SUPPLEMENTARY DATA - Bosma *et al.*

Table S1. Growth, end product formation and genetic accessibility of selected isolates.

			Sugars supporting growth[3]				Products (g/L)[4]										
Strain	**Identification[1]**	**Isol.[2]**	**G**	**S**	**X**	**A**	**Lac**	**Ace**	**Suc**	**Mal**	**Eth**	**For**	**2,3-BDO**	**Prop**	**Total**	**OD_{600}**	**CFU[5]**
ET 138	***B. smithii***	**3**	**+**	**+**	**+**	**+/-**	**6.74 ±*0.18***	**0.81 ±*0.02***	**0.07 ±*0.00***	**nd**	**nd**	**nd**	**nd**	**nd**	**7.61 ±*0.16***	**1.425 ±*0.058***	**3**
DSM 1	*B. coagulans*	T	+[7]	-[7]	-[7]	-[7]	6.37 ±*0.76*	0.36 ±*0.05*	0.13 ±*0.05*	0.18 ±*0.30*	0.04 ±*0.07*	nd	0.10 ±*0.02*	nd	7.17 ±*1.16*	1.467 ±*0.147*	20[6]
ET 236	*G. thermodenitrificans*	5	+/-	+	+	+	5.37 ±*0.60*	1.15 ±*0.07*	0.13 ±*0.01*	0.17 ±*0.11*	0.04 ±*0.02*	0.07 ±*0.04*	0.05 ±*0.01*	nd	6.97 ±*0.85*	0.833 ±*0.006*	0
ET 239	*1: A. pallidus* *2: B. coagulans*	5	+/-	+/-	++	+/-	5.03 ±*0.22*	0.87 ±*0.19*	0.15 ±*0.04*	nd[1]	0.07 ±*0.00*	nd	0.04 ±*0.03*	nd	6.68 ±*0.27*	0.928 ±*0.041*	1: 0 2: bg
ET 224	*B. coagulans*	5	+/-	+/-	+	+/-	4.47 ±*0.12*	0.72 ±*0.02*	0.20 ±*0.00*	nd[1]	nd	nd	0.06 ±*0.01*	nd	6.20 ±*0.03*	0.852 ±*0.011*	0
ET 251	***G. thermodenitrificans***	**5**	**+**	+/-	+/-	+/-	**4.89 ±*0.03***	**0.85 ±*0.02***	**0.13 ±*0.00***	**0.11 ±*0.01***	**0.13 ±*0.02***	**0.00 ±*0.00***	**0.07 ±*0.01***	**nd**	**6.18 ±*0.03***	**0.876 ±*0.002***	**4**
ET 131	*1: G. caldoxylosilyticus* *2: G. thermodenitrificans*	2	++	+/-	++	+/-	4.71 ±*0.37*	0.91 ±*0.00*	0.14 ±*0.04*	0.12 ±*0.02*	nd	0.02 ±*0.01*	nd	nd	5.91 ±*0.39*	0.538 ±*0.076*	1/2: 0
DSM 2542	*G. thermoglucosidans*	T	+[8]	+[8]	+[8]	-[8]	4.39 ±*0.19*	0.92 ±*0.03*	0.21 ±*0.05*	0.03 ±*0.02*	0.09 ±*0.13*	0.14 ±*0.15*	nd	0.02 ±*0.01*	5.81 ±*0.26*	0.695 ±*0.195*	2300
ET 130	*G. caldoxylosilyticus*	2	++	++	+	+/-	4.59 ±*0.37*	0.76 ±*0.04*	0.18 ±*0.01*	0.08 ±*0.01*	nd	0.04 ±*0.00*	nd	nd	5.64 ±*0.40*	0.578 ±*0.048*	1: bg 2: 0
ET 186	*G. thermodenitrificans*	4	+/-	+	+/-	+/-	4.12 ±*0.39*	0.92 ±*0.01*	0.21 ±*0.07*	0.07 ±*0.03*	nd	0.02 ±*0.00*	0.04 ±*0.06*	nd	5.37 ±*0.49*	0.803 ±*0.168*	0
ET 144	*1: B. thermocopriae* ***2: G. thermodenitrificans***	**3**	**+/-**	**+/-**	**+**	**+/-**	**3.63 ±*0.34***	**1.02 ±*0.03***	**0.15 ±*0.02***	**0.12 ±*0.10***	**0.21 ±*0.18***	**0.11 ±*0.02***	**0.08 ±*0.01***	**nd**	**5.32 ±*0.06***	**0.817 ±*0.101***	1: 0 **2: 6**
ET 241	*G. thermodenitrificans*	5	+/-	+/-	+	+/-	3.99 ±*0.89*	0.98 ±*0.07*	0.16 ±*0.05*	0.01 ±*0.01*	nd	0.06 ±*0.05*	0.03 ±*0.05*	nd	5.23 ±*1.00*	0.662 ±*0.018*	0
ET 050	*G. thermodenitrificans*	1	+/-	+	++	-	3.72 ±*0.49*	1.01 ±*0.24*	0.12 ±*0.02*	0.09 ±*0.04*	nd	0.02 ±*0.00*	0.05 ±*0.07*	nd	5.00 ±*0.85*	0.611 ±*0.125*	bg

ET 208	*G. caldoxylosilyticus*	4	+/-	+/-	-	+/-	3.14 ±0.80	1.06 ±0.14	0.57 ±0.20	0.16 ±0.03	nd	0.04 ±0.01	nd	nd	4.98 ±1.18	0.881 ±0.081	bg
ET 129	*1: G. caldoxylosilyticus* *2: G. thermodenitrificans*	2	++	+++	+	-	3.56 ±0.48	0.99 ±0.06	0.16 ±0.04	0.08 ±0.02	nd	0.02 ±0.01	nd	nd	4.81 ±0.36	0.579 ±0.082	1: 0 2: bg
ET 143	*B. thermocopriae*	3	+	+	+	+/-	2.81 ±0.33	1.07 ±0.17	0.15 ±0.04	nd	0.26 ±0.15	0.20 ±0.12	0.01 ±0.01	0.09 ±0.08	4.60 ±0.90	0.719 ±0.095	res
ET 072	*G. thermodenitrificans*	2	+	+	++	-	3.48 ±0.41	0.81 ±0.18	0.10 ±0.01	nd	nd	0.01 ±0.00	0.11 ±0.15	nd	4.52 ±0.45	0.356 ±0.020	1/2: bg
ET 157	*G. thermodenitrificans*	3	+	+/-	+/-	-	3.45 ±0.03	0.94 ±0.06	0.07 ±0.01	nd	nd	0.01 ±0.00	0.03 ±0.02	nd	4.49 ±0.03	0.553 ±0.028	0
ET 136	*G. thermodenitrificans*	3	+	++	+	+	2.46 ±0.07	1.14 ±0.01	0.16 ±0.03	nd	0.50 ±0.42	0.16 ±0.04	nd	0.03 ±0.02	4.45 ±0.58	0.564 ±0.090	0
ET 244	*B. thermocopriae*	5	+	+/-	+/-	+/-	3.46 ±0.38	0.36 ±0.02	0.01 ±0.00	nd[1]	nd	nd	0.04 ±0.00	0.02 ±0.00	3.89 ±0.41	0.403 ±0.054	0
ET 226	*B. thermocopriae*	5	+/-	+/-	+++	+/-	3.41 ±0.18	0.38 ±0.01	0.02 ±0.01	nd	nd	nd	0.01 ±0.01	0.02 ±0.00	3.84 ±0.21	0.663 ±0.020	0
ET 225	*A. pallidus*	5	+/-	+/-	+	+/-	3.07 ±0.87	0.63 ±0.00	0.07 ±0.03	nd	nd	nd	nd	0.03 ±0.01	3.79 ±0.89	0.558 ±0.033	bg
ET 039	*G. thermodenitrificans*	1	+	+	+	+	2.57 ±0.08	0.92 ±0.07	0.07 ±0.03	0.04 ±0.01	nd	0.02 ±0.00	nd	nd	3.62 ±0.19	0.481 ±0.010	bg
ET 261	*1+2: B. thermocopriae* *3: G. thermodenitrificans*	5	+	+/-	+	+	1.96 ±0.17	1.14 ±0.10	0.06 ±0.02	nd	0.23 ±0.01	0.20 ±0.09	nd	0.03 ±0.00	3.62 ±0.02	0.613 ±0.014	1: bg 2/3: 0
ET 145	*G. thermodenitrificans*	3	+	+/-	+	+/-	1.91 ±0.03	1.18 ±0.09	0.11 ±0.06	nd	0.24 ±0.05	0.10 ±0.02	0.01 ±0.01	nd	3.54 ±0.14	0.505 ±0.029	0
ET 200	*G. thermodenitrificans*	4	+	+/-	+	+/-	2.65 ±0.01	0.64 ±0.25	0.16 ±0.04	0.07 ±0.05	nd	nd	nd	nd	3.53 ±0.34	0.729 ±0.055	0
ET 036	*G. thermodenitrificans*	1	+	+	+	+	2.50 ±1.41	0.80 ±0.31	0.08 ±0.07	0.12 ±0.11	nd	0.02 ±0.01	nd	nd	3.52 ±1.69	0.456 ±0.052	bg
ET 156	*G. thermodenitrificans*	3	++	++	+	+/-	2.34 ±1.02	0.90 ±0.20	0.16 ±0.11	0.02 ±0.02	nd	0.01 ±0.01	0.04 ±0.05	nd	3.46 ±1.36	0.448 ±0.083	0
ET 042	*G. thermodenitrificans*	1	+	+	+	+	2.36 ±0.21	0.89 ±0.07	0.03 ±0.01	0.07 ±0.03	nd	0.02 ±0.01	nd	nd	3.37 ±0.27	0.627 ±0.106	0

ET 229	*B. coagulans*	5	+/-	+/-	+	+/-	2.83 *±1.61*	0.47 *±0.03*	0.03 *±0.02*	nd	nd	nd	nd	0.02 *±0.00*	3.35 *±1.67*	0.373 *±0.045*	0
ET 267	*1+2: G. thermodenitrificans*	5	+	+/-	+	+/-	2.19 *±0.90*	0.83 *±0.00*	0.05 *±0.03*	0.11 *±0.15*	0.03 *±0.04*	0.02 *±0.00*	nd	nd	3.22 *±1.13*	0.432 *±0.048*	1/2: 0
ET 011	*1+2: G. thermodenitrificans*	1	+	+	+	+	2.00 *±0.91*	0.79 *±0.22*	0.08 *±0.03*	0.08 *±0.01*	0.14 *±0.20*	0.02 *±0.00*	nd	nd	3.11 *±1.35*	0.539 *±0.117*	1: 0 2: bg
ET 081	*G. thermodenitrificans*	2	+	+	++	-	2.03 *±0.20*	0.93 *±0.11*	0.07 *±0.00*	0.06 *±0.02*	nd	0.01 *±0.00*	nd	nd	3.10 *±0.08*	0.740 *±0.091*	bg
ET 230	*1+2: B. coagulans*	5	+/-	+/-	++	+++	2.34 *±1.00*	0.66 *±0.00*	0.04 *±0.01*	nd	nd	nd	nd	0.02 *±0.00*	3.06 *±1.01*	0.419 *±0.072*	1/2: bg
ET 263	*B. thermocopriae*	5	+	+/-	+	+/-	1.72 *±0.54*	0.85 *±0.16*	0.03 *±0.01*	nd	0.24 *±0.10*	0.05 *±0.00*	nd	nd	2.89 *±0.80*	0.600 *±0.098*	0
ET 159	*G. thermodenitrificans*	4	+/-	+/-	+/-	+/-	0.74 *±0.02*	0.67 *±0.06*	nd	nd	nd	0.05 *±0.01*	nd	0.01 *±0.00*	1.46 *±0.06*	0.253 *±0.001*	0
ET 160	*G. thermodenitrificans*	4	+/-	+/-	-	+/-	0.69 *±0.03*	0.53 *±0.03*	nd	nd	nd	0.04 *±0.06*	nd	0.00 *±0.01*	1.27 *±0.11*	0.315 *±0.103*	bg

[1] The selection based on products was made prior to making pure cultures – the identification shown is that after making pure cultures; in cases where the pure culture was split in multiple species both are shown because in this selection round it was still a mixed culture. In the CFU-column, the identification for the subcultures is provided after the corresponding strain sub-number (e.g. 1, 2, or 3) as this was performed after making pure cultures.

[2] Isol: isolation condition (see Table 1).

[3] Sugars used in the selection step in the isolation procedure (see Table 1), prior to making pure cultures. Abbreviations: G: glucose; S: sucrose; X: xylose; A: arabinose; na: not applicable.

[4] Cultures were analyzed by HPLC after 48 h of growth at their isolation temperature (55 or 65°C) in 15 mL screw-capped tubes with 8 mL TMM + 0.5 g/L yeast extract + 10 g/L glucose + $CaCO_3$. Transformable strains are shown in bold. Two genetically accessible *Bacillus* type strains known from literature for green chemical or fuel production were taken along as reference strains, e.g. *G. thermoglucosidans* DSM 2542^T (1) and *B. coagulans* DSM 1^T (2, 3). Data are the average of duplicates; standard deviations are indicated in italics after the '±'. nd = not detected; nd[1] = a very high malate peak with a large shoulder was observed, indicating the presence of another product besides or instead of malate but this was not further evaluated. Abbreviations: Lac: lactate; Ace: acetate; Suc: succinate; Mal: malate; Eth: ethanol; For: formate; 2,3-BDO: 2,3-butanediol; Prop: propionate.

[5] CFU = colony forming units per µg DNA after electrotransformation with pNW33n. Transformation was confirmed by isolating plasmid material and subsequent PCR and restriction analysis (Figure S1). Res = naturally resistant to chloramphenicol; nr = not reproducible, bg = background colonies after electroporation, also when no DNA was added, while these strains did not grow on chloramphenicol prior to transformation – colonies by these strains did not test positive in PCR. Results are shown for pure cultures, e.g. 239-1 and 239-2 are '1:0 2:bg' meaning 239-1 showed 0 colonies and 239-2 showed background colonies.

[6] As reported by (3).

[7] Acid production from these sugars as determined by (4).

[8] Acid production from these sugars as determined by (5).

Table S2. Optimization of electrotransformation for *B. smithii* ET 138.

Nr	Parameter changed	Buffer[1]	Final OD[2]	Hrs of growth[3]	kV	µF	Ω	µg DNA	Cuvette mm	Rec. medium[4]	Cm rec[5]	CFU fresh[6]	CFU -80[6]
1	Cm rec+sett.	SG-5	0.483	1.75	1.5	25	600	1	1	RG	N	3	nd
2	Cm rec+sett.	SG-5	0.500	1.5	1.5	25	600	1	1	RG	Y	8	nd
3	Cm rec+sett.	SG-5	0.483	1.75	2.0	25	200	1	2	RG	N	33	nd
4	Cm rec+sett.	SG-5	0.500	1.5	2.0	25	200	5	2	RG	Y	3	nd
5	Settings	SG-5	0.424	2.9	2.0	25	200	1	2	RG	N	12	nd
6	Settings	SG-5	0.424	2.9	1.5	25	200	0.7	1	RG	N	nd	113
7	Settings	SG-5	0.424	2.9	2.0	25	200	0.7	2	RG	N	nd	7
8	Settings	SG-5	0.424	2.9	2.0	25	400	0.7	2	RG	N	nd	157
9	OD	SG-5	0.452	1.4	2.0	25	400	1	2	RG	N	70	nd
10	OD	SG-5	0.678	1.4	2.0	25	400	1	2	RG	N	11	nd
11	Rec. medium	SG-5	0.574	2.1	2.0	25	400	1	2	LB2	N	712	1149
12	Rec. medium	SG-5	0.574	2.1	2.0	25	400	1	2	RG	N	0	nd
13	Rec. medium	SG-5	0.514	2.1	2.0	25	400	1	2	LB2	N	406	443
14	Rec. medium	SG-5	0.514	2.1	2.0	25	400	1	2	RG	N	1	nd
15	Glycerol, rec.	SG-10	0.446	2.5	2.0	25	400	1	2	LB2	N	796	476
16	Glycerol, rec.	SG-10	0.446	2.5	2.0	25	400	1	2	RG	N	36	nd
17	Rec. medium	SG-5	0.522	2.1	2.0	25	400	1	2	LB2	N	590	nd
18	Rec. medium	SG-5	0.522	2.1	2.0	25	400	1	2	RG	N	0	nd
19	Rec. medium	SG-5	0.519	1.75	2.0	25	400	1	2	LB2	N	960	nd
20	Rec. medium	SG-5	0.519	1.75	2.0	25	400	1	2	RG	N	5	nd
21	Buffer	SM-10	0.454	2.5	2.0	25	400	1	2	LB2	N	40	0
22	Buffer	SM-10	0.454	2.5	2.0	25	400	1	2	RG	N	0	nd
23	Fast growth[7]	SG-5	0.539	1	2.0	25	400	2.5	2	LB2	N	1900	nd
24	Fast growth[7]	SG-5	0.512	1.25	2.0	25	400	1	2	LB2	N	nd	1122
25	µg DNA[7]	SG-5	0.617	1.3	2.0	25	400	0.2	2	LB2	N	2409	nd
26	µg DNA[7]	SG-5	0.617	1.3	2.0	25	400	0.02	2	LB2	N	5118	nd

Lines indicate different experiments. Nr. 1 is the original protocol as described by Rhee *et al.*(6). Nr.3 are settings based on based on (7), which are the settings as used for screening of *Geobacillus* strains.

[1] 5 or 10 indicates the % of glycerol. SG = sucrose glycerol buffer from *Bacillus* protocol (6); SM = sorbitol mannitol buffer from *Geobacillus* protocol (8).

[2] Final OD_{600} after the indicated number of hours ([3]) when growing cells prior to making them competent.

[3] Number of hours cells had grown before making them competent.

[4] RG is LB with 121 g/L sucrose and 10 g/L glucose (6).

[5] Whether or not 1/1000 diluted chloramphenicol was added after 2h of recovery. Y = yes; N = no.

[6] CFU: colony forming units per µg DNA. Cells were either electroporated directly after making them competent ('fresh') or after storage in -80°C ('-80'). nd = not determined.

[7] In these experiments, after overnight growth, cells were transferred to 500 mL Erlenmeyer flasks or 1 L bottles (having a similar bottom surface) instead of to a 250 mL Erlenmeyer to allow for more aeration.

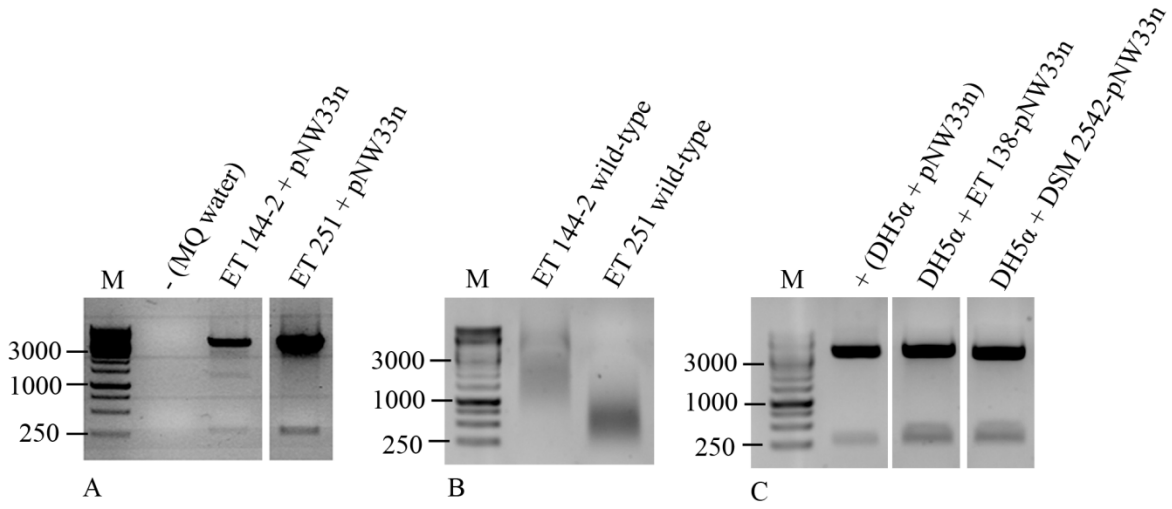

Figure S1. Restriction analysis of plasmid DNA extracted from isolates and control strain transformed with pNW33n. Restriction was performed with StuI and HindIII; pNW33n digested with StuI and HindIII yields bands of 282 and 3955 bp. M: Fermentas 1 kb DNA ladder. For each digestion reaction, 550-650 ng isolated plasmid DNA was used. **A:** Plasmids isolated from *G. thermodenitrificans* strains ET 144-2 and ET 251 transformed with pNW33n. **B:** Plasmids isolated from *G. thermodenitrificans* strains ET 144-2 and ET 251 prior to transformation with pNW33n (negative control) **C:** Plasmids isolated from *E. coli* DH5α transformed pNW33n (original source) and after retransformation with plasmids isolated from *B. smithii* ET 138 transformants and *G. thermoglucosidans* DSM 2542 transformants.

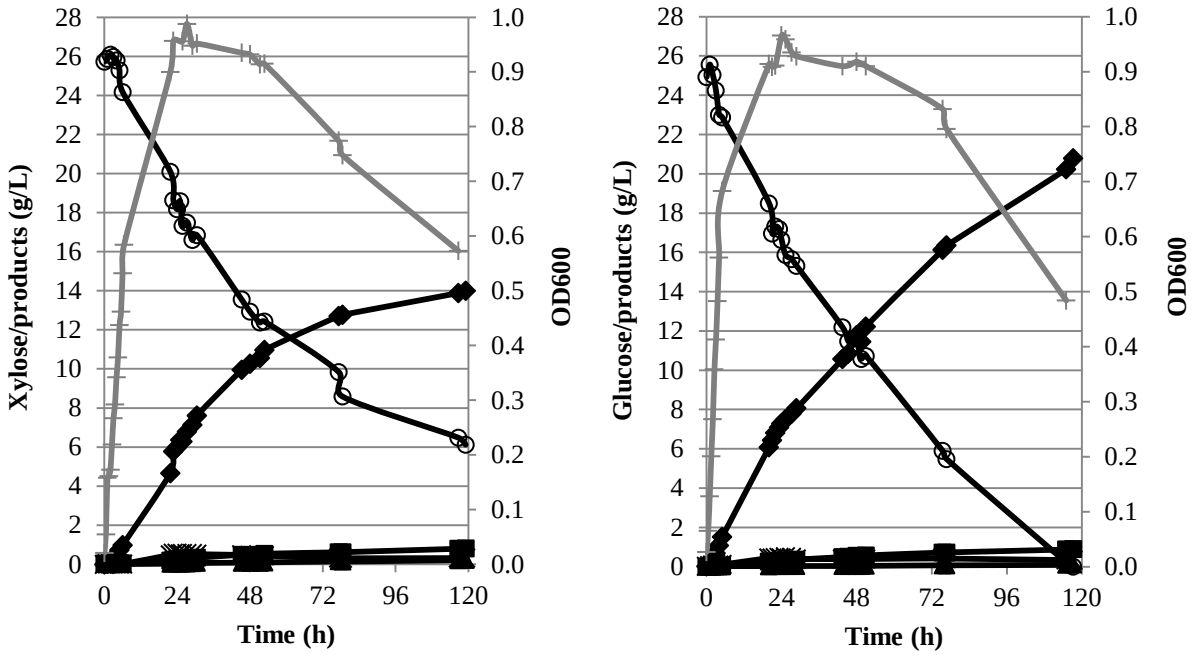

Figure S2. Fermentation of *B. smithii* ET 138 on 25 g/L xylose and glucose. Fermentation was carried out in 1 L TVMY supplemented with 25 g/L xylose or glucose, at 55°C, pH 6.5, 150 rpm and without any gas addition. Grey line with plus-sign: OD600; open circles: xylose or glucose; filled diamonds: lactate; closed squares: acetate; crosses: malate and succinate; closed triangles: pyruvate. During the xylose fermentation, severe browning of the medium was observed after approximately 70h.

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