COMPARATIVE ANALYSIS OF TWO ATTACHMENT VARIANTS OF BUTYRIVIBRIO FIBRISOLVENS

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Appendix A. GC chromatograms and analysis of proportions of monosaccharides.

A.1 Example monosaccharide GC chromatograms of various EP fractions.

A.2 Monosaccharide GC chromatograms of S and L fraction N EP.

A.3 Monosaccharide composition of S and L fraction N EP.

A.4 Monosaccharide GC chromatograms of S and L fraction I EP.

A.5 Monosaccharide composition of S and L fraction I EP.

A.6 Monosaccharide GC chromatograms of S and L fraction M EP.

A.7 Monosaccharide composition of S and L fraction M EP.

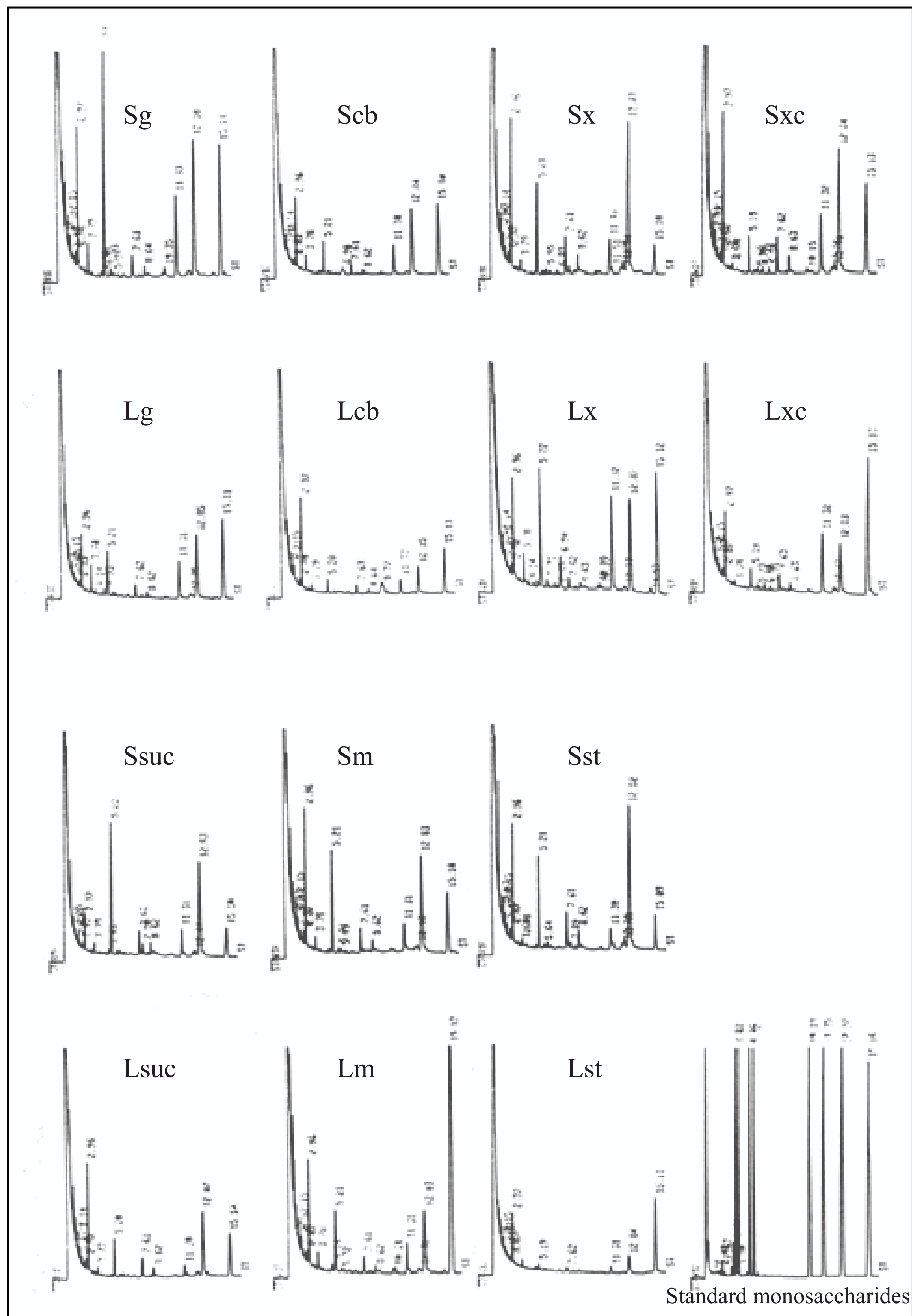
A.8 Monosaccharide GC chromatograms of S and L fraction PD EP.

A.9 Monosaccharide composition of S and L fraction PD EP.

A.1 Example monosaccharide GC chromatograms of various EP fractions.

S and L indicate *B. fibrisolvens* E14 variants S and L, respectively. Sg and Lg indicate EP samples isolated from S and L variants grown in glucose, respectively. Fractions M and PD were obtained from cell free medium, fraction N was obtained from plates, and fraction I was obtained from both methods (Figure 6.1). u1, a, b, c, u2, d, e, f, g, y and i indicate unknown 1, rhamnose, fucose, ribose, unknown2, arabinose, mannose, galactose, glucose, acidic monosaccharide and internal standard inositol. u1 and u2 may be glycerol and ribose phosphate. For fractions N and M the peak patterns were identical. Not much monosaccharide was seen in fraction I. Phenol extraction seemed to pull most of the monosaccharide of fraction M, especially galactose (f) and glucose (g), as seen in fraction PD. The chromatograms and the corresponding major peaks proportions of each fraction for S and L variants EP isolated from various carbon sources grown cultures are shown in Appendix A2 – A9.

A.2 Monosaccharide GC chromatograms of S and L fraction N EP



Fraction N was obtained from plates (Figure 6.1). Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst). The peaks are identified as described in Appendix A.1. The proportions of major peaks are shown in Appendix A.3.

A.3 Monosaccharide composition of S and L fraction N EP.

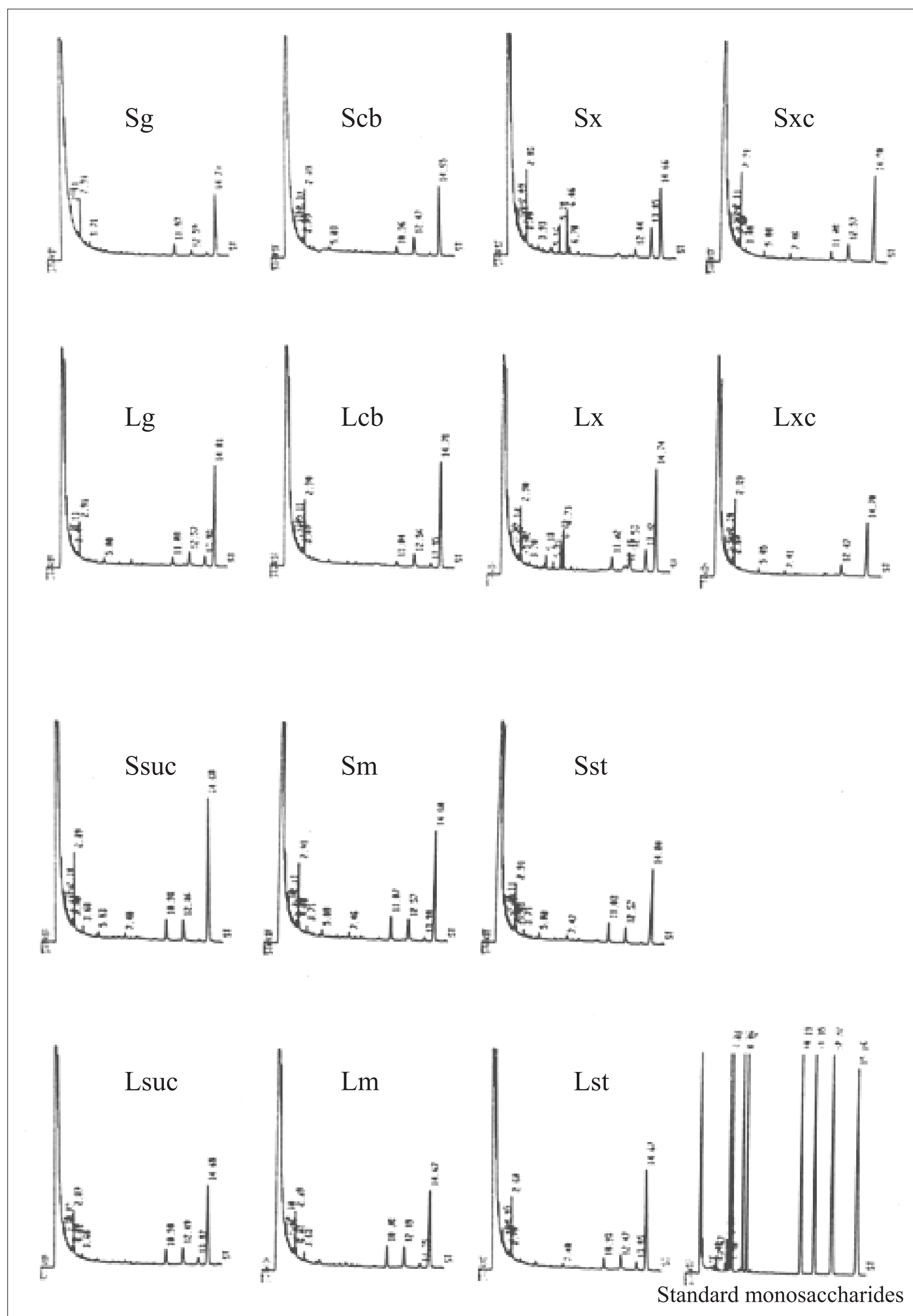
Sample	Number of Experiment	Monosaccharides (nmol/mg EP sample)			
		U1	U2	Galactose	Glucose
Sg	3	143 ± 15	336 ± 29	259 ± 8	526 ± 37
Lg	2	134 ± 42	146 ± 12	196 ± 15	499 ± 15
Scb	2	135 ± 14	145 ± 25	204 ± 19	591 ± 67
Lcb	2	206 ± 58	79 ± 13	126 ± 11	322 ± 6
Sx	2	647 ± 48	809 ± 44	502 ± 10	2510 ± 53
Lx	2	151 ± 33	276 ± 14	533 ± 15	505 ± 80
Sxc	3	209 ± 19	92 ± 5	273 ± 12	701 ± 68
Lxc	2	100 ± 20	53 ± 12	182 ± 13	262 ± 86
Ssuc	3	170 ± 24	1135 ± 35	378 ± 10	2235 ± 306
Lsuc	2	321 ± 33	269 ± 25	101 ± 8	877 ± 91
Sm	2	311 ± 22	451 ± 11	212 ± 11	1127 ± 273
Lm	2	45 ± 15	42 ± 8	26 ± 2	79 ± 10
Sst	3	448 ± 29	785 ± 43	256 ± 21	2184 ± 116
Lst	2	90 ± 8	18 ± 2	65 ± 16	207 ± 71

Fraction N was obtained from plates (Figure 6.1). Small amounts of rhamnose, mannose and acidic monosaccharide were not included.

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 or 3 independent experiments (number of experiments as shown in the table). The chromatograms are shown in Appendix A.2.

A.4 Monosaccharide GC chromatograms of S and L fraction I EP.



Fraction I was obtained from both plates and cell free medium (Figure 6.1). Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst). The peaks are identified as described in Appendix A.1. The proportions of major peaks are shown in Appendix A.5.

A.5 Monosaccharide composition of S and L fraction I EP.

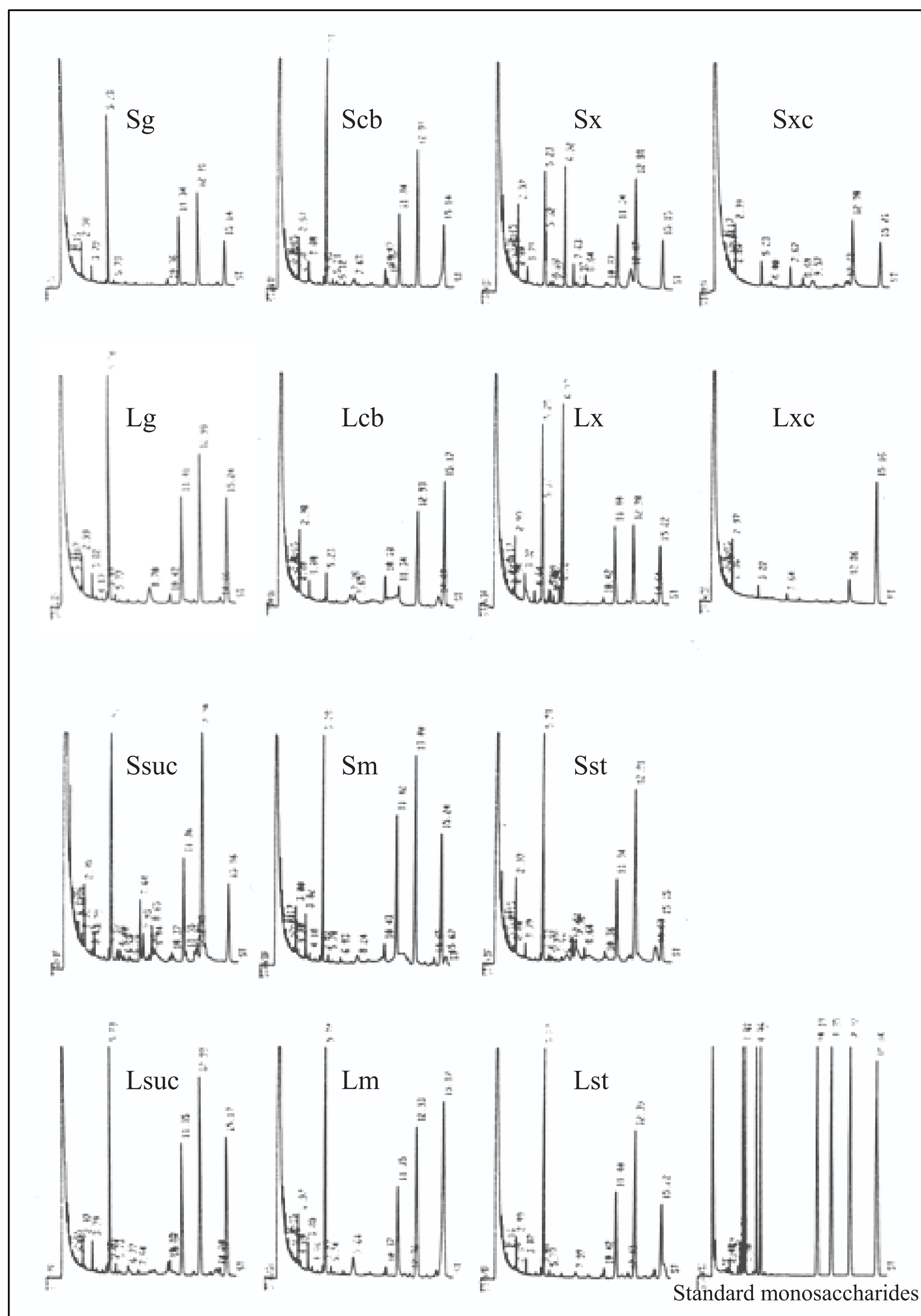
Sample	Number of experiment	Monosaccharides (nmol/mg EP sample)			
		U1	U2	Galactose	Glucose
Sg	2	88 ± 4	27 ± 3	81 ± 7	55 ± 2
Lg	3	4 4 ± 2	25 ± 4	48 ± 5	80 ± 2
Scb	2	102 ± 7	32 ± 5	46 ± 4	147 ± 8
Lcb	3	87 ± 10	25 ± 3	20 ± 1	80 ± 15
Sx	2	138 ± 14	46 ± 8	60 ± 5	77 ± 12
Lx	3	89 ± 16	27 ± 7	63 ± 6	122 ± 36
Sxc	2	114 ± 11	<	40 ± 5	80 ± 12
Lxc	2	155 ± 10	<	34 ± 3	129 ± 20
Ssuc	2	58 ± 7	32 ± 5	63 ± 10	128 ± 43
Lsuc	3	70 ± 7	29 ± 2	71 ± 2	159 ± 19
Sm	2	66 ± 10	<	88 ± 3	90 ± 22
Lm	3	82 ± 13	<	122 ± 14	142 ± 10
Sst	2	72 ± 7	24 ± 2	105 ± 13	96 ± 18
Lst	2	86 ± 3	22 ± 5	50 ± 7	97 ± 16

Fraction I was obtained from both plate and cell free medium methods (Figure 6.1). After dialysis, it appeared as insoluble flocculent material, and was easily separated by centrifugation. Small amounts of rhamnose, mannose and acidic monosaccharides were not included.

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean and standard error of 2 or 3 independent experiments (numbers of experiment as shown in the table), < represents less than 1. The chromatograms are shown in Appendix A.4.

A.6 Monosaccharide GC chromatograms of S and L fraction M EP.



Fraction M was obtained from cell free medium (Figure 6.1). Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst). The peaks are identified as described in Appendix A.1. The proportions of major peaks are shown in Appendix A.7.

A.7 Monosaccharide composition of S and L fraction M EP.

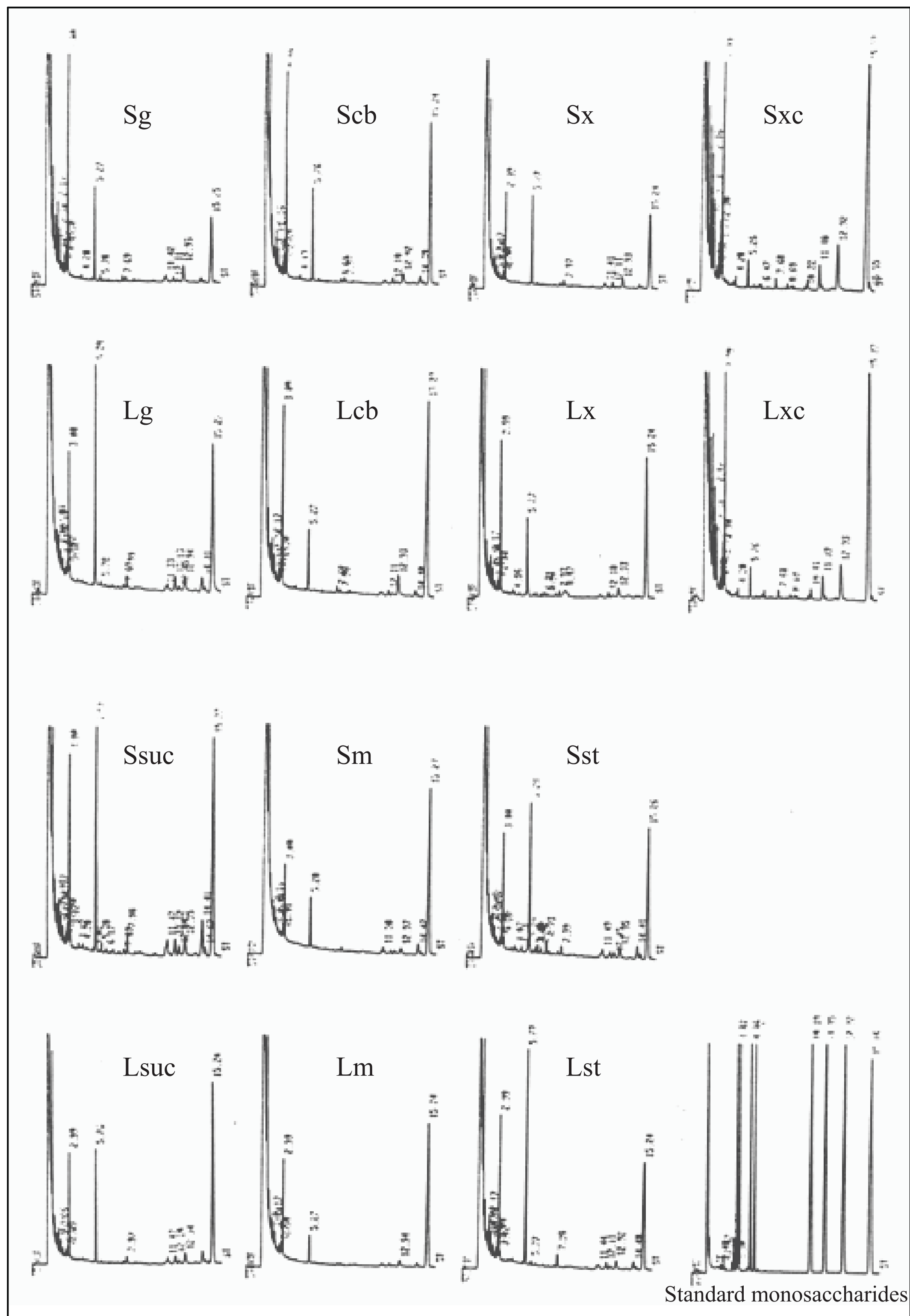
Sample	No. of experiment	Monosaccharides (nmol/mg EP sample)			
		U1	U2	Galactose	Glucose
Sg	3	73 ± 23	701 ± 95	639 ± 25	967 ± 23
Lg	2	65 ± 32	661 ± 65	445 ± 18	717 ± 17
Scb	2	45 ± 20	621 ± 45	451 ± 75	979 ± 190
Lcb	2	96 ± 17	48 ± 4	556 ± 86	864 ± 114
Sx	2	240 ± 45	648 ± 84	568 ± 49	1222 ± 103
Lx	2	156 ± 14	690 ± 93	528 ± 15	1707 ± 113
Sxc	2	197 ± 10	212 ± 32	31 ± 4	1032 ± 231
Lxc	2	76 ± 21	30 ± 2	6 ± 1	1184 ± 220
Ssuc	3	102 ± 7	1197 ± 44	576 ± 40	2217 ± 157
Lsuc	3	43 ± 9	559 ± 38	408 ± 33	698 ± 56
Sm	2	72 ± 19	604 ± 141	497 ± 24	822 ± 25
Lm	2	39 ± 8	297 ± 39	217 ± 42	413 ± 76
Sst	2	218 ± 15	1488 ± 137	842 ± 14	2147 ± 125
Lst	2	74 ± 4	1107 ± 206	571 ± 19	1343 ± 229

Fraction M was obtained from cell free medium (Figure 6.1). Small amounts of rhamnose, mannose and acidic monosaccharide were not included.

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 or 3 independent experiments (number of experiments as shown in the table). The chromatograms are shown in Appendix A.6.

A.8 Monosaccharide GC chromatograms of S and L fraction PD EP.



Fraction PD was obtained from cell free medium (Figure 6.1). Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst). The peaks are identified as described in Appendix A.1. The proportions of major peaks are shown in Appendix A.9.

A.9 Monosaccharide composition of S and L fraction PD EP.

Sample	Number of experiment	Monosaccharides (nmol/mg EP sample)			
		U1	U2	Galactose	Glucose
Sg	2	426 ± 40	294 ± 14	78 ± 6	83 ± 12
Lg	2	185 ± 45	410 ± 81	47 ± 5	37 ± 6
Scb	2	160 ± 14	114 ± 7	28 ± 4	30 ± 1
Lcb	2	156 ± 13	79 ± 2	22 ± 3	69 ± 5
Sx	2	310 ± 148	324 ± 65	38 ± 4	75 ± 11
Lx	3	209 ± 36	241 ± 13	30 ± 5	69 ± 27
Sxc	2	169 ± 17	17 ± 1	24 ± 1	50 ± 4
Lxc	2	220 ± 44	30 ± 7	29 ± 3	50 ± 7
Ssuc	2	167 ± 40	445 ± 56	65 ± 7	67 ± 24
Lsuc	2	146 ± 79	366 ± 28	28 ± 3	73 ± 39
Sm	2	115 ± 48	76 ± 11	21 ± 2	99 ± 7
Lm	2	172 ± 73	60 ± 16	20 ± 3	67 ± 40
Sst	2	190 ± 64	260 ± 22	39 ± 7	102 ± 58
Lst	2	270 ± 88	427 ± 106	53 ± 5	92 ± 37

Fractions PD was obtained from cell free medium (Figure 6.). Small amounts of rhamnose, mannose and acidic monosaccharide were not included.

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 or 3 independent experiments (number of experiments as shown in the table). The chromatograms are shown in Appendix A.8.

Appendix B. GC chromatograms and analysis of proportions of fatty acids.

B.1 Example fatty acid GC chromatograms of various EP fractions.

B.2 Fatty acid GC chromatograms of S and L fraction N EP.

B.3 Major fatty acid composition of S and L fraction N EP.

B.4 Fatty acid GC chromatograms of S and L fraction I EP.

B.5 Major fatty acid composition of S and L fraction I EP.

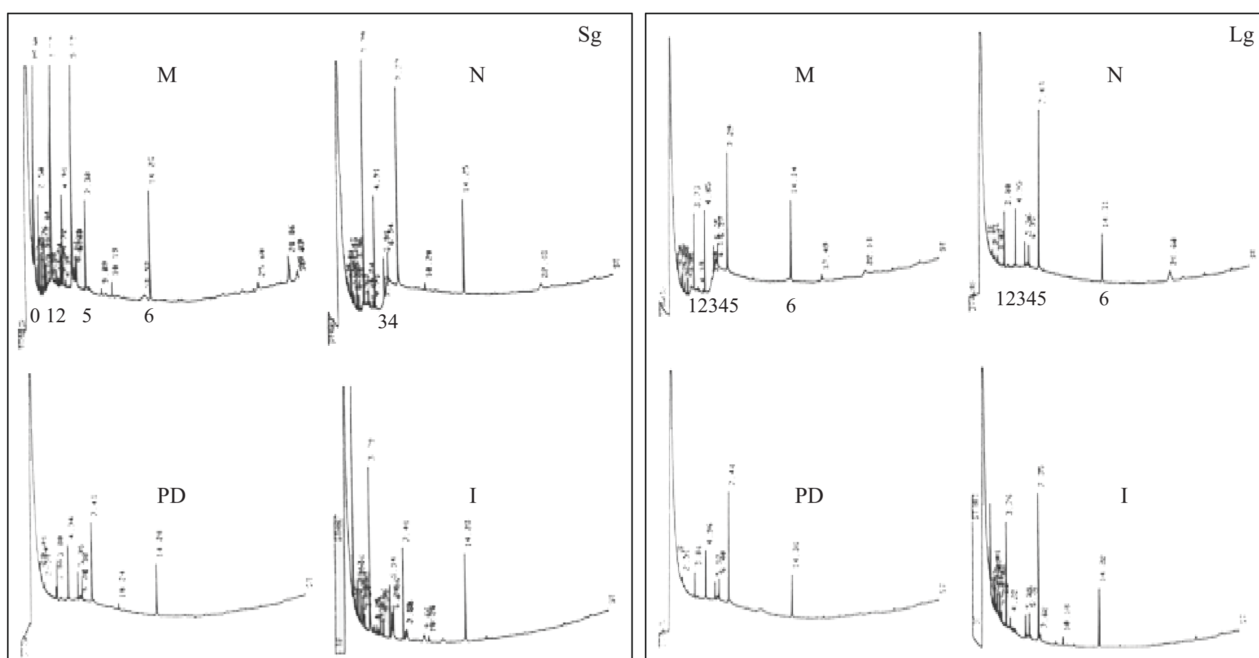
B.6 Fatty acid GC chromatograms of S and L fraction M EP.

B.7 Major fatty acid composition of S and L fraction M EP.

B.8 Fatty acid GC chromatograms of S and L fraction PD EP.

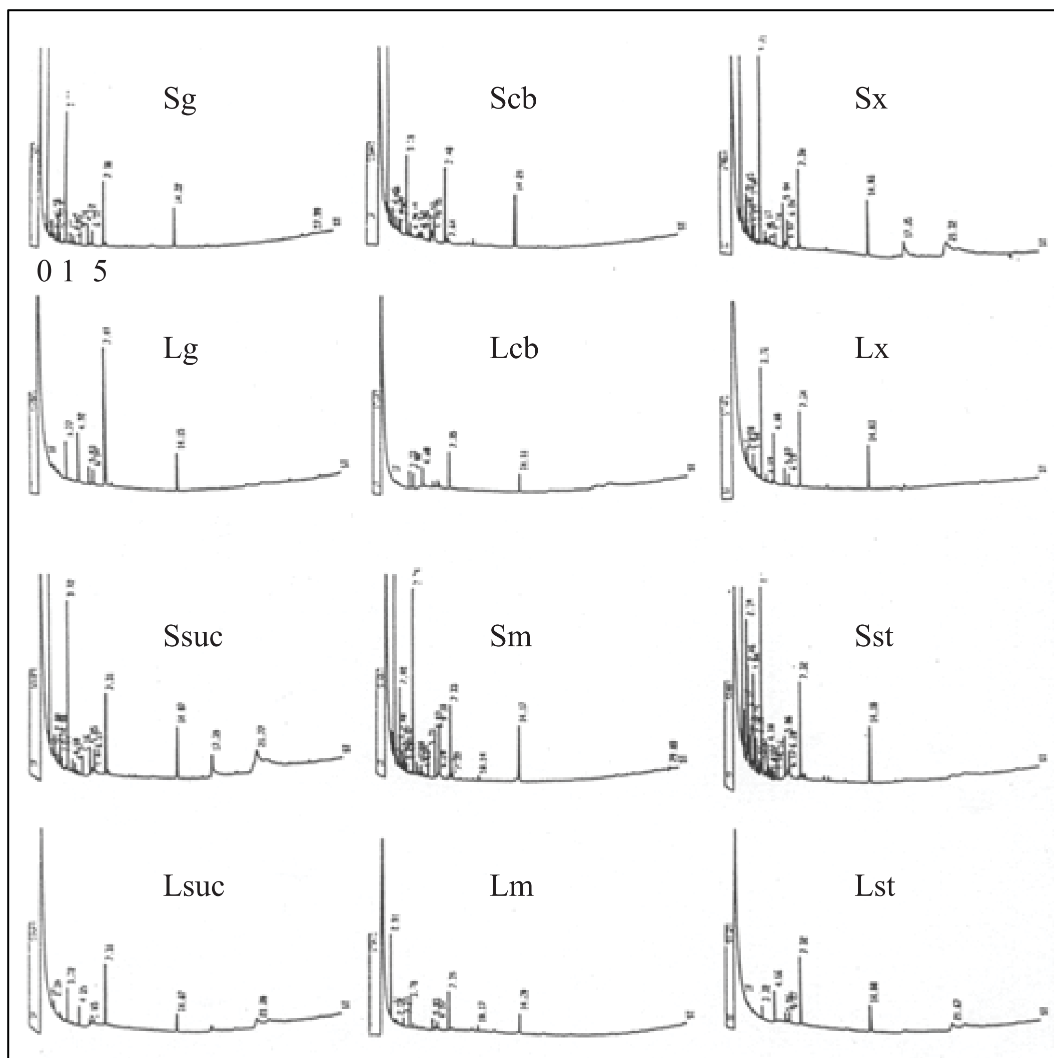
B.9 Major fatty acid composition of S and L fraction PD EP.

B.1 Example fatty acid GC chromatograms of various EP fractions.



S and L indicate *B. fibrisolvens* E14 variants S and L, respectively. Sg and Lg indicate EP samples isolated from S and L variants grown in glucose, respectively. Fractions M and PD fractions were obtained from cell free medium, fraction N was obtained from plate, and fraction I was obtained from both methods (Figure 6.1). 0, 1, 2, 3, 4, 5 and 6 indicate antioxidant, C16:0, u3, C18:0, C18:1, internal standard C19 and u4, respectively. The chromatograms and the corresponding proportions of each fraction for S and L variants EP isolated from cultures grown in various carbon sources are shown in Appendix B2 – B9.

B.2 Fatty acid GC chromatograms of S and L fraction N EP.



Fraction N was obtained from plates (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

0, 1 and 5 indicate antioxidant, C16:0 (palmitic acid) and internal standard C19 peaks, respectively.

Other peaks are identified as described in Appendix B.1. The proportions of major peaks are shown in Appendix B.3.

B.3 Major fatty acid composition of S and L fraction N EP.

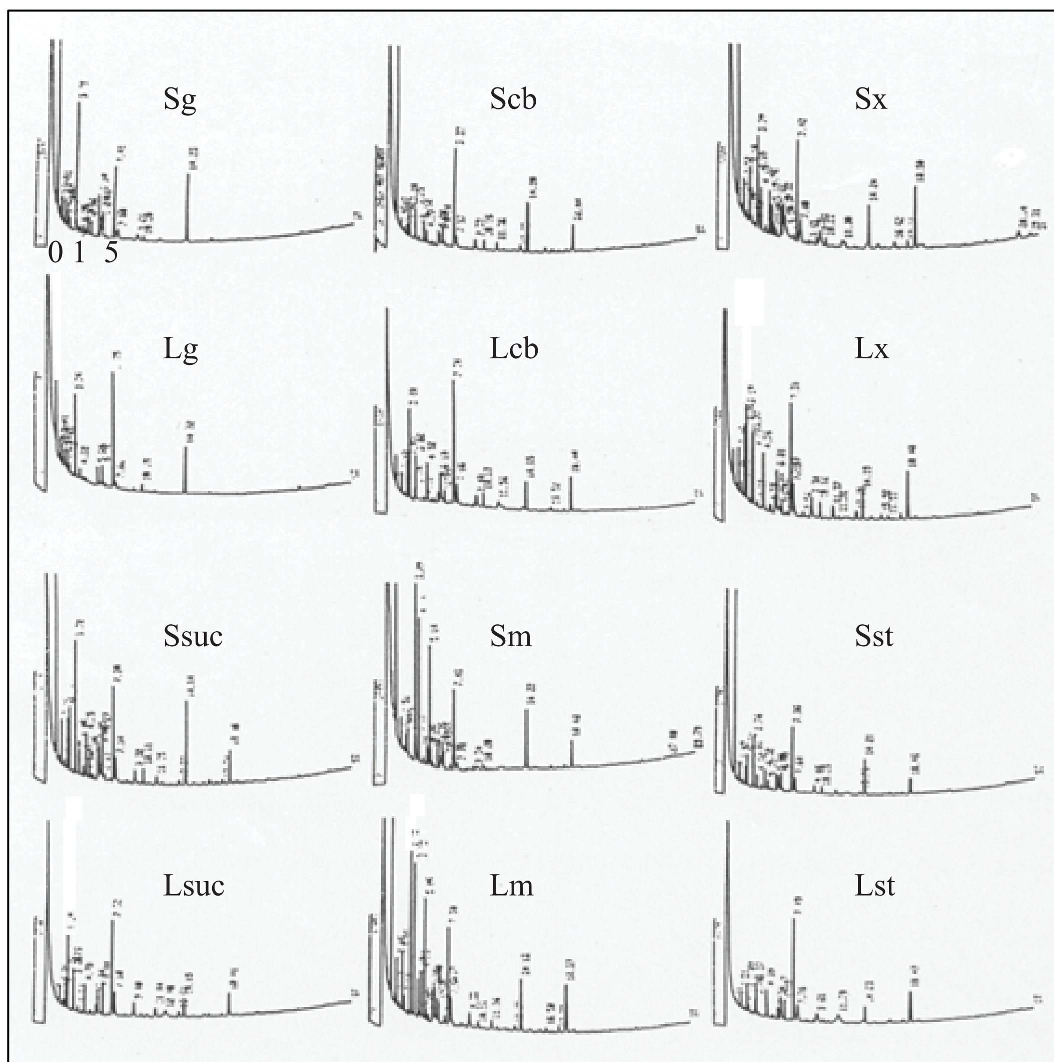
Sample	Number of Experiment	Fatty acids (pmol/mg EP sample)				
		3.76	4.88	5.99	6.31	14.21
		C16:0	U3	C18:0	C18:1	U4
Sg	2	328 ± 40	<	18 ± 3	10 ± 2	50 ± 3
Lg	2	56 ± 6	21 ± 3	9 ± 2	6 ± 1	30 ± 8
Scb	2	58 ± 2	2 ± <	14 ± 3	22 ± 5	55 ± 3
Lcb	2	45 ± 2	25 ± 3	9 ± 1	10 ± 1	38 ± 6
Sx	2	120 ± 8	1 ± <	31 ± 4	18 ± 1	56 ± 4
Lx	2	59 ± 3	35 ± 3	12 ± 2	7 ± 1	42 ± 9
Sxc	-	not determined				
Lxc	-	not determined				
Ssuc	2	191 ± 8	2 ± <	18 ± 1	13 ± 2	53 ± 3
Lsuc	2	25 ± 2	1 ± <	15 ± 2	15 ± 3	33 ± 7
Sm	2	504 ± 39	<	34 ± 1	32 ± 8	59 ± 14
Lm	2	27 ± 1	2 ± <	15 ± 4	14 ± 3	39 ± 2
Sst	2	157 ± 15	2 ± <	21 ± 3	18 ± 5	50 ± 2
Lst	2	38 ± 11	25 ± 3	8 ± 2	8 ± 2	35 ± 2

Fraction N was obtained from plates (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 independent experiments, and < represents less than 1. The chromatograms are shown in Appendix B.2.

B.4 Fatty acid GC chromatograms of S and L fraction I EP.



Fraction I was obtained from both plates and cell free medium methods (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

0, 1 and 5 indicate antioxidant, C16:0 (palmitic acid) and internal standard C19 peaks, respectively.

Other peaks are identified as described in Appendix B.2. The proportions of major peaks are shown in Appendix B.5.

B.5 Major fatty acid composition of S and L fraction I EP.

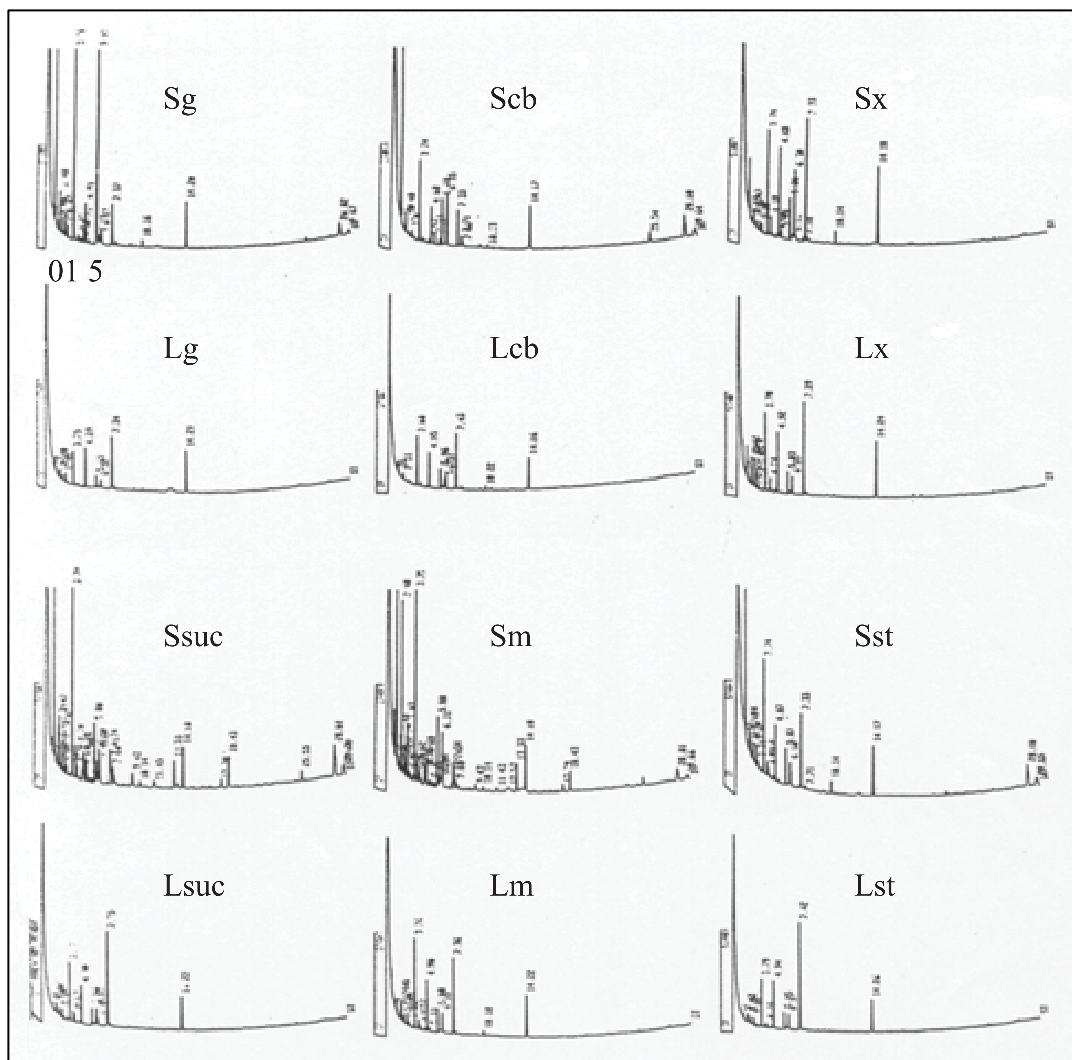
Sample	Number of experiment	Fatty acids (pmol/mg EP sample)				
		3.76	4.88	5.99	6.31	14.21
		C16:0	U3	C18:0	C18:1	U4
Sg	1	80	18	32	31	80
Lg	1	30	4	9	9	34
Scb	1	18	13	9	6	38
Lcb	1	60	22	29	7	48
Sx	1	32	24	16	13	30
Lx	1	17	29	14	5	22
Sxc	-	not determined				
Lxc	-	not determined				
Ssuc	1	86	15	23	25	75
Lsuc	1	19	18	14	20	15
Sm	1	131	14	16	14	46
Lm	1	85	25	13	10	43
Sst	1	30	17	14	13	42
Lst	1	16	20	8	9	14

Fraction I was obtained from both plates and cell free medium (Figure 6.1). After dialysis, it appeared as insoluble flocculent material, and it was easily separated by centrifugation.

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Due to low yield, the determination was performed once. Samples were collected at 3 different times. The chromatograms are shown in Appendix B.4.

B.6 Fatty acid GC cfromatograms of S and L fraction M EP.



Fraction M was obtained from cell free medium (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

0, 1 and 5 indicate antioxidant, C16:0 (palmitic acid) and internal standard C19 peaks, respectively.

Other peaks are identified as described in Appendix B.1. The proportions of major peaks are shown in Appendix B.7.

B.7 Major fatty acid composition of S and L fraction M EP.

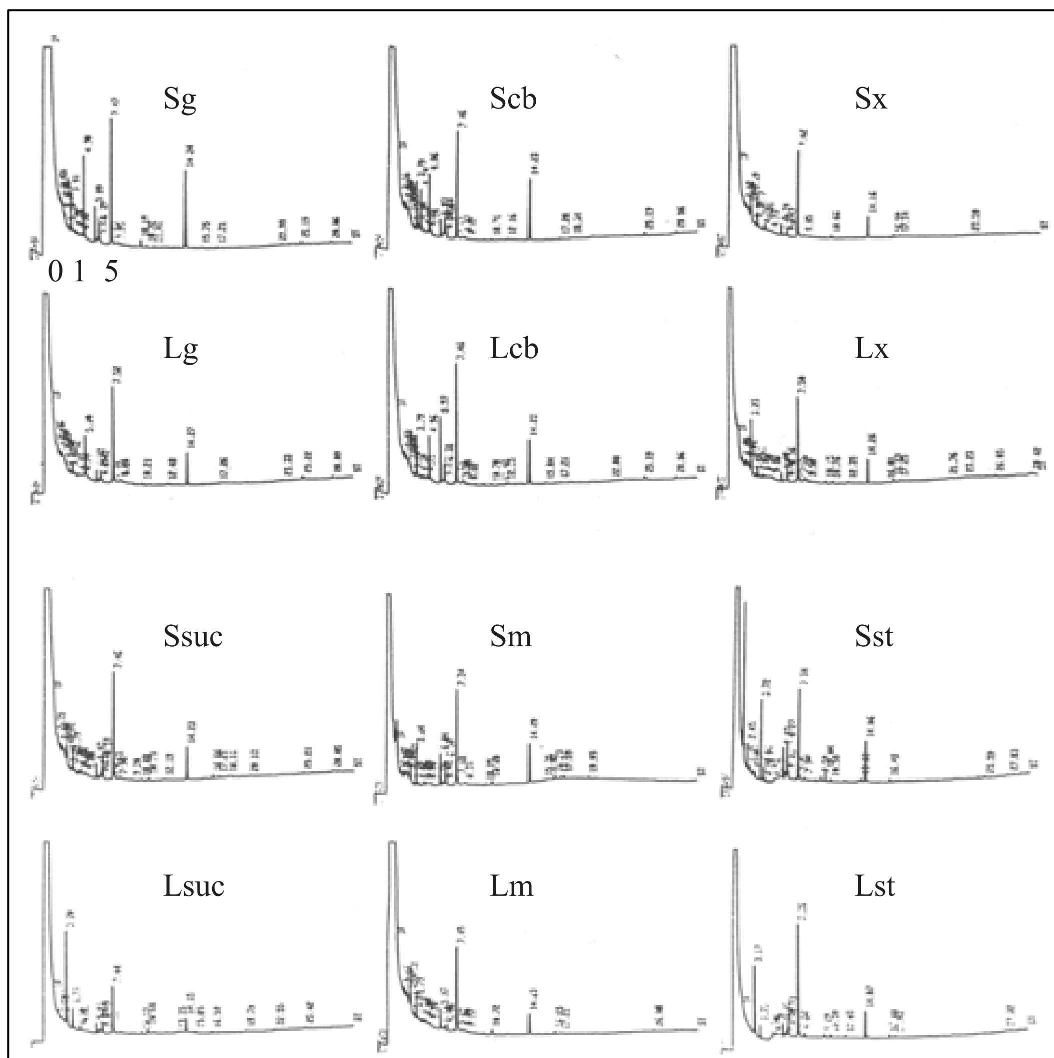
Sample	Number of Experiment	Fatty acids (pmol/mg EP sample)				
		3.76	4.88	5.99	6.31	14.21
		C16:0	U3	C18:0	C18:1	U4
Sg	2	295 ± 14	51 ± 4	432 ± 20	31 ± 4	99 ± 7
Lg	2	38 ± 2	37 ± 3	28 ± 1	17 ± 1	72 ± 5
Scb	2	95 ± 19	51 ± 4	70 ± 3	75 ± 45	91 ± 22
Lcb	2	36 ± 2	34 ± 3	19 ± 1	16 ± 1	48 ± 4
Sx	2	138 ± 12	37 ± 2	21 ± 8	35 ± 4	57 ± 3
Lx	2	42 ± 8	35 ± 3	18 ± 5	15 ± 1	62 ± 9
Sxc	-	not determined				
Lxc	-	not determined				
Ssuc	2	259 ± 26	37 ± 6	115 ± 10	60 ± 12	91 ± 11
Lsuc	2	46 ± 22	21 ± 1	10 ± <	19 ± 8	31 ± 4
Sm	2	421 ± 32	34 ± 10	159 ± 10	175 ± 19	136 ± 22
Lm	2	50 ± 3	35 ± 2	18 ± 1	17 ± 3	49 ± 3
Sst	2	168 ± 15	38 ± 2	39 ± 2	21 ± 5	59 ± 3
Lst	2	22 ± 4	26 ± 3	34 ± 5	11 ± 3	25 ± 2

Fraction M was obtained from cell free medium (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 independent experiments, and < represents less than 1. The chromatograms are shown in Appendix B.6.

B8. Fatty acid GC chromatograms of S and L fraction PD EP.



Fraction PD was obtained from cell free medium (Figure 6.1).

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

0, 1 and 5 indicate antioxidant (not present), C16:0 (palmitic acid) and internal standard C19 peaks, respectively.

Other peaks are identified as described in Appendix B.1. The proportions of major peaks are shown in Appendix B.9.

B.9 Major fatty acid composition of S and L fraction PD EP.

Sample	Number of experiment	Fatty acids (pmol/mg EP sample)				
		3.76	4.88	5.99	6.31	14.21
		C16:0	U3	C18:0	C18:1	U4
Sg	2	50 ± 4	36 ± 3	17 ± 1	11 ± 2	50 ± 4
Lg	2	28 ± 2	23 ± 1	16 ± 8	8 ± 1	28 ± 2
Scb	2	44 ± 3	29 ± 2	11 ± 1	10 ± 2	44 ± 3
Lcb	2	32 ± 5	20 ± 1	34 ± 2	13 ± 3	32 ± 5
Sx	2	19 ± 2	<	8 ± 1	15 ± 1	19 ± 2
Lx	2	22 ± 3	<	7 ± 1	13 ± 2	22 ± 3
Sxc	-	not determined				
Lxc	-	not determined				
Ssuc	2	28 ± 5	1 ± <	10 ± 2	20 ± 6	28 ± 5
Lsuc	2	21 ± 1	1 ± <	13 ± 2	15 ± 4	21 ± 1
Sm	2	28 ± 4	<	21 ± 2	17 ± 1	28 ± 4
Lm	2	20 ± 1	1 ± <	14 ± 1	11 ± 6	20 ± 1
Sst	2	39 ± 4	<	21 ± 3	27 ± 1	39 ± 4
Lst	2	18 ± 2	9 ± 2	8 ± 2	11 ± 1	18 ± 2

Fraction PD was obtained from cell free medium (Figure 6.1)

Sg, Scb, Sx, Sxc, Ssuc, Sm and Sst indicate EP samples isolated from S variant grown in glucose, cellobiose, xylan, cellulose, sucrose, maltose and starch, respectively. Similarly for EP isolated from L variant (Lg, Lcb, Lx, Lxc, Lsuc, Lm and Lst).

Values represent the mean plus standard error of 2 independent experiments, and < represents less than 1. The chromatograms are shown in Appendix B.8.

Appendix C. Medium, buffers and reagents

C.1 LB (Luria Bertani) Medium
C.2 Brain Heart Infusion (BHI) medium
C.3 Defined medium
C.4 Solid medium
C.5 Selective/antibiotic containing medium
C.6 Hemin + 1,4-Naphtoquinone solution
C.7 Mineral solution I
C.8 Mineral solution II
C.9 Trace elemen solution
C.10 Volatile fatty acid (VFA) mixture
C.11 Vitamin solution
C.12 Bradford Reagent
C.13 3 M Sodium acetate pH 5.2 stock solution
C.14 1 M Dithiothreitol (DTT) stock solution
C.15 100 mM Phenylmethyl sulfonyl fluoride (PMSF) stock solution
C.16 4 x 1.5M Tris-HCL pH 8.8 containing 0.4%SDS
C.17 4 X 0.5M Tris-HCl pH 6.8 containing 0.4%SDS
C.18 30 % Acrylamide-0.8 % bis-acrylamide stock solution
C.19 Resolving gel (10 % acrylamide)
C.20 Stacking gel (3.9 % acrylamide)
C.21 2 x SDS Sample buffer
C.22 5 x SDS/Electrophoresis Buffer (stock)
C.23 Coomassie Blue staining solution
C.24 Protein Gel destaining solution
C.25 TE buffer pH 6.8; 7.4; 7.6 or 8.0
C.26 TE saturated Phenol
C.27 50 x TAE buffer
C.28 6 x Agarose gel loading buffer
C.29 Ethidium bromide stock solution
C.30 Solution I for plasmid isolation
C.31 Solution II for plasmid isolation
C.32 Solution III for plasmid isolation
C.33 20 x SSC
C.34 Prehybridisation solution
C.35 Hybridisation solution
C.36 Somogyi copper reagent
C.37 Nelson (Arsenomolybdate) reagent
C.38 CMC stock solution
C.39 Phosphate-Buffered Saline (PBS) pH 7.6
C.40 Fixative solution
C.41 Spurr's resin
C.42 Water saturated phenol
C.43 Ampicillin stock solution
C.44 Erythromycin stock solution
C.45 Electroporation (EB) Buffer

C.1 LB (Luria Bertani) Medium /100 ml

Ingredients

Tryptone ……………………………. 1.0 g

Yeast Extract ………………………… 0.5 g

NaCl …………………………………. 1.0 g

The pH was adjusted to 7.5 with NaOH, and volume was made up to 100 ml with H_2O.

C.2 Brain Heart Infusion (BHI) medium

Ingredients /100 ml

BHI powder .... 3.7 g

Hemin + 1,4-Naphtoquinon solution .... 1 ml

L-cysteine hydrocloride ....… 0.05 g

The medium was brought to a volume of 100 ml with H_2O, boiled and placed in a coy anaerobic hood (95% CO_2/5% H_2 atmosphere) for 4 hours. The medium was then dispensed into Hungate tubes, sealed and autoclaved.

C.3 Defined medium (Nili and Brooker 1995)

Ingredients /100 ml

Mineral solution I ……………………………………… 6.0 ml

Mineral solution I ………………………………………. 6.0 ml

Trace elements ………………………………………….. 0.5 ml

Hemin +1,4-Naphtoquinon solution …………………….. 1.0 ml

Resazurin (0.1%) ……………………………………….. 0.05 ml

L-Cysteine-HCl ………………………………………… 0.05 g

Vitamin solution ………………………………………… 4.0 ml

VFA mixture …………………………………………….. 0.33 ml

Sodium carbonate 8% (w/v) ……………………………….. 5.0 ml

Carbon source ^ ………………………………………… 0.5 g

NH_4Cl ^^ …………………………………………… 0.37g

^ = glucose, cellobiose, xylan, crystalline cellulose, sucrose, maltose or starch

^^ = or casein (0.5 g) or casein (0.25 g) + ammonium chloride (0.25 g)

The medium was brought to a volume of 100 ml with H_2O (the pH was adjusted to 7.7-7.8 before adding sodium carbonate and vitamin solution), it was then boiled and placed in a Coy anaerobic hood (95% CO_2/5% H_2 atmosphere) for 4 hours. The medium was then dispensed into Hungate tubes or Schott bottles , sealed and autoclaved.

C.4 Solid medium

For agar plate (solid) medium, agar powder was added to liquid medium (final concentration 1.4 %) before autoclaving.

C.5 Selective/antibiotic containing medium

The solid or liquid medium was autoclaved, cooled and the appropriate amount antibiotic stock solution was added medium temperature was about 50-60^oC.

C.6 Hemin + 1,4-Naphtoquinone solution (Caldwell and Byrant 1966, Gomez-Alarcon *et al.* 1982)

Ingredients	g/l
Hemin	0.5
1,4-Naphtoquinone	0.1

The ingredients were dissolved in 10 ml of 1M NaOH, and the volume was brought to a final volume of 1 liter with H_2O. The solution was stored at 4^oC.

C.7 Mineral solution (Atlas 1993, Nili 1996)

Ingredients	g/l
K_2HPO_4...	11.84

The solution was dissolved in H_2O to a final volume of 1 liter. The solution was stored at 4^oC.

C.8 Mineral solution II (Atlas 1993, Nili 1996)

Ingredients	g/l
NaCl	1.78
Na_2SO_4	8.30
KH_2PO_4	7.08
$CaCl_2.2H_2O$	3.19
$CoCl_2.6H_2O$	0.02
$MnCl_2.6H_2O$	0.20
$MgSO_4.7H_2O$	3.75

To prevent precipitation, $CaCl_2.2H_2O$ was dissolved in H_2O separately, and it was then added to the rest of the mineral solution II. The solution was stored at 4^oC.

C.9 Trace element solution (Gomez-Alarcon *et al*. 1982, Nili and Brooker 1995)

Ingredients	g/l
$FeSO_4$	0.1
H_3BO_3	0.1
$NiCl_2.6H_2O$	0.05
$CuSO_4.5H_2O$	0.05
$ZnSO_4.7H_2O$	0.1
$Na_2MoO_4.2H_2O$	0.1
$Al_2(SO_4)_3.12H_2O$	0.02

The ingredients were dissolved in H_2O to final volume of 1 liter. The solution was stored at 4^oC.

C.10 Volatile fatty acid (VFA) mixture (Caldwell and Byrant 1966)

Ingredients	
Acetic acid	17.0
Butyric acid	4.0
n-valeric acid	1.0
Propionic acid	6.0
Iso-valeric acid	1.0
Iso-butyric acid	1.0
DL-α-methylbutyric acid	1.0

The fatty acids were pipetted into a tight-fighting-closure container, and the pH was adjusted to 7.0 using 10 M NaOH. The solution was stored at 4°C.

C.11 Vitamin solution (Cotta and Hespell 1986)

Ingredients	mg/l
Biotin	8.3
Folic acid	8.3
Riboflavin	67.0
Lipoic acid	6.7
Nicotinamide	67.0
Pyridoxamine	67.0
Cyanocobalamin	8.3
p-amino benzoic acid	8.3
Calcium pantothenate	67.0
Thiamine hydrocloride	67.0

The ingredients were dissolved in H_2O, and the pH was adjusted to 7.0. The solution was stored in aliquots of 10 ml at -20°C.

C.12 Bradford Reagent (Bradford 1976)

Ingredients	/l
Coomassie Brilliant Blue G-250 ……………….	0.1g
95% Ethanol …………………………………..	50.0 ml
85% Phosphoric acid …………………………..	100.0 ml

The volume was brought up to 1 liter with H_2O, mixed and filtered through a Whatman No.1 filter paper. The solution was stored in a dark bottle at 4°C.

C.13 3 M Sodium acetate pH 5.2 stock solution (Sambrook *et al.* 1989)

Ingredients /100 ml

Sodium acetate 3 H_2O …………………………… 40.81 g

The sodium acetate was dissolved in 80 ml of H_2O and the pH was adjusted to 5.2 with glacial acetic acid. The volume was then brought up to 100 ml with H_2O and sterilized by autoclaving.

C.14 1 M Dithiothreitol (DTT) stock solution (Sambrook *et al.* 1989)

Ingredients /20 ml

Dithiothreitol ……………………………………… 3.09 g

0.01 M Sodium Acetate pH 5.2 …………………… 20 ml

The solution was filter-sterilized, dispensed into 1-ml aliquots and it was then stored at –20°C.

C.15 100 mM Phenylmethyl sulfonyl fluoride (PMSF) stock solution (Sambrook *et al.* 1989)

Ingredients /ml

Phenylmethyl sulfonyl fluoride ……………………. 17.4 mg

Isopropanol ………………………………………… 1 ml

Stored at –20°C.

C.16 4 x 1.5M Tris-HCl pH 8.8 containing 0.4%SDS

Ingredients	/500 ml
Tris base ……………………………………….	91.0 g
SDS ………………………………………..……	2.0 g

The Tris base was dissolved in 300 of H_2O, the pH was adjusted to 8.8 with 1 M HCl, and the volume was brought up to 500 ml. The solution was filtered trough 0.45 µm filter, SDS was then added and mixed by stirring. The solution was stored at 4°C.

C.17 4 X 0.5M Tris-HCl pH 6.8 containing 0.4%SDS

Ingredient	/500 ml
Tris base ………………………………………	30.25 g
SDS ……………………………………………	2.0 g

The Tris base was dissolved in 300ml of H_2O. The pH was adjusted to 6.8 with concentrated HCl, and the volume was brought up to 500ml. The solution was filtered through a 0.45µm filter. SDS was then added and mixed The solution was stored at 4°C.

C.18 30 % Acrylamide-0.8 % bis-acrylamide stock solution

Ingredients	/100 ml
Acrylamide ………………………………….. .	30.0 g
N, N'-methylene bis-acrylamide ……………….	0.8 g

The ingredients were dissolved in H_2O to final volume of 100 ml. The solution was filtered through a 0.45um filter and stored at 4°C in dark bottle.

C.19 Resolving gel (10 % acrylamide)

Ingredients	/15ml
30% acrylamide / 0.8% bis-acrylamide stock solution	5.0 ml
4 x Tris-HCl/SDS, pH 8.8 ……..………………………	3.75 ml
H_2O ……….……………………………………….	6.25 ml
10% Ammonium persulfate * …..……………..…...	0.05 ml
TEMED* ………………………………………...	0.01 ml

* These reagents were added after degassing the solution for 10- 15 minutes.

C.20 Stacking gel (3.9 % acrylamide)

Ingredient	/5 ml
30% acrylamide/ 0.8% bis-acrylamide stock solution	0.65 ml
4 x Tris-HCl. pH 6.8 ………………...…………	1.25 ml
H_2O ……………………………………………….	3.05 ml
10% Ammonium persulfate * solution …………….	0.025 ml
TEMED* ………………………………………….	0.005 ml

* These reagents were added after degassing the solution for 10 - 15 minutes.

C.21 2 x SDS Sample buffer

Ingredient	/100 ml
4 X Tris-HCl/SDS buffer, pH 6.8 …………….	25.0 ml
Glycerol ………………………………………	20.0 ml
SDS ……………………………………………	4.0 g

Bromophenol Blue ……………………………. 1.0 mg

The ingredients were dissolved in H_2O to final volume of 100 ml, and mixed by stirring. The solution was stored at –20°C.

C.22 5 x SDS/Electrophoresis Buffer (stock)

Ingredients	/l
Tris base ………………………………….…….	15.1 g
Glycine ……………………………………….	72.0 g
SDS …………………………………………..	5.0g

The ingredients was dissolved in H_2O to final volume of 1 liter and mixed by stirring, and was then stored at 4°C. The stock solution was diluted to 1 x in H_2O, prior to use.

C.23 Coomassie Blue staining solution

Ingredients	/l
Coomassie Brilliant Blue R-250 ……………………	2.0g
Methanol ……………………………………………	500.0 ml
Glacial acetic acid …………………………………..	100.0 ml

The solution was brought up to final volume of 1 liter with H_2O, and mixed by stirring for 30-40 minutes. The solution was filtered through Whatman No.1 filter paper and stored at room temperature.

C.24 Protein Gel destaining solution

Ingredients	/100ml
Methanol …………………………………………….	25.0 ml

Glacial acetic acid ……………………………………… 10.0 ml

H_2O ……………………………….……...……………… 65.0 ml

C.25 TE buffer pH 6.8; 7.4; 7.6 or 8.0 (Sambrook *et al.* 1989)

Ingredients

EDTA pH 8.0 .... 10.0 mM

Tris-HCl, pH 6.8; 7.4; 7.6 or 8.0 ……........... 100.0 mM

C.26 TE saturated Phenol

Phenol was melted at 68°C and 8-hydroxyquinoline was added to a concentration of 0.1%. The mixture was then equilibrated with an equal volume of 1.0 M Tris-HCl pH 8.0 overnight with stirring. The upper layer (aqueous phase) was removed and an equal volume of 0.1 M Tris-HCl pH 8.0 was added and equilibrated until the pH of the aqeous phase was 7.6. The equilibrated phenol was stored (in dark bottle) under the 0.01 M Tris-HCl pH 7.6 at 4°C.

C.27 50 x TAE buffer (Sambrook *et al.* 1989)

Ingredients /l

Tris base (Trizma) .... 242.0 g

Glacial acetic acid .... 57.1 ml

EDTA (0.5 M, pH 8.0) .... 100.0 ml

C.28 6 x Agarose gel loading buffer (Sambrook *et al.* 1989)

Ingredients	/10 ml
Glycerol	30 %
Xylene cyanol	0.25 %
Bromophenol Blue	0.25 %

C.29 10 mg/ml Ethidium bromide stock solution (Sambrook *et al.* 1989)

Ingredients	/10 ml
Ethidium bromide ……………………………………..	1 g

The dye was dissolved in 100 ml of H_2O and mixed by stirring on magnetic stirrer. The solution was stored in dark bottle at room temperature.

C.30 Solution I for plasmid isolation

Ingredients	
Tris-HCl pH 8.0 ………………………………….	10 mM
EDTA pH 8.0 …………………………………..	10 mM
NaCl ……………………………………………..	50 mM
Sucrose ……………………………………………..	20 % (w/v)

C.31 Solution II for plasmid isolation

Ingredients	
NaOH (freshly diluted from 10 N stock) ………………	200 mM
SDS (freshly diluted from 10 % stock) ………………...	1 % (w/v)

C.32 Solution III for plasmid isolation

Ingredients

5 M Potassium acetate ……………………………..	60 ml
glacial acetic acid ………………………………….	11.5 ml
H_2O …………………………………………………...	28.5 ml

C.33 20 x SSC (Sambrook *et al.* 1989)

Ingredients	g/l
Sodium citrate	88.2
Sodium chloride	175.3

The ingredients were dissolved in 800 ml of H_2O and the pH was adjusted to 7.0 with a few drops of a 10 N NaOH. The volume was brought up to 1 liter with H_2O and then sterilized by autoclaving.

C. 34 Prehybridisation solution

Ingredients	/10 ml
Skim milk powder (Blotto) ………………………	1.0g
20 x SSC ……………………………………………	2.5 ml
10%SDS ……………………………………………	0.5 ml
1% (w/v) Calf thymus DNA ……………………..	0.1 ml
H_2O ……………….……………………………..	6.9 ml

The solution was freshly made and heated at 65°C, prior to use.

C.35 Hybridisation solution

Ingredients	/10 ml
Skim milk powder (Blotto) ………………………	0.1g
20 x SSC ……………………………………….	2.5 ml
40% Polyethylene glycol (PEG) …………………	1.0 ml
H_2O ……….……………………………………...	6.5 ml

The solution was freshly made and heated at 65^oC, prior to use.

C36. Somogyi copper reagent (Ashwell 1957, Wood and Bhat 1988)

Ingredients	/liter
Disodium phosphate ($Na_2HPO_4.12H_2O$) …..…………..	70.5g
Sodium potassium tartrate ………………..……………	40.0g
1N Sodium hydroxide …………………………………..	100.0 ml
10% aqueous solution of copper sulphate($CuSO_4.5H_2O$)	180.0 ml
Sodium sulphate decahydrate ($Na_2SO_4.10H_2O$) ………	180.0 g

Disodium phosphate and sodium potassium tartrate were first dissolved in 350ml of H_2O. 1N NaOH was then added slowly, followed by 10% $CuSO_4$ and Na_2SO_4 solutions. The volume was brought up to 1 liter with H_2O. The solution was incubated at 37^oC for 48h. It was filtered through Whatman No.1 and stored in a dark (dark bottle) at 27^oC. For a working solution, the stock solution was diluted 1:2.8 with H_2O.

C37 Nelson (Arsenomolybdate) reagent (Ashwell 1957, Wood and Bhat 1988)

Ingredients	/l
Ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$]	25.0g
Concentrated sulphuric acid (H_2SO_4) ………………….	25.0 ml
12% (w/v) aqueous solution of sodium arsenate ($Na_2HAsO_4.7H_2O$) ……………………	25.0 ml

Ammonium heptamolybdate was first dissolved in 450ml of warm H_2O. After cooling, sulphuric acid was added slowly and the solution allowed to cool before adding sodium arsenate solution. The solution was incubated at 37°C for 48h, filtered through Whatman No.1 and stored in a dark (dark bottle) at room temperature.

C.38 CMC stock solution (Ashwell 1957, Wood and Bhat 1988)

2 g of CMC powder was dissolved in 100 ml of phosphate buffer pH 6.8, and mixed by heating and vigorous stirring with a magnetic stirring bar. The solution was then sterilized by autoclaving and stored at 4°C.

C.39 Phosphate-Buffered Saline (PBS) pH 7.6 (Smitberg and Krieg 1994, Nili and Brooker 1995)

Ingredients	g/l
NaCl	8.50
$NaH_2PO_4.H_2O$	0.18
Na_2HPO_4, anhydrous	1.236

C.40 Fixative solution

Ingredients

Paraformaldehyde .... 4.0 g

Glutaraldehyde .... 1.25 g

PBS plus 4% (w/v) sucrose .... 100 ml

Paraformaldehyde powder was dissolved in sucrose-containing PBS buffer by heating to 60-70°C and stirring. One to three drops of 1 M NaOH were added with stirring until the solution became clear, cooled and glutaraldehyde-containing PBS buffer was added, and then brought to final volume of 100 ml with PBS buffer.

C.41 Spurr’s resin

Ingredients

ERL (Vinyl cyclohexane dioxide) .... 10.0 g

NSA (Nonenyl succinic anhydride) .... 26.0 g

DER (Diglycidyl ether of polypropylene glycol) .... 5.0 g

DMAE (2, Dimethylaminoethanol) .... 0.2 ml

C.42 Water saturated phenol

The water saturated phenol was prepared as in Appendix C.26, except H_2O was used instead of Tris-HCl.

C.43 Ampicillin stock solution (Sambrook *et al.* 1989)

50 mg of ampicillin was dissolved 1ml of H_2O and then filter sterilized. The solution was stored at –20°C. 100 µl was added to 100 ml of medium, to obtain 50 µg/ml (final concentration).

C.44 Erythromycin stock solution

10 mg of erythromycin was dissolved 1ml of 70 % ethanol and then filter sterilized (for precaution) in anaerobic hood. The solution was made prior to use. 100 µl was added to 100 ml of medium, to obtain 10 µg/ml (final concentration). For anaerobic liquid medium, it was added separately inside an anaerobic hood, prior to inoculation.

C.45 Electroporation Buffer (for B. fibrisolvens, for *E. coli* as described in the methods or in Sambrook *et al.* 1989)

Ingredients	/400 ml
Sorbitol	21 g
0.1 % resazurin	0.4 ml
1 M Dithiothreitol	0.4 ml

Sorbitol and resazurin were brought to a volume of 400 ml with H_2O, boiled and placed in a coy anaerobic hood (95 % CO_2 / 5 % H_2 atmosphere) for 4 hours. The solution was autoclaved, cooled to room temperature in an anaerobic chamber, and 0.4 ml of 1 M (filter sterilized) DTT was added to the solution and then leaved further until the solution was colourless. The solution was stored in a tight fitting bottle at 4°C.

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