidyl transferase	-2.2	0.009
348	YP_001307539.1	6.6	Sporulation stage II, protein R	-3.3	0.012
358	YP_001310793.1	6.0	Hypothetical protein	-3.1	0.012
370	YP_001311478.1	5.6	Hypothetical protein	-2.0	0.037
374	YP_001310974.1	5.9	Short-chain dehydrogenase/reductase SDR	-5.0	0.017
376	YP_001307888.1	5.3	Deoxyribose-phosphate aldolase	-2.4	0.004
380	YP_001309075.1	5.7	Hypothetical protein	-3.8	0.061
389	YP_001309653.1	5.8	ExsB protein	-2.1	0.020

394	YP_001311520.1	6.1	L-Ribulose-5-phosphate 4-epimerase	-3.0	0.026
395	YP_001308657.1	5.7	Hypothetical protein	-1.8	0.017
405	YP_001308594.1	5.7	Hypothetical protein	-2.7	0.037
434	YP_001307336.1	5.3	Fumarate hydratase	-1.7	0.010
436	YP_001311457.1	7.0	Hypothetical protein	-1.7	0.023
439	YP_001310387.1	5.3	Hypothetical protein	-1.9	0.039
444	YP_001307345.1	6.4	Cell wall hydrolase, SleB	-2.3	0.010
454	YP_001310870.1	7.0	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	-1.7	0.039
465	YP_001308205.1	5.8	Hypothetical protein	-4.1	0.002
466	YP_001308171.1	5.7	Carbonic anhydrase	-2.3	0.023
471	YP_001308947.1	5.2	Hypothetical protein	-2.6	0.014
511	YP_001309731.1	6.2	Hypothetical protein	2.4	0.010

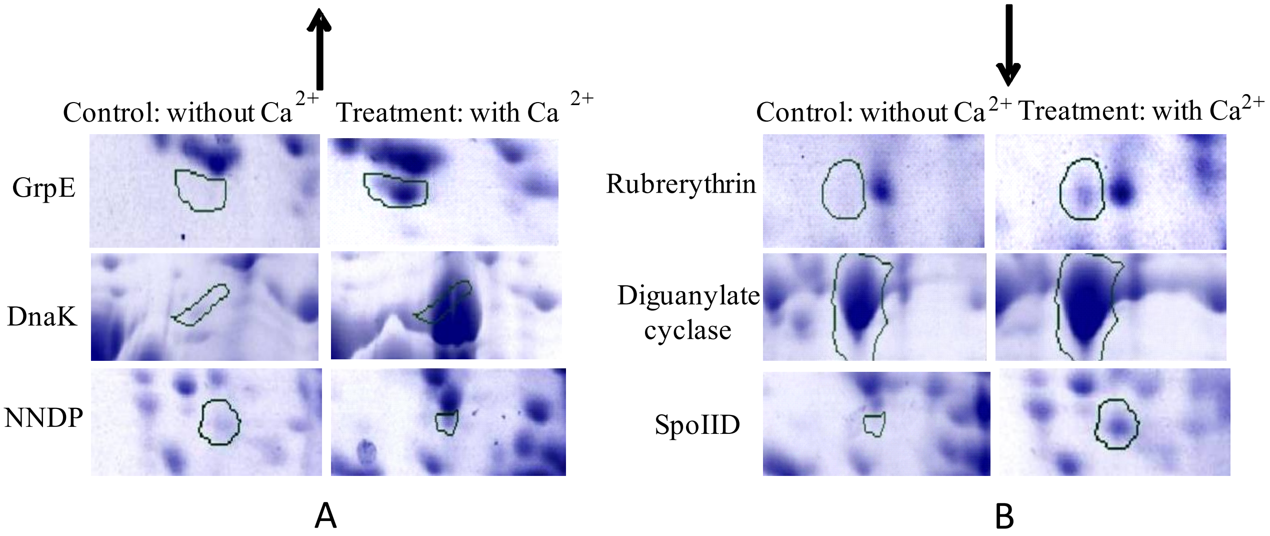

Figure S1: Representative expression levels of selected proteins of *C. beijerinckii* 8052, which increased (A) or decreased (B) in Ca^{2+}-treated cells compared to the untreated cells. NNDP - nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase; SpoIID - sporulation stage II protein D.

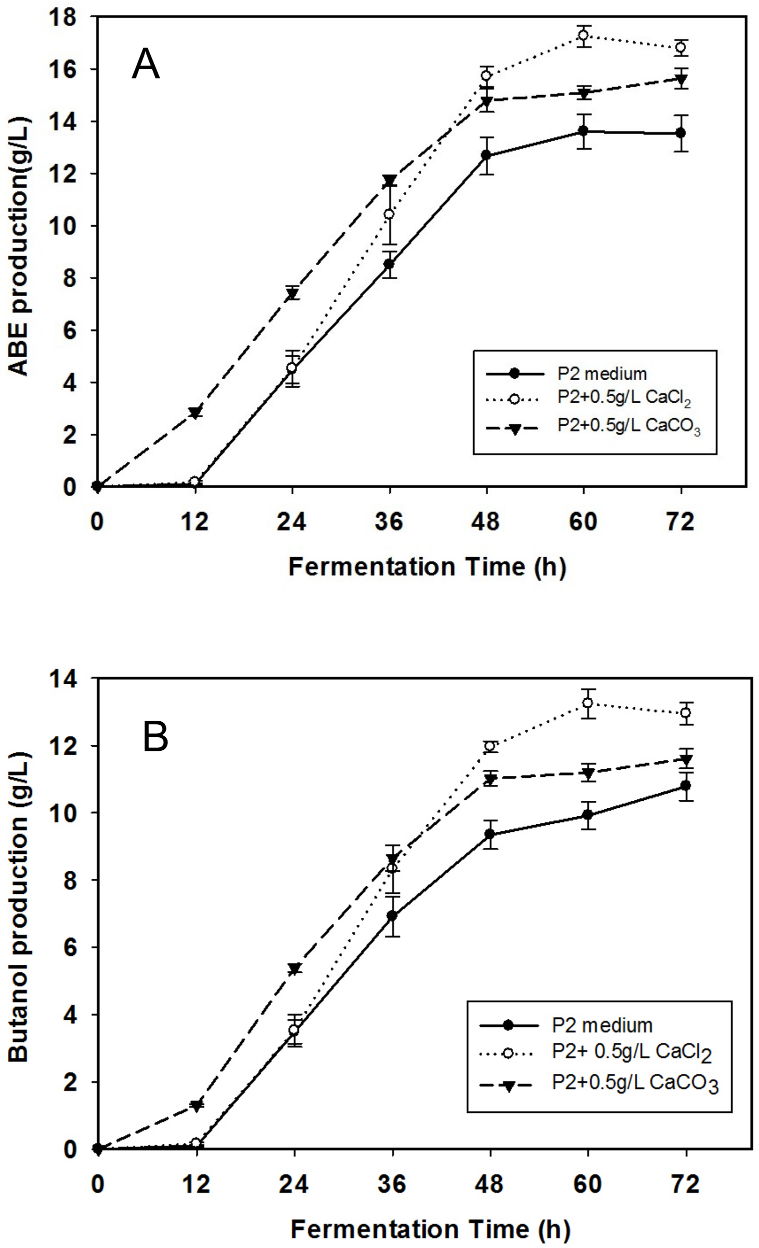

Figure S2: Comparison of total ABE (A) and butanol (B) profiles between batch *C. beijerinckii* NCIMB 8052 fermentations conducted in P2 medium supplemented with 0.5 g/L $CaCl_2$ and 0.5 g/L $CaCO_3$.