

SUPPLEMENTAL MATERIAL

for manuscript entitled

“Polysaccharides and proteins added to flowing drinking water at microgram-per-liter levels promote the formation of biofilms predominated by *Bacteroidetes* and *Proteobacteria*”

Eveline L.W. Sack, Paul W.J.J. van der Wielen,* and Dick van der Kooij

Running Title

Biopolymer utilization at $\mu\text{g l}^{-1}$ levels by biofilms

Affiliation Authors

KWR Watercycle Research Institute
P.O. Box 1072, 3430 BB Nieuwegein
The Netherlands

*** Corresponding Author**

KWR Watercycle Research Institute
P.O. Box 1072, 3430 BB Nieuwegein
The Netherlands

Telephone number: +31 (0)30 60 69 642

Email address: Paul.van.der.Wielen@kwrwater.nl

SECTION: RESULTS

Table S1. Experiments (A – D) conducted to assess biofilm formation on glass cylindrical rings exposed to continuously flowing tap water with added biopolymers at $\mu\text{g C l}^{-1}$ levels in a biofilm monitor.

Expt no.	Compound(s) added	Duration and concentration of addition	
		<i>Period (day)</i>	<i>Concentration</i>
A1	Blank	0 – 69	No compounds added
A2	Maltose	0 – 32	10 $\mu\text{g mal-C l}^{-1}$
A3 <i>Phase 1</i>	Amylopectin ^a or	0 – 39	10 $\mu\text{g amp-C l}^{-1}$
<i>Phase 2</i>	Maltose & amylopectin	39 – 69	10 $\mu\text{g mal-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$
A4	Maltose & amylopectin	0 – 32	10 $\mu\text{g mal-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$
B1	Blank	0 – 86	No compounds added
B2 <i>Phase 1</i>	Acetate (ac)	0 – 57	3.0 $\mu\text{g ac-C l}^{-1}$
<i>Phase 2</i>		57 – 86	4.5 $\mu\text{g ac-C l}^{-1}$
B3	Amylopectin	0 – 86	10 $\mu\text{g amp-C l}^{-1}$
B4 <i>Phase 1</i>	Acetate	0 – 31	3.0 $\mu\text{g ac-C l}^{-1}$
<i>Phase 2</i>	Acetate & amylopectin	31 – 57	3.0 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$
<i>Phase 3</i>	Acetate & amylopectin	57 – 86	4.5 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$
C1	Blank	0 – 52	No compounds added
C2	Acetate	0 – 52	10 $\mu\text{g ac-C l}^{-1}$
C3	Amylopectin	0 – 52	10 $\mu\text{g amp-C l}^{-1}$
C4 <i>Phase 1</i>	Acetate	0 – 20	10 $\mu\text{g ac-C l}^{-1}$
<i>Phase 2</i>	Acetate & amylopectin	20 – 52	10 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$
D1	Blank	0 – 88	No compounds added
D2	Caseinate ^a	0 – 82	10 $\mu\text{g cas-C l}^{-1}$
D3	Gelatin ^a	0 – 88	10 $\mu\text{g gel-C l}^{-1}$
D4	Laminarin ^a	0 – 82	10 $\mu\text{g lam-C l}^{-1}$

^a Amylopectin from corn; casein sodium salt from bovine milk; gelatin type B from bovine skin; laminarin from *Laminaria digitata* (Sigma-Aldrich, Germany).

Table S2. Parameters of biofilm formation with organic compounds added to tap water at $\mu\text{g C l}^{-1}$ levels ^a.

Exp no.	Added compound(s) & concn	Fe accumulation rate	FeC _{max}		Avg temp of	Avg ATP content
		during biofilm formation \pm SE	day	day	feed water \pm SD ^f	in feed water \pm SD ^f
		($\mu\text{g Fe pg ATP}^{-1}$)	($\mu\text{g Fe cm}^{-2}$)		($^{\circ}\text{C}$)	(ng l^{-1})
A1	None	39 \pm 4	12 ^e	69		
A2	10 $\mu\text{g mal-C l}^{-1}$	4.7 \pm 0.4	8.8	18		
A3.1	10 $\mu\text{g amp-C l}^{-1}$	n.d. ^b	2.6	39		
A3.2	10 $\mu\text{g mal-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$	4.1 \pm 1.1	50	69	18.1 \pm 1.2 (n = 39)	4.4 \pm 1.4 (n = 39)
A4	10 $\mu\text{g mal-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$	4.7 \pm 0.5; 3.8 \pm 0.5 ^c	47	28		
B1	None	29 \pm 3	12	86		
B2.1	3.0 $\mu\text{g ac-C l}^{-1}$		n.d.	-		
B2.2	4.5 $\mu\text{g ac-C l}^{-1}$	6.3 \pm 0.7 ^d	28	86		
B3	10 $\mu\text{g amp-C l}^{-1}$	n.d. ^b	11	86	15.6 \pm 1.0 (n = 23)	3.9 \pm 1.02 (n = 23)
B4.1	3.0 $\mu\text{g ac-C l}^{-1}$		n.d.	-		
B4.2	3.0 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$	4.8 \pm 0.6 ^d	n.d.	-		
B4.3	4.5 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$		31	86		
C1	None	13 \pm 0.1	2.2	46		
C2	10 $\mu\text{g ac-C l}^{-1}$	1.6 \pm 0.2	29	48		
C3	10 $\mu\text{g amp-C l}^{-1}$	11 \pm 1	2.1	46	11.8 \pm 1.0 (n = 20)	3.8 \pm 1.4 (n = 20)
C4.1	10 $\mu\text{g ac-C l}^{-1}$					
B4.2	10 $\mu\text{g ac-C l}^{-1}$ & 10 $\mu\text{g amp-C l}^{-1}$	1.7 \pm 0.2 ^d	28	48		

Table S2. Continued

D1	None	34 ± 1.9	12	88		
D2	10 $\mu\text{g cas-C l}^{-1}$	8.3 ± 1.4	31	75	14.6 ± 1.6	3.9 ± 1.1
D3	10 $\mu\text{g gel-C l}^{-1}$	6.7 ± 0.8	22	88	(n = 48)	(n = 48)
D4	10 $\mu\text{g lam-C l}^{-1}$	9.2 ± 1.4	22	75		

^a Iron accumulation as a function of biofilm concentration, with standard error (SE); FeC_{max} , maximum iron concentration on glass ring, with standard deviation (SD), and day on which FeC_{max} was measured; r^2 , correlation coefficient of linear regression.

^b Not determined because not enough data were available for calculation.

^c Fe accumulation before and after day 12.

^d Fe accumulation during all two or three phases of the experiment.

^e Fe concentration was approximately 2 to 3 $\mu\text{g Fe cm}^{-2}$ between day 14 to 30 and 6 $\mu\text{g Fe cm}^{-2}$ around day 40.

^f n = number of measurements used to calculate the average and SD.

Table S3. Classification of OTUs clustering with bacteria obtained from biofilms formed with blank tap water (BBL) and tap water with added maltose (BMAL), amylopectin (BAP), maltose & amylopectin (BMA+AP), acetate (BAC), acetate and amylopectin (BAC+AP), gelatin (BGEL), caseinate (BCAS), or laminarin (BLAM) at $\mu\text{g C l}^{-1}$ levels. Single-clone OTUs present in one out of nine biofilm types have been excluded.

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Actinobacteria; Miscellaneous										
Uncultured actinobacterium clone Elev_16S_853 (EF019692) / uncultured Acidothermaceae bacterium clone Elev_16S_1288 (EF019917)	99/98	2	-	-	-	-	-	-	-	-
Uncultured bacterium clone V201-13 (HQ114028) / Uncultured actinobacterium AUVE_14A12 (EF651635)	95/91	1	-	1	-	-	-	-	-	-
Uncultured bacterium GC55 (AJ271048) / Uncultured actinobacterium clone AUVE_14A12 (EF651635)	97/92	-	1	1	-	-	-	-	-	-
Uncultured bacterium clone AK4DE1_05H (GQ397037) / Uncultured actinobacterium clone AL5.80 (GU047499)	99/99	-	-	-	-	-	1	-	-	1
Uncultured Actinobacteria bacterium clone QEEA1AG12 (CU918623)	93	-	-	-	-	-	-	1	-	1
Uncultured actinobacterium clone Elev_16S_853 (EF019692)	98	-	-	-	-	-	-	1	-	1
Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae										
Uncultured Bacteroidetes clone MEsp06cnp11A7 (FJ828175) / Uncultured bacterium clone Granotes_C-B3-9O7R (FN297643) / Uncultured <i>Emticicia</i> sp. clone cuticle_12 (HQ111160)	99/99/97	-	7	-	5	-	-	-	-	-
Uncultured bacterium clone Granotes_C-B3-9O7R (FN297643) / Uncultured Bacteroidetes clone MEsp06cnp11C11 (FJ828202) / <i>Emticicia</i> sp. DC2b-1 (AB552862)	99/99/93	-	2	-	-	-	-	-	-	-
Uncultured bacterium clone 01F_F1_E25 (GU568965) / <i>Leadbetterella</i> sp. HME6675 (HM590831)	99/97	-	3	-	-	-	-	-	-	-
Uncultured bacterium clone mdt15e10 (AY536973) / <i>Pontibacter</i> sp. strain R-36965 (FR691450)	98/85	-	-	-	-	-	-	-	2	-

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae & Flavobacteriaceae										
<i>Flavobacterium</i> sp. HME6012 (HM149211)	99	-	3	-	6	-	-	-	-	-
<i>Flavobacterium</i> sp. CL1.152 (AM934685) / <i>Flavobacterium succinicans</i> strain DSM 4003 (AM230493)	99/98	-	-	-	5	-	-	-	-	1
Uncultured <i>Flavobacterium</i> sp. clone L-1 (JF703584)	98	-	-	-	-	-	-	1	1	-
Uncultured Bacteroidetes bacterium clone MA194T1-3r5_E10 (GQ469392) / <i>Flavobacterium</i> sp. KOPRI 25152 (FJ544919)	97/97	-	-	-	-	-	2	-	-	-
<i>Flavobacterium</i> sp. HME6133 (HM776988) / <i>Flavobacterium aquatile</i> NBRC 15052 (AB517711)	98/97	-	-	-	-	-	-	-	2	-
Uncultured <i>Fluviicola</i> sp. clone cuticle_2.22 (HQ111170)	98	-	-	-	-	-	-	4	-	-
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Chitinophagaceae & Saprospiraceae										
Saprospiraceae bacterium clone MS-Wolf2-H (AJ786323) / <i>Haliscomenobacter hydrossis</i> strain DSM 1100 (AJ784892)	99/98	-	6	-	1	-	-	-	-	4
Uncultured bacterium clone 71021-ONK-SLIME-RF4; clone 71021-ONK-SLIME-RF1 (FJ037622; FJ037621) / <i>Haliscomenobacter hydrossis</i> strain DSM 1100 (AJ784892)	99;99/97	-	10	-	-	-	-	-	-	11
Uncultured bacterium clone F23 (FJ230926) / Uncultured <i>Haliscomenobacter</i> sp. clone SPS-H30 (AM990052)	97/95	-	-	-	-	-	-	-	2	-
Uncultured Bacteroidetes bacterium clone MEsp06cnp11F12 (FJ828234) / Uncultured <i>Haliscomenobacter</i> sp. clone 2G9 (GU074028)	91/86	-	-	-	-	-	-	-	-	4
Uncultured Bacteroidetes clone AS59 (EU283378) / Uncultured Saprospiraceae bacterium clone epr8 (EU177765)	99/98	-	1	1	-	-	-	2	5	6

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Bacterioidetes; Miscellaneous										
Uncultured bacterium clone MBR-8_HF_BF48 (FM201200) / Uncultured Bacteroidetes bacterium clone IRD18G07 (AY947969)	99/93	-	-	-	-	2	-	-	-	-
Uncultured bacterium clone 6-37 (HM163138) / Uncultured Bacteroidetes bacterium clone TR1C12 (FJ916091)	98/93	-	-	-	-	-	-	-	2	-
Chloroflexi; Miscellaneous										
Uncultured bacterium clone P060905_D09 (HQ385575) / Uncultured Chloroflexi bacterium clone CARS52 (FJ902461)	97/95	1	-	-	-	-	-	-	2	-
Uncultured bacterium clone HM49 (AM909917) / Uncultured Uncultured Chloroflexi bacterium clone HG-B01104 (JN409095)	99/99	-	-	-	-	-	2	-	-	-
Cyanobacteria; Miscellaneous										
Uncultured bacterium clone S15D-MN45 (AJ583205) / Uncultured Cyanobacterium clone HrhB65 (AM159280)	99/88	1	-	-	-	1	-	-	-	4
Fibrobacteres/Acidobacteria group; Fibrobacteres & Acidobacteria; Miscellaneous										
Uncultured bacterium clone HAVOmat06 (EF032778) / Uncultured <i>Acidobacterium</i> sp. clone sw-xj104 (GQ302574)	98/98	-	-	2	-	-	-	-	-	-
Uncultured Acidobacteria bacterium clone GASP-MA2S2_H09 (EF662908) / Uncultured Firmicutes bacterium clone GASP- KB2S2_F01 (EU298134)	97/97	3	-	-	-	-	-	-	-	-
Uncultured bacterium clone TX5A_08 (FJ152716) / Uncultured Acidobacteria bacterium clone AMAG3 (AM935828)	98/97	2	-	2	-	-	-	-	-	-
Uncultured bacterium clone GASP-OKB-629-E07 (EU043666) / Uncultured Acidobacteria bacterium clone KBS_T8_R2_149282_g1 (HM061919)	97/96	-	-	1	-	-	-	-	2	-
Uncultured bacterium clone STU23 (EU700165) / Uncultured Acidobacteria bacterium clone AUVE_15D10 (EF651730)	93/94	-	-	-	-	-	-	3	-	-

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Firmicutes; Miscellaneous										
Uncultured bacterium clone Hb_42(28) (HM534459) / Uncultured Firmicutes bacterium clone GASP-MB1S3_B09 (EF664669)	100/98	-	-	2	-	-	-	-	-	-
Uncultured bacterium clone Biofilm_818d_c18 (DQ058684) / Uncultured Firmicutes bacterium clone GASP-KC1S2_G02 (EU299215)	98/97	-	-	2	-	-	-	-	-	-
Uncultured bacterium clone 0702007K01DGUTF1915 (GU369037) / Uncultured Firmicutes bacterium clone A352 (HQ162813)	98/97	-	-	-	-	2	-	-	-	-
Gemmatimonadetes; Gemmatimonadetes; (class); Gemmatimonadales; Gemmatimonadaceae										
Uncultured bacterium clone AA142 (GQ859798) / Uncultured Gemmatimonadetes bacterium clone AMFC3 (AM935288)	95/95	-	-	-	-	-	-	-	1	1
Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae										
Uncultured bacterium clone Inlet_March02_c17 (DQ058674) / Uncultured <i>Nitrospira</i> sp. clone Jy04A40 (AB591407)	99/98	7	1	4	-	3	-	4	7	9
Uncultured bacterium clone pLW-66 (DQ067038) / Uncultured <i>Nitrospira</i> sp. clone Jy04A40 (AB591407)	99/99	1	-	7	-	2	3	5	1	-
Uncultured bacterium clone KA613O121 (HM445534) / Uncultured <i>Nitrospira</i> sp. clone GASP-MA4S1_A06	99/99	1	-	1	-	-	-	-	-	-
Uncultured bacterium clone TA3_51 (EU746700) / Uncultured <i>Nitrospira</i> sp. clone Jy04A32 (AB591401)	99/99	1	-	3	-	1	-	5	-	1
Uncultured Nitrospirae bacterium clone 167 (AB252937) / Uncultured <i>Nitrospira</i> sp. clone Jy04A34 (AB591403)	99/99	-	1	-	1	-	-	-	-	1
Uncultured bacteria clone c13 (FN687453) / Uncultured <i>Nitrospira</i> sp. clone 3 (DQ414437)	99/99	-	-	-	-	-	-	2	-	-

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Uncultured bacterium clone MACA-RR18 (GQ500753) / Uncultured <i>Nitrospira</i> sp. clone Jy04A40 (AB591407)	99/99	-	-	-	-	-	-	-	4	-
Planctomycetes; Miscellaneous										
Uncultured planctomycete clone GASP-WB1W2_B12 (EF073662) / Uncultured <i>Gemmata</i> sp. clone OTU33/APA (AM902601)	90/89	2	-	-	-	-	-	-	-	-
Uncultured <i>Pirellula</i> sp. clone Ivry3 (FJ236060)	99	-	-	1	-	-	1	-	-	-
Uncultured bacterium clone TG_FD_0.7_May_09_B109 (HQ532961) / Uncultured <i>Planctomyces</i> sp. clone Joinville4 (FJ236055)	99/95	1	1	-	-	-	-	-	-	-
Uncultured Crater Lake bacterium CL500-4 (AF316776) / Uncultured <i>Planctomyces</i> sp. clone S3-41 (HQ674917)	99/90	3	-	-	-	-	-	-	-	2
Uncultured Planctomycetes bacterium clone QEDN8AH04 (CU927823) / Uncultured <i>Planctomyces</i> sp. clone B07-08B (FJ543033)	90/90	-	-	-	-	-	-	1	-	1
Proteobacteria; α-Proteobacteria; Rhizobiales; Hyphomicrobiaceae & Rhizobiaceae										
Uncultured bacterium clone AS253O1O5 (HM445062) / Uncultured <i>Pedomicrobium</i> sp. clone GASP-KC3S1_H03 (EU300117)	99/97	2	-	-	-	-	-	-	-	-
Uncultured alpha proteobacterium clone b10-4 (FN594639) / Uncultured <i>Pedomicrobium</i> sp. clone GASP-KB1W3_G11 (EU297964)	98/98	1	-	-	-	1	-	-	-	-
Uncultured bacterium clone GP28366hO3 (HM445358) / <i>Pedomicrobium manganicum</i> strain ATCC 33121 (GU269549)	98/98	2	-	-	-	-	-	-	1	1
Uncultured bacterium clone MACA-EFT23 (GQ500792) / Uncultured <i>Hyphomicrobium</i> sp. clone ENR16 (FJ536923)	97/95	1	-	-	-	-	-	-	1	-
Uncultured alpha proteobacterium clone QEDN1CF08 (CU927121) / <i>Hyphomicrobium</i> sp. Is-B219 (EF35007)	95/96	-	-	-	-	1	-	2	1	1

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Uncultured bacterium clone MP104-SW-b25 (DQ088788) / Uncultured <i>Hyphomicrobium</i> sp. clone YJQ-3 (AY569281)	99/99	-	-	1	-	4	2	-	-	-
Uncultured bacterium clone MACA-EFT28 (GQ500796) / <i>Hyphomicrobium</i> sp. clone as1-44 (GU257506)	96/95	-	-	1	-	-	-	2	-	-
Uncultured Hyphomicrobiaceae bacterium clone GASP_WA2W3_G08 (EF073040) / Uncultured <i>Rhizobium</i> sp. clone Cvi68 (FJ12876) / <i>Devosia</i> sp. strain P-39; strain P-37 (AM411922; AM411923)	99/98/ 98;98	-	9	1	1	-	-	2	-	1
Uncultured Hyphomicrobiaceae bacterium clone Elev_16S_1899 (EF020323) / Uncultured <i>Rhodoplanes</i> sp. clone CMME11 (AM935954)	99/98	-	-	2	-	-	-	-	-	-
Proteobacteria; α-Proteobacteria; Rhizobiales; Miscellaneous										
Uncultured bacterium clone ar1g416 (HM921128) / Uncultured <i>Nitratireductor</i> sp. clone CL3.D14 (FM175760)	100/99	1	-	3	-	-	-	1	1	-
Uncultured Rhizobiales bacterium clone AMGE5 (AM935400) / Uncultured <i>Parvularcula</i> sp. clone REG_R2P5_D3 (FJ933364)	99/95	-	-	-	-	-	-	3	2	-
Uncultured bacterium clone RRB034 (EU284540) / Uncultured <i>Methylocapsa</i> sp. clone CL2.C17 (FM175364)	99/98	-	-	-	-	-	-	-	2	-
Uncultured α -proteobacterium clone Br_42 (26) (HM534221) / Uncultured Rhizobiales clone A6_100 (AM940599)	99/98	2	-	-	-	-	-	-	-	-
Proteobacteria; α-Proteobacteria; Sphingomonadales; Sphingomonadaceae										
Uncultured bacterium clone DUP6D09 (HQ178928) / <i>Sphingomonas</i> sp. clone DOL79 (HM224434) / <i>Sphingopyxis</i> sp. clone 03SUJ8 (HQ436495)	99/98/98	-	-	-	-	-	-	4	-	2
Uncultured bacterium clone L2-10 (HM460661) / <i>Sphingopyxis</i> sp. clone 03SUJ8 (HQ436495) / <i>Sphingomonas</i> sp. P1S1 (AJ620198)	98/98/98	-	-	-	-	-	-	-	3	-

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Proteobacteria; α-Proteobacteria; Miscellaneous										
Uncultured α -proteobacterium clone FTL75 (AF529112) / <i>Caedibacter caryophila</i> BGD19 (AJ238683)	98/92	-	-	-	-	-	2	-	1	-
Uncultured bacterium clone MBR-30_LF_AS77 (FM200963) / Uncultured α -proteobacterium clone PSB011.C21_D24 (GU300176)	96/94	3	-	1	-	-	-	-	-	-
Uncultured bacterium clone L65 (EU834774) / α -proteobacterium ASRW6-4 (AB299782)	98/97	-	-	-	-	-	2	-	-	-
Proteobacteria; β-Proteobacteria; Burkholderiales; Burkholderiaceae										
Uncultured bacterium clone 298EGG26 (FJ593728) / <i>Ralstonia solanacearum</i> strain LYP (HQ1766322)	99/98	4	-	-	-	-	1	-	-	2
Uncultured bacterium clone IS-56 (GQ339155) / Uncultured Burkholderiaceae bacterium clone Elev_16S_775 (EF019630)	99/98	-	1	-	-	-	1	-	-	-
Proteobacteria; β-Proteobacteria; Burkholderiales; Comamonadaceae										
Uncultured bacterium clone cs-62 (HQ115556) / <i>Hydrogenophaga defluvii</i> strain hyd1 (AM942546)	99/98	-	2	1	7	-	-	-	-	-
Uncultured Comamonadaceae bacterium clone GC1m-3-13 (EU641295) / <i>Hydrogenophaga caeni</i> strain EMB71 (DQ372983)	99/99	-	-	-	4	3	6	-	-	1
Uncultured Comamonadaceae bacterium clone GC12m-3-55 (EU641050) / <i>Hydrogenophaga</i> sp. EMB75 (DQ413154)	97/96	-	-	-	-	2	-	-	-	-
Uncultured bacterium clone MA101C05 (FJ694402) / Uncultured <i>Rhodoferrax</i> sp. clone CL4.E36 (FM175916)	99/99	-	2	-	1	7	13	-	-	-
<i>Rhodoferrax</i> sp. Asd M2A1 (FM955857)	97	-	-	-	-	-	1	-	-	1
Uncultured Burkholderiales bacterium clone con40-3 (FJ517721) / <i>Curvibacter</i> sp. HME6817 (HQ167730) / Uncultured <i>Rhodoferrax</i> sp. Clone spike_1 (HQ1111491)	99/99/99	-	-	-	2	-	-	-	-	-

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Bacterium IRO1 (AY928215) / <i>Curvibacter</i> sp. IPC7 (HQ108174) / Uncultured <i>Rhodofera</i> sp. clone AL_35 (HQ008554)	99/98/98	-	-	-	-	1	2	-	-	-
Uncultured β -proteobacterium clone LNA_100608-96 (HM037382) / <i>Curvibacter</i> sp. ATCC 700892 (HM357758) / <i>Aquamonas fontana</i> strain AQ11 (AB120965)	99/99/99	-	-	-	-	1	2	-	-	-
Glacier bacterium FXS1 (AY315177) / Uncultured <i>Polaromonas</i> sp. clone IC3028 (HQ595202)	99/99	-	-	-	-	3	1	-	-	-
Uncultured bacterium clone EpiUMB27 (FJ849279) / Uncultured <i>Polaromonas</i> sp. clone B6_50; B6_25 (AM940813; AM940793)	97/96	-	-	-	-	4	1	-	-	-
Uncultured β -proteobacterium clone 0G8 (EU499554) / Uncultured <i>Comamonas</i> sp. R15-4; R15-54 clone (JF808871; JF808872)	99/99/99	-	-	-	-	1	1	-	-	-
Uncultured <i>Ottowia</i> sp. clone CL2.C46 (FM175385)	97	-	-	-	-	1	1	-	2	-
Uncultured bacterium clone pLW-2 (DQ066963) / <i>Leptothrix</i> <i>ginsengisoli</i> strain OTSz_A_210 (FM886840)	99/98	-	-	-	-	-	1	-	-	1
Proteobacteria; β-Proteobacteria; Burkholderiales; Oxalobacteraceae & unclassified Burkholderiales										
Uncultured β -proteobacterium clone A9 (AJ867902) / <i>Undibacterium</i> sp. HME6890 (HQ665007)	99/98	-	-	-	-	-	-	2	-	-
Uncultured bacterium clone 1-60 (HM163102) / Uncultured Oxalobacteraceae bacterium clone AMJD9 (AM934886)	98/95	-	-	3	-	-	-	-	-	-
Uncultured bacterium clone MBR-8_HF_AS40 (FM201235) / Oxalobacteraceae NR164 (DQ520826)	99/91	-	-	-	-	-	2	-	-	-
Proteobacteria; β-Proteobacteria; Rhodocyclales; Rhodocyclaceae										
Uncultured β -proteobacterium clone GASP-MA4W2_B12 (EF664309) / Uncultured Rhodocyclaceae bacterium clone Amb_16S_1454 (EF018945) / Uncultured Burkholderiaceae bacterium clone Amb_16S_495 (EF018160)	100/98/98	1	-	1	-	-	-	-	-	1

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Uncultured Rhodocyclaceae bacterium clone B-UP-T0_OTU10 (FM204987) / <i>Dechloromonas aromatica</i> RCB (CP000089) / <i>Ferribacterium limneticum</i> strain cda-1 (Y17060)	99/99/99	-	-	-	-	7	1	-	-	-
Uncultured bacterium clone DUR_11 (EF565162) / <i>Azospira restricta</i> strain SUA2 (DQ974114)	98/97	-	-	-	-	-	3	-	-	-
Proteobacteria; β-Proteobacteria; Miscellaneous										
Uncultured bacterium clone Field-198 (EU499698) / Uncultured <i>Methylibium</i> sp. clone GASP-KC2W1_E01 (EU299892)	98/98	2	-	-	-	-	-	-	-	-
Uncultured <i>Thiobacillus</i> sp. clone 25-91-ArvAB (AB425068)	99	-	2	-	-	-	-	-	-	-
Proteobacteria; γ-Proteobacteria; Alteromonadales; Alteromonadaceae										
Uncultured bacterium clone 0702002P16BMCFH8850 (GU369524) / <i>Microbulbifer</i> sp. Th/B/38 (AY224196)	98/88	-	-	-	-	-	-	5	-	1
Proteobacteria; γ-Proteobacteria; Legionellales; Coxiellaceae & Legionellaceae										
Uncultured bacterium clone Toolik_Jun2005_shruborg_73 (DQ509966) / <i>Legionella oakridgensis</i> (AB571648)	95/86	-	-	1	-	-	-	-	-	1
Proteobacteria; γ-Proteobacteria; Pseudomonadales; Pseudomonadaceae										
Uncultured bacterium clone ncd266b09c1 (HM270455) / <i>Cellvibrio</i> sp. KY-CR-1 (EF692633)	96/95	-	16	1	28	-	-	-	-	-
Uncultured bacterium clone lmu-b143 (JN023132) / <i>Cellvibrio</i> sp. KY- YJ-3 (EF692636)	96/96	-	7	-	16	-	1	-	-	-
Uncultured bacterium clone StLS75 (EU219027) / Uncultured <i>Cellvibrio</i> sp. clone Plot18-B10 (FJ889293)	98/96	-	-	-	-	-	-	-	-	2

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Proteobacteria; γ-Proteobacteria; Thiotrichales; Thiostreptaceae										
Uncultured bacterium clone V201-103 (HQ114118) / Uncultured <i>Beggiatoa</i> sp. clone CL5.H29 (FM176297)	99/95	-	1	1	-	-	-	-	-	-
Uncultured bacterium clone GP27685f11 (HM445483) / Uncultured <i>Beggiatoa</i> sp. clone CL5.H29 (FM176297)	99/96	-	-	1	-	1	-	-	-	-
Uncultured bacterium clone cs-12 (HQ115542) / <i>Thiothrix</i> <i>fructosivorans</i> strain Q (GU269554)	99/99	-	-	-	-	12	7	-	-	-
Proteobacteria; γ-Proteobacteria; Xanthomonadales; Xanthomonadaceae										
Uncultured bacterium clone AK1DE2_11A (GQ397024) / Uncultured <i>Lysobacter</i> sp. clone MKT-G24 (AM990045)	100/98	-	-	-	-	-	-	2	7	-
Uncultured γ -proteobacterium clone QEDN8DD06 (CU926385) / Uncultured Xanthomonadaceae bacterium clone GASP-MA4S2_C04 (EF664034)	99/96	-	-	-	-	-	-	1	1	-
Uncultured bacterium clone DP3.5.36 (FJ612198) / Uncultured <i>Dyella</i> sp. clone CL6-7.L193 (FM176830)	99/99	-	-	-	-	-	-	-	2	-
Proteobacteria; γ-Proteobacteria; Miscellaneous										
Uncultured bacterium clone SS_LKC22_UB116 (AM490696) / Uncultured γ -proteobacterium clone SM1D02 (AF445671)	87/85	-	-	5	-	-	-	-	-	-
Uncultured bacterium clone 6134Rh (GU201120) / Uncultured γ - proteobacterium clone LHCHWD146 (GU016234)	97/96	-	-	-	-	-	-	2	-	-
Proteobacteria; δ-Proteobacteria; Myxococcales										
Uncultured Polyangiaceae bacterium clone cuticle_16 (HQ111161)	98	-	-	-	-	1	-	-	-	1

Table S3. Continued

Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	No. of clones per OTU in biofilm sample								
		BBL t82	BMAL t24	BAP t86	BMA+ AP t24	BAC t86	BAC+ AP t86	BGEL t88	BCAS t82	BLAM t82
Proteobacteria; δ-Proteobacteria; Miscellaneous										
Uncultured bacterium clone ORSFAM_h07 (EF393289) / Uncultured <i>Desulfofustis</i> sp. clone CL5.H8 (FM176284)	96/96	-	-	2	-	-	-	-	-	-
Unidentified Bacteria; Miscellaneous										
Uncultured bacterium clone Crozet_s_864 (FM214345)	91	-	-	2	-	-	-	-	-	-
Uncultured organism clone Jsed45 (GQ994853) / Uncultured bacterium clone MBIOS-06 (EU369122)	87/85	-	-	3	-	-	-	-	-	5

Table S4. Identity of dominant bacterial strains isolated from biofilms formed with tap water with added maltose (BMAL), amylopectin (BAP), maltose & amylopectin (BMA+AP), acetate (BAC), acetate and amylopectin (BAC+AP), gelatin (BGEL), caseinate (BCAS), or laminarin (BLAM) at $\mu\text{g C l}^{-1}$ levels. Symbols: +, isolated from biofilm; -, not isolated from biofilm.

Strain no.	Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	Biofilm					
			BMAL	BAP	BMA+ AP	BGEL	BCAS	BLAM
Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae								
BF-BP1	<i>Flavobacterium</i> sp. HME6012 (HM149211)	99	+	+	+	-	-	-
BF-BP2	Uncultured <i>Flavobacterium</i> sp. clone L-1 (JF703584)	99	-	-	-	+	+	-
BF-BP3	Uncultured bacterium clone ncd555e08c1 (HM278469) / <i>Flavobacterium columnare</i> strain Ga-6-93 (AY842901)	99/98	-	-	-	-	+	-
BF-BP4	<i>Flavobacterium</i> sp. HME6133 (HM776988) / <i>Flavobacterium aquatile</i> NBRC 15052 (AB517711)	98/97	-	-	-	-	+	-
BF-BP5	<i>Flavobacterium</i> sp. CL1.152 (AM934685) / <i>Flavobacterium</i> <i>succinicans</i> strain DSM 4003 (AM230493)	99	-	-	-	-	-	+
BF-BP6	<i>Flavobacterium</i> sp. R-36233 (FR682719)	98	-	-	-	-	-	+
Proteobacteria; α-Proteobacteria; Caulobacterales; Caulobacteraceae								
BF-BP7	<i>Brevundimonas</i> sp. strain AKB-20080JO103 (AM988997)	99	-	-	-	+	+	-
BF-BP8	<i>Caulobacter</i> sp. tsz32 (FJ979860) / <i>Brevundimonas</i> sp. strain AKB- 20080JO103 (AM988997)	99/99	-	-	-	+	-	-
Proteobacteria; β-Proteobacteria; Burkholderiales; Oxalobacteraceae								
BF-BP9	Uncultured β -proteobacterium clone A9 (AJ867902) / <i>Undibacterium</i> sp. HME6890 (HQ665007)	99/98	-	-	-	+	-	-

Table S4. Continued

Strain no.	Organism(s) with highest similarity (GenBank accession no.) classified according to lineage	Similarity (%)	Biofilm					
			BMAL	BAP	BMA+ AP	BGEL	BCAS	BLAM
Proteobacteria; γ-Proteobacteria; Pseudomonadales; Pseudomonadaceae								
BF-BP10	Uncultured bacterium clone ncd266b09c1 (HM270455) / <i>Cellvibrio</i> sp. KY-CR-1 (EF692633)	96/95	+	-	+	-	-	-
BF-BP11	Uncultured bacterium clone lmu-b143 (JN023132) / <i>Cellvibrio</i> sp. KY-YJ-3 (EF692636)	96/96	+	-	+	-	-	-
Proteobacteria; γ-Proteobacteria; Xanthomonadales; Xanthomonadaceae								
BF-BP12	Uncultured bacterium clone AK1DE2_11A (GQ397024) / Uncultured <i>Lysobacter</i> sp. clone MKT-G24 (AM990045)	100/98	-	-	-	+	-	-
BF-BP13	Uncultured bacterium clone ncd2676d01c1 (JF232474) / Uncultured <i>Xanthomonas</i> sp. clone AUVE_10A09 (EF651335)	100/100	-	-	-	-	+	-
BF-BP14	<i>Lysobacter</i> sp. RB64 (FJ898299) / <i>Lysobacter daecheongensis</i> (AB249682)	99/99	-	-	-	+	+	-