

SUPPLEMENTARY INFORMATION

Supporting and additional information on our results. This file contains images and data to give a better impression of our experiments and findings as well as the results of the accompanying and evaluating experiments.

Journal name: Scientific Reports

Article title: Comparative analysis of the influence of BpfA and BpfG on biofilm development and current density in *Shewanella oneidensis* under oxic, fumarate- and anode-respiring conditions

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28 **S1 Strains used in this study.**

Strain number	Strain	Source
JG918	<i>S. oneidensis</i> MR-1 $\Delta\lambda$	Bursac <i>et al.</i> ¹
JG1738	<i>S. oneidensis</i> MR-1 $\Delta\lambda$ <i>bpfG</i> (C116S)	This study
JG1739	<i>S. oneidensis</i> MR-1 $\Delta\lambda$ <i>bpfA</i> (P_{cymA})	This study

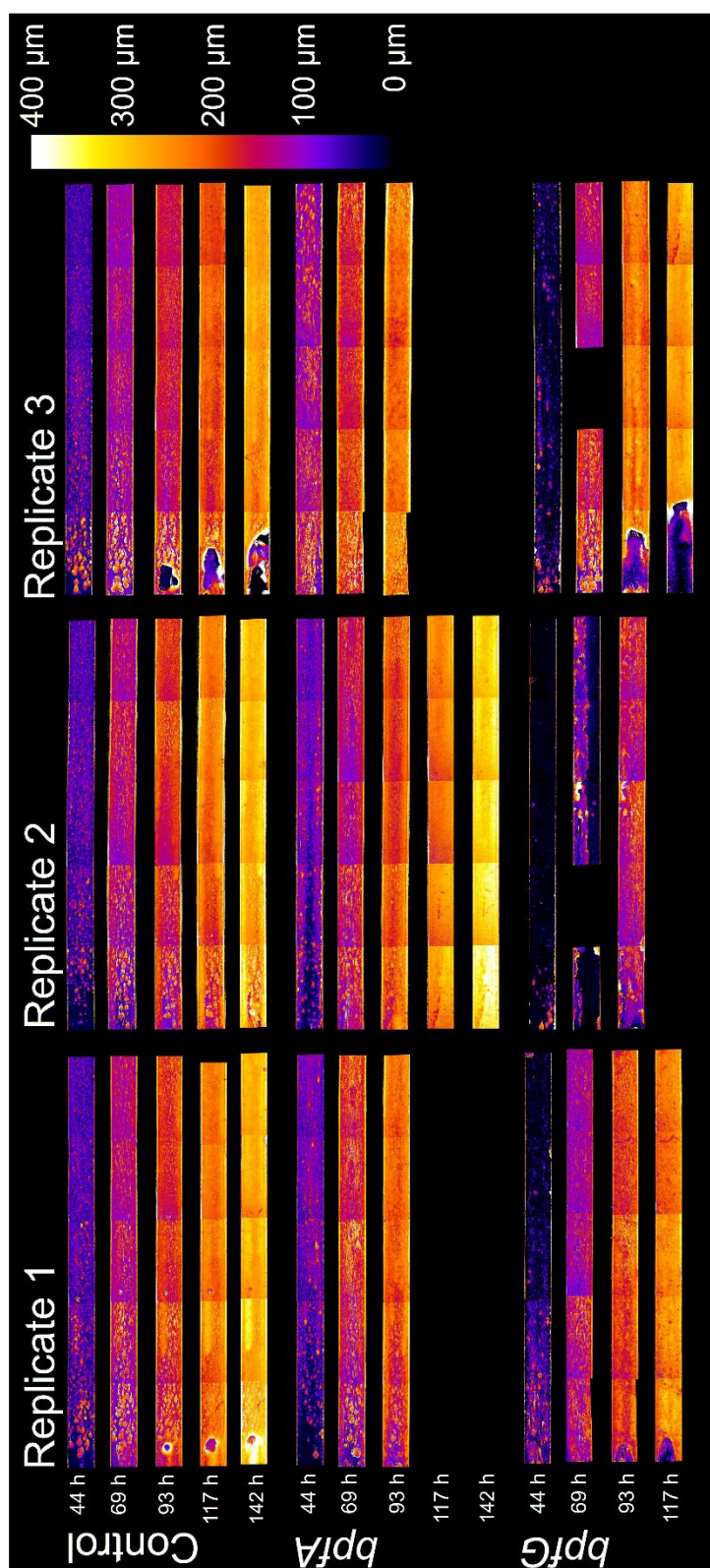
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30 All strains used in this study can be found in Table S1.

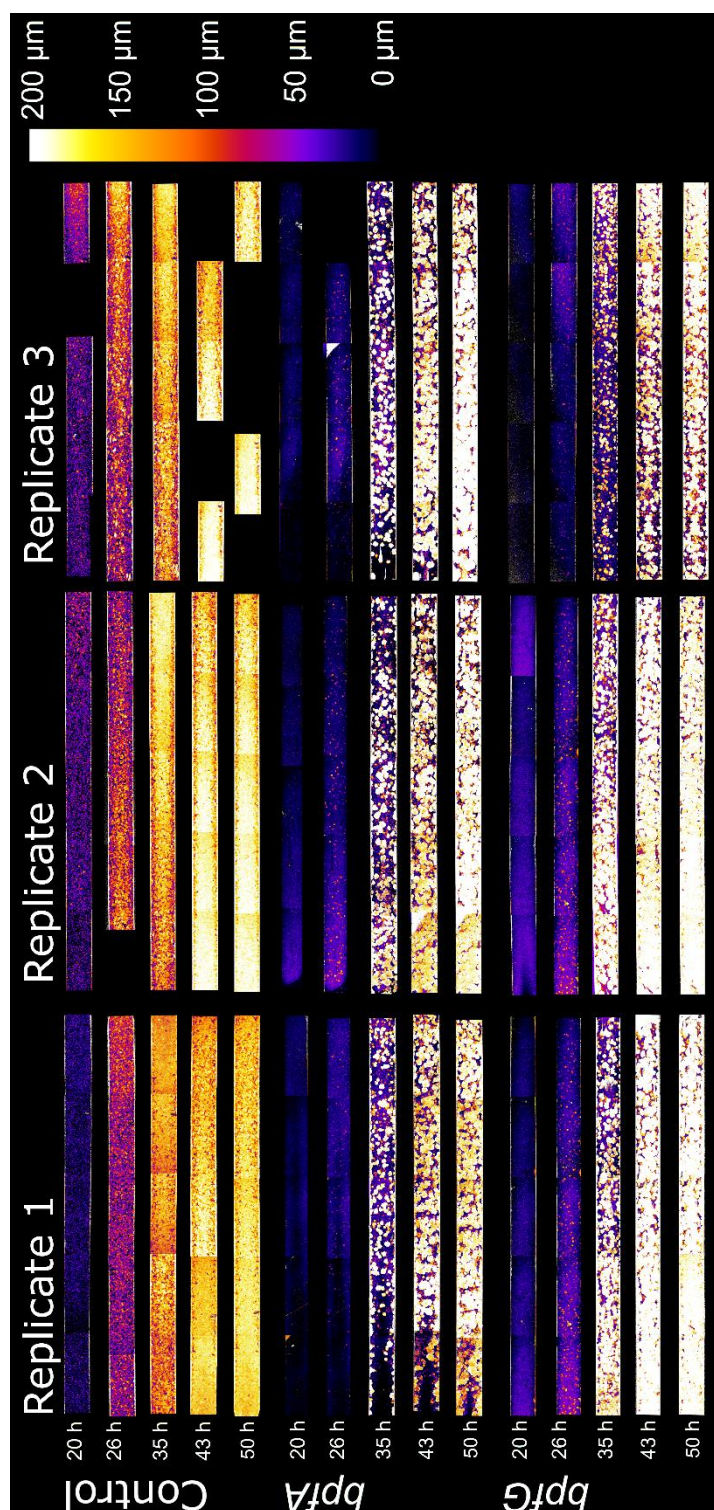
31 To construct the marker-less mutants of *S. oneidensis*, primers as listed in Table S2 were used.

32 **S2 Primers used in this study.**

#	Name	Sequence (5'-3')	Purpose
1	Primer_3474_bpfG_500up_F	AGTGCCAAGCTTGCATGCCTGCAGG TCGAGTGTCAATTGAAGCCCG	Gibson ligation: $\Delta bpfG$
2	Primer_3475_bpfG_500up_R	TCTGTCTAAACAGTGTCTCTTGGG TGAGCCTACTCTATTGGTGT	
3	Primer_3476_bpfG_500down_F	ACACCAATTAACACCAATAGAGTA GGCTCACCAAGATGACACT	
4	Primer_3477_bpfG_500down_R	TACGAATTCGAGCTCGGTACCCGGG GATCGATGGCGACCAAAAAC	
5	Primer_3490_bpfA_PromoterDeletion_500Up_F	AGTGCCAAGCTTGCATGCCTGCAGG TCGAACACCATGGGCTTATG	Gibson ligation: $\Delta bpfA$
6	Primer_3491_bpfA_PromoterDeletion_500Up_R	TAATTACCGATCCCATTAAGAAAC ATATTTATAACAAATTATTC	
7	Primer_3492_bpfA_PromoterDeletion_500Down_F	ATAATTTGTTATAAATATGTTTCTT TAATGGGATCGGTAATTACA	
8	Primer_3493_bpfA_PromoterDeletion_500Down_R	AGCTCGGTACCCGGGGATCGTATTG CTGTCGTTGGCTAGTATAGAAGGTG	
9	Primer_3502_bpfGC116S_R	TGAATCTCCACCATTGAC	Point mutation <i>bpfG</i> (C116S)
10	Primer_3503_bpfGC116S_F2	TCAGAAGATTTTTCAATCGC	
11	Primer_3494_bpfA500Up_CymAP_R	AATGATTAAGTACCACCTTAATAAA GAAACATATTTATAACAAATTATTC	Gibson ligation: P_{cymA}
12	Primer_3495_CymAPromoter_F	TTTGTTATAAATATGTTTCTTTATT AAGGTGGTACTTAATCATTT	
13	Primer_3496_500Down_CymAP_F	TTTGACAATATTTTGGAGATAGAG TAATGGGATCGGTAATTACA	
14	Primer_3497_CymAPromoter_R	TTGATGTAATTACCGATCCCATTTAC TCTATCTCCAAAAATATTGT	
15	Primer_3498_bpfAP_Deletion_Test_R	TCGCTATCATTGTTGAGC	Test primers: ΔP_{bpfA}
16	Primer_3499_bpfAP_Deletion_Test_F	TACAAACAGCGAGTCTC	
17	Primer_3500_bpfG_Deletion_Test_F	TCAATATTGACGAGATGACC	Test primers: $\Delta bpfG$
18	Primer_3501_bpfG_Deletion_Test_R	CATGTTGAGATAGGGCA	
19	Primer_3504_bpfG_C116S_Test_F	TCGATGACATAAAATTATGGG	Test primers: <i>bpfG</i> (C116S)
20	Primer_3505_bpfG_C116S_Test_R	GTGATCCGCATCTTATCT	
21	Primer_0041_pMQ150for	CTGGCGAAAGGGGGATGTG	Test primers: pMQ test
22	Primer_0042_pMQ150rev	CATTAGGCACCCAGGCTTTAC	



S3 Height maps of biofilms that were cultivated using microfluidic continuous flow reactors with lactate as electron donor. Cultivation was carried out under oxic conditions and the retention time was 5.23 min at a flow rate of 4 mL h⁻¹. After 44, 69, 93, 117 and 142 h five images were taken of each cultivation channel, converted into color-coded height maps by processing and combined into one image by stitching. The cultivation channels shown have a size of 3 x 45 mm and correspond to a top view of the biofilm, with a flow direction from left to right.



S4 Height maps of biofilms that were cultivated using microfluidic continuous flow reactors with lactate as electron donor and fumarate as electron acceptor. Cultivation was carried out under anoxic conditions and the retention time was 5.23 min at a flow rate of 4 mL h⁻¹. After 20, 26, 35, 42 and 50 h five images were taken of each cultivation channel, converted into color-coded height maps by processing and combined into one image by stitching. The cultivation channels shown have a size of 3 x 45 mm and correspond to a top view of the biofilm, with a flow direction from left to right.

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88 Biofilms were studied using a newly developed microfluidic cultivation platform operating under
 89 laminar flow conditions ². To enable (semi-)automated OCT imaging, a gantry robot (DLE-RG-0003,
 90 igus® GmbH, Cologne, Germany) was used to capture images. Development of the biofilm could be
 91 monitored throughout the experiment with high reproducibility and low workload. OCT images were
 92 acquired with a Ganymede™ spectral domain system (GAN611C1-SP1, Thorlabs GmbH, Dachau,
 93 Germany) and analyzed. To describe and quantify the biofilms, the acquired datasets were
 94 processed in several consecutive steps. Tables S5-7 contain the mean values and standard deviations
 95 of Figure 2. Tables S8-10 contain the mean values and standard deviations of Figure 4. Furthermore,

significant differences to the control strain are marked by colored boxes. Additionally, Figure S3 shows height maps of the biofilms over time as color-coded maps for all three replicates of Figure 1. Figure S4 shows height maps of the biofilms over time as color-coded maps for all three replicates of Figure 3.

S5 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biovolume. Cultivation was conducted in microfluidic flow cells under oxic conditions with lactate as electron donor and atmospheric oxygen as electron acceptor. Biovolume in mm³ cm⁻² is given over time. Mean values and standard deviations were calculated from individual replicates (n = 3). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. Colored boxes indicate significant differences to the control strain (unpaired t-tests: green = p < 0.01). An asterisk (*) indicates, that there were two reactors remaining and ** indicates that only one reactor remained.

		44.5 h	70.3 h	93.1 h	117.1 h	143.2 h
JG918	$\Delta\lambda$	6,0 ± 0,3	11,3 ± 0,5	15,4 ± 1,1	19,4 ± 1,5	33,0 ± 2,7
JG1739	$\Delta\lambda$ <i>bpfA</i> (P _{cymA})	7,4 ± 2,5	13,2 ± 2,3	19,1 ± 1,4	28,8**	39,7**
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	1,8 ± 0,8	9,2 ± 3,8	15,8 ± 3,2	18,5* ± 0,9	

S6 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biofilm height. Cultivation was conducted in microfluidic flow cells under oxic conditions with lactate as electron donor and atmospheric oxygen as electron acceptor. Biovolume in µm is given over time. Mean values and standard deviations were calculated from individual replicates (n = 3). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. Colored boxes indicate significant differences to the control strain (unpaired t-tests: green = p < 0.01; red = p < 0.05). An asterisk (*) indicates, that there were two reactors remaining and ** indicates that only one reactor remained.

		44.5 h	70.3 h	93.1 h	117.1 h	143.2 h
JG918	$\Delta\lambda$	79,7 ± 5,4	145,6 ± 5,1	175,4 ± 10,6	211,2 ± 14,4	290,7 ± 16,8
JG1739	$\Delta\lambda$ <i>bpfA</i> (P _{cymA})	96,1 ± 41,4	168,0 ± 15,7	214,6 ± 8,6	261,2**	345,8**
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	27,0 ± 14,3	124,8 ± 37,7	185,1 ± 27,7	197,7* ± 28,7	

S7 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biofilm porosity. Cultivation was conducted in microfluidic flow cells under oxic conditions with lactate as electron donor and atmospheric oxygen as electron acceptor. Biofilm porosity in % is given over time. Mean values and standard deviations were calculated from individual replicates (n = 3). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. An asterisk (*) indicates, that there were two reactors remaining and ** indicates that only one reactor remained.

		44.5 h	70.3 h	93.1 h	117.1 h	143.2 h
JG918	$\Delta\lambda$	23,9 ± 1,5	21,7 ± 0,8	12,4 ± 1,2	8,4 ± 1,2	11,7 ± 16,6
JG1739	$\Delta\lambda$ <i>bpfA</i> (P _{cymA})	28,4 ± 2,4	22,1 ± 8,9	11,5* ± 5,3		
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	23,8 ± 7,9	29,5 ± 12,8	16,1 ± 5,2	18,9* ± 4,4	

S8 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biofilm height. Cultivation was conducted in microfluidic flow cells under anoxic conditions with lactate as electron donor and fumarate as electron acceptor. Biovolume in μm is given over time. Mean values and standard deviations were calculated from individual replicates ($n = 3$). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. Colored boxes indicate significant differences to the control strain (unpaired t-tests: green = $p < 0.01$; red = $p < 0.05$).

		18.17 h	23.7 h	33.4 h	40.7 h	47.6 h
JG918	$\Delta\lambda$	27.3 $\pm$ 9.9	81.1 $\pm$ 13.9	141.7 $\pm$ 10.4	175.0 $\pm$ 14.5	180.3 $\pm$ 14.6
JG1739	$\Delta\lambda$ <i>bpfA</i> (P_{cymA})	10.2 $\pm$ 2.7	18.8 $\pm$ 4.5	114.3 $\pm$ 24.3	161.4 $\pm$ 19.3	185.6 $\pm$ 27.1
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	17.6 $\pm$ 8.6	31.4 $\pm$ 10.7	199.4 $\pm$ 16.0	236.9 $\pm$ 43.6	314.9 $\pm$ 78.6

S9 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biovolume. Cultivation was conducted in microfluidic flow cells under anoxic conditions with lactate as electron donor and fumarate as electron acceptor. Biovolume in $\text{mm}^3 \text{cm}^{-2}$ is given over time. Mean values and standard deviations were calculated from individual replicates ($n = 3$). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. Colored boxes indicate significant differences to the control strain (unpaired t-tests: green = $p < 0.01$; red = $p < 0.05$).

		18.17 h	23.7 h	33.4 h	40.7 h	47.6 h
JG918	$\Delta\lambda$	2.4 $\pm$ 0.9	7.6 $\pm$ 1.8	11.5 $\pm$ 1.2	15.5 $\pm$ 1.4	16.1 $\pm$ 1.4
JG1739	$\Delta\lambda$ <i>bpfA</i> (P_{cymA})	1.0 $\pm$ 0.2	1.7 $\pm$ 0.4	7.2 $\pm$ 1.2	9.5 $\pm$ 0.9	11.8 $\pm$ 1.3
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	1.8 $\pm$ 0.6	3.4 $\pm$ 0.4	21.1 $\pm$ 12.0	22.2 $\pm$ 4.3	22.6 $\pm$ 4.7

S10 Impact of *bpfG* (C116S) as well as overexpression of *bpfA* on biofilm porosity. Cultivation was conducted in microfluidic flow cells under anoxic conditions with lactate as electron donor and fumarate as electron acceptor. Biofilm porosity in % is given over time. Mean values and standard deviations were calculated from individual replicates ($n = 3$). Significant differences of the *bpfA* and *bpfG* mutants were calculated in comparison to JG918. Colored boxes indicate significant differences to the control strain (unpaired t-tests: green = $p < 0.01$; red = $p < 0.05$).

		18.17 h	23.7 h	33.4 h	40.7 h	47.6 h
JG918	$\Delta\lambda$	11.1 $\pm$ 2.7	7.8 $\pm$ 8.1	19.2 $\pm$ 3.8	11.9 $\pm$ 0.9	10.9 $\pm$ 0.4
JG1739	$\Delta\lambda$ <i>bpfA</i> (P_{cymA})	8.6 $\pm$ 0.4	6.9 $\pm$ 3.1	35.3 $\pm$ 6.2	41.0 $\pm$ 2.7	24.3 $\pm$ 17.3
JG1738	$\Delta\lambda$ <i>bpfG</i> (C116S)	6.6 $\pm$ 0.1	7.7 $\pm$ 2.0	41.0 $\pm$ 0.0	27.5 $\pm$ 3.0	26.4 $\pm$ 1.1

References

- Bursac, T., Gralnick, J. A. & Gescher, J. Acetoin production via unbalanced fermentation in *Shewanella oneidensis*. *Biotechnol Bioeng* **114**, 1283–1289 (2017).
- Klein, E. M., Wurst, R., Rehnland, D. & Gescher, J. Elucidating the Development of Cooperative Anode-Biofilm-Structures. *Biofilm* **7**, 100193 (2024).