biology

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Supplementary Materials

Production of D-Tagatose by Whole-Cell Conversion of Recombinant *Bacillus subtilis* in the Absence of Antibiotics

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Table S1 . Comparison of characteristics of L-AIs from various microorganisms.

Source of L-arabinose isomerase	Reaction condition	Metal ion or boric acid requirement(mol/L)	con- cen- tra-	ose con- centra-	Conversion yield(%)
Mesophilic					
Lactobacillus sakei	pH7.4,50°C	Mn^{2+}（1.0）	54	45.9	85（48 h）
Lactobacillus brevis PC16	pH6.5,67°C	Mn^{2+}（1.0）	125	99.6	79.7(28 h)
Lactobacillus fermentum CGMCC 2921	pH6.5,65°C	Mn^{2+}（1.0）	9	4.95	55(12 h)
Lactobacillus fermentum CGMCC 2921	pH6.5,70°C	Mn^{2+}（1.0）	100	75	75(24 h)
Pediococcus pentosaceus PC-5	pH6.0,50°C	Mn^{2+}（0.6）Co2+(0.8)	5	2.6	52(24 h)
*Enterococcus faecium D*BFIQE36	pH5.5,50°C	Mn^{2+}（0.3）	90	40.5	45(48 h)
Lactobacillus plantarum WU14	pH7.17,50°C	Mn^{2+}（10.0）	108	41.4	38.31(28 h)
Bacillus coagulan NL01	pH7.5,60°C	Mn^{2+}（0.5）Co2+(0.5)	20	10.6	53(20 h)
Thermophilic					
Geobacillus therodenitrificans	pH8.5,50°C	No	300	165	55(2 h)
Geobacillus therodenitrificans	pH8.5-9.0, 60°C	boric acid (1110.0)	500	370	74(24 h)
Bacillus thermoglucosidasius KCTC 1828	pH7.4,40°C	Mn^{2+}（1.0）	36	16.42	45.6(48 h)
Acidothermus cellulolytic ATCC 43068	pH7.5,75°C	Mn^{2+}（1.0）Co^{2+}(0.5)	9	4.8	53(12 h)
Alicyclobacillus acidocaldarius	pH6.0,60°C	Mn^{2+}（1.0）Co^{2+}(1.0)	18	7.9	44(12 h)
Alicyclobacillus hesperidium URH17-3-68	pH7.0,70°C	Co^{2+}(1.0)	9	3.9	43(27 h)
Hyperthermophilic					
Thermotoga neapolitana 5068	pH7.0,80°C	Mn^{2+}（1.0）Co^{2+}(1.0)	1.8	1.2	68(20 h)
Thermotoga Maritima	pH7.5,70°C	Mn^{2+}（5.0）	500	190	38(242 h)

Host expressing system	Reference
Lactobacillus plantarum	[16]
B. subtilis DB403	[15]
E. coli	[14]
B. subtilis 168	[13]
E. coli	[12]
E. coli	[11]
Lactococcus lactis N79000	[10]
E. coli	[9]
Corynebacterium glutamicum	[8]
E. coli	[7]
E. coli	[6]
E. coli	[5]
E. coli	[4]
E. coli	[3]
E. coli	[2]
E. coli	[1]

Table S2. List of bacterial strains and plasmids used in this study.

Strains and plasmids	Characteristics	Source and reference
E. coli K-12	β-galactosidase and arabinose isomerase gene provider	Invitrogen
B. subtilis 168	trpC, expression host of *B. subtilis*	Takara
168 D1P1(*alrA*-, Zeo^r)	168 derivate, △*alrA*, lox71-Zeo^r-lox66	This study
168 D1P2(*alrA*-, Kan^r)	168 derivate, △*alrA*, lox72, Kan^r, pTSC	This study
168 D1(*alrA*-)	168 derivate, △*alrA*	This study
168 D1/pMA5-*alrA*	The recombinant 168 D1 strain harboring the plasmid pMA5-*alrA*	This study
168 D1/pMA5-*alrA-araA*	The recombinant 168 D1 strain harboring the plasmid pMA5-*alrA-araA*	This study
168 D1/pMA5-*alrA-lacZ*	The recombinant 168 D1 strain harboring the plasmid pMA5-*alrA-lacZ*	This study
168 D1/pMA5-*alrA-lacZ-araA*	The recombinant 168 D1 strain harboring the plasmid pMA5-*alrA-lacZ-araA*	This study
168 D2P1(Zeo^r)	168 derivate, *lacZ*, △*alrA*, lox71-Zeo^r-lox66	This study
168 D2P2(Kan^r)	168 derivate, *lacZ*, △*alrA*, lox72, Kan^r, pTSC	This study
168 D2(*alrA*-)	168 derivate, *lacZ*, △*alrA*	This study
168 D2/pMA5-*alrA*	The recombinant 168 D2 strain harboring the plasmid pMA5-*alrA*	This study
168 D2/pMA5-*alrA-araA*	The recombinant 168 D2 strain harboring the plasmid pMA5-*alrA-araA*	This study
Plasmids		
pMA5	Kan^r, Zeo^r, containing the *Hpa*Ⅱ promoter	Takara
p7Z6	pMD18-T ligated with lox71-Zeo^r-lox66 cassette	Takara
pTSC	Kan^r, temperature sensitive in *B. subtilis* containing Pspac-cre expression cassette	Takara
pMA5-*alrA*	*B. subtilis* expression vector containing *alrA* gene	This study
pMA5-*alrA-araA*	*B. subtilis* expression vector containing *alrA* and *araA* genes	This study
pMA5-*alrA-lacZ*	*B. subtilis* expression vector containing *alrA* and *lacZ* genes	This study
pMA5-*araA*	*B. subtilis* expression vector containing *araA* gene	This study
pMA5-*alrA-lacZ-araA*	B. *subtilis* co-expression vector containing *alraA, araA* and *lacZ* gene	This study

Zeo^r: bleomycin resistance; Kan^r: kanamycin resistance;.

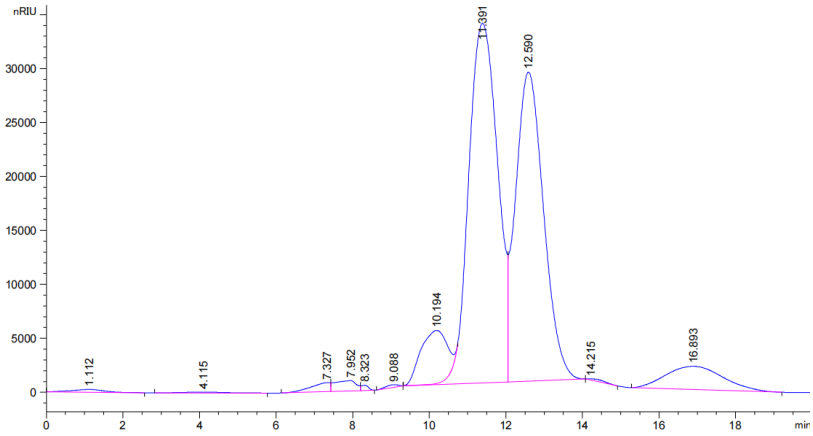

Figure S1. HPLC results of conversion products. The conversion using *B.subtilis* 168 D1/pMA5-*alrA-lacZ-araA* whole-cells. Bioconversion was performed at 40 °C, pH 8 (0.2 mol/L citric acid-Na_2HPO_4), Mn^{2+} concentration of 0.1 mol/L, and 0.1% TritonX-100, and cell concentration resulting in an OD of 40 at 600 nm. 10.194 min: lactose, 11.391 min: glucose, 12.590min: galactose, 16.893 min: D-tagatose.

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