

Supplementary information of:

The role of extracellular polymeric substances of fungal biofilms in mineral attachment and weathering

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1. Supplementary Methods

Method description A: Analysis protocol of X-ray photoelectron spectroscopy

Sample analysis was performed at the Bundesanstalt für Materialforschung und -prüfung, Germany using a Kratos AXIS Ultra DLD with a monochromatic Al anode ($E=1486.6$ eV) with a power of 150 W. This was done in combination with a neutralizer to compensate for charging effects. The electron binding energies were referenced to the aliphatic C1s peak at 285.0 eV. The quantification was done with Unifit 2021 using the peak areas, element-specific Scofield factors ¹ and the spectrometer-dependent transmission function. The peak areas were obtained after subtracting a modified Tougaard background. All samples were analysed in the hybrid mode with a pass energy of 80 eV for survey spectra and 20 eV for high-resolution spectra.

Method description B: Extended set-up and procedure of dissolution experiments

Dissolution experiments were carried out in batch reactor set-ups to quantify the abiotic dissolution of olivine and the effect of both the wild type fungus and its pigment mutants on olivine dissolution. The batch experiment is comprised of a 500 ml polycarbonate reactor flask (Corning) filled with olivine powder, nutrient medium solution and growing fungal biomass. The medium remained in the reactor and the dissolution rate was quantified based on the temporal evolution of the concentration of accumulating released metals. The air phase of the reactors was in contact with the atmosphere as the batch reactors were closed with caps with built-in air filters (Corning). Oxygenated conditions are therefore assumed. To avoid chemical and microbiological contamination, all flasks were cleaned by washing them once with 1M HCl (for one day), once with 1M HNO₃ (for one day), rinsing them four times with MilliQ water, and then autoclaving them. Before autoclaving, 4 (± 0.001) g of cleaned olivine was added to each batch reactor. After autoclaving the batch reactors were filled with 400 (± 0.5) ml of the sterile CNPS nutrient solution. Biotic experiments were inoculated with a hemocytometer-quantified aliquot of the *K. petricola* culture (WT or mutant).

From the reactors, 5.0 ± 0.1 ml samples were taken at regular time points with a volumetric pipet. To keep the solid-liquid ratio constant, sampling was done whilst manually shaking the flasks keeping all solids in suspension. Therefore, each sampling event decreased the mass of olivine in the reactor by ca. 1.25% (i.e., 5 ml sample / 400 ml total volume). These samples were filtered (0.22 μm); 1 ml was used for pH measurement and 4 ml was acidified with HNO₃ (Merck, suprapur[®] grade) to a pH <2 and stored in acid-cleaned, polypropylene falcon tubes at 4°C prior to dissolved element analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES). At the end of experimental runs, the dried biomass was weighed, and samples were taken for SEM and grade of attachment analyses.

Method description C: Inductively coupled plasma optical emission spectroscopy: sample preparation, analysis protocol, quality control standards and medium blanks

To determine the elemental concentrations in the medium samples, they were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 720-ES). Sample preparation and analyses were performed in the Helmholtz Laboratory for the Geochemistry of the Earth Surface (HELGES) at GFZ Potsdam ². The analytical procedure and instrumentation are described in Schuessler et al. ³ and was followed with minor adaptations. First, medium solution samples were filtered (<0.2 µm), acidified with HNO₃ (Merck, suprapur® grade) to a pH <2 and stored in acid cleaned, polypropylene falcon tubes at 4°C. One day before analysis, samples were diluted 1:2 or 1:3 with quartz distilled HNO₃ containing Cs as ionisation buffer to achieve matrix matching to the calibration standards. In and Sc were added as internal recovery standards. The final concentrations in diluted sample solutions were 0.3 M HNO₃, 1000 ppm Cs, 1.3 ppm In and 1.4 ppm Sc, matching the composition of the ICP-OES calibration standards. Linear calibration range was verified by measurements of 4 to 8 calibration standard solutions, covering the range of element concentrations in the diluted samples. Calibration standards were prepared from multi element ICP standards (Merck CertiPUR, traceable to NIST reference materials) diluted in 0.3 M HNO₃ containing ca. 1 mg g⁻¹ Cs prepared from CsNO₃ salt (99.999% purity). Uncertainties from calibration were typically below 4% relative. The detection limit was 0.1169 ± 0.0015 ppm for Mg, 0.0619 ± 0.0012 for Si and 0.0055 ± 0.0002 for Fe. In each analytical session, blanks and quality control standards (QCs) (Multi-element ICP-MS standard mixed with the medium solution,) were analysed after every 10th sample. The analytical uncertainty used to interpret the sample results is conservatively estimated based on the accuracy and the precision of the repeated measurement of these QCs. Analytical results of these QCs are reported in Supplementary Table 1 and their element concentrations are given in Supplementary Table 2. To elucidate the element contributions from the pure medium to the measured Mg concentrations in the samples, medium blanks were analysed. Medium blanks constitute samples taken from the medium solution used in the batch reactors. The Mg, Si and Fe concentration values of the medium blanks (i.e., 0.018 ± 0.019 ppm, 0.275 ± 0.017 ppm, and 0.004 ± 0.002 ppm, respectively) were subtracted from the respective Mg, Si and Fe concentrations from the batch samples.

2. Supplementary Tables

Supplementary Table 1: Results of quality control standards analysed by ICP-OES during analysis of samples. All analyses were performed in the Helmholtz Laboratory for the Geochemistry of the Earth Surface (HELGES) at GFZ Potsdam. A QC standard stock solution was prepared in dilute HNO₃. QC standard 1 contained the CNPS medium components (at the same final concentrations as used in the dissolution experiments) mixed with several ICP single-element standard solutions (Merck CertiPUR). Before analysis, all standard stock solutions were diluted ~3 times with 0.45M HNO₃ containing ca. 1500 ppm Cs, ca. 2 ppm In and ca. 2 ppm Sc. Note that concentration values are corrected for dilution. The diluted concentrations of the QC standards prepared from stock before each analytical session are given in Supplementary Table 2. The mean results of n replicates measurements are given together with 2 standard deviations (2SD). The measured deviation from the reference value is a quantitative indication of accuracy.

Name	Mg ($\mu\text{g g}^{-1}$)	Si ($\mu\text{g g}^{-1}$)	Fe ($\mu\text{g g}^{-1}$)	In ($\mu\text{g g}^{-1}$)	Sc ($\mu\text{g g}^{-1}$)
QC Standard 1 ^a : (Merck CertiPUR ICP standards + CNPS medium solution) reference value	3.74	3.83	3.74	3.98	3.98
measured value (n=12)	3.67	3.74	3.68	3.87	3.98
2SD	0.03	0.03	0.04	0.04	0.04
Measured deviation from reference value (%)	-1.9%	-2.3%	-1.8%	-3.0%	0.0%

Supplementary Table 2: Element concentrations ($\mu\text{g g}^{-1}$) in the diluted QC standard (with CNPS medium addition).

Element	concentration ($\mu\text{g g}^{-1}$) in diluted QC standard 1
Al	1.26
C	517
Ca	1.27
Cr	1.26
Cu	1.25
Fe	1.25
In	1.33
K	4.31
Mg	1.25
Mn	1.24
Na	4.38
Ni	1.25
P	1.71
S	121
Sc	1.33
Si	1.28
Sr	1.24
Ti	1.29
Cs	1008
HNO ₃ (mol l ⁻¹)	0.34

Supplementary Table 3: Overview of testing the lectins library of Thomas Neu on the EPS of the *K. petricola* WT and/or pigment-free $\Delta pks1/\Delta phd1$ mutant (following Neu and Kuhlicke ⁴). A presence of a fluorescent signal is indicated with a “+”, the absence - by a “-”.

Lectin	AAL	AIA	AMA	ASA	BAN	BPA	Calsepa	CSA	DBA	DSA	ECA	GNA	GS-I	HHA
WT	+	-	-	-	+	-	-	+	-	-	-	+	-	+
$\Delta pks1/\Delta phd1$	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Lectin	HMA	MNA-G	MPA	NPA	PMA	PSA	PTA	RCA	SNA	STA	UDA	UEA-I	WFA	WGA
WT	-	-	+	+	+	+	-	+	-	-	+	-	-	+
$\Delta pks1/\Delta phd1$	+	+	+	-	-	+	+	+	+	+	+	+	+	+

Supplementary Table 4: XPS analysis of EPS matrices of freeze-dried biofilms of the WT and all pigment mutant strains of *K. petricola*. Note that only C, O and N could be detected. Given are the mean values with twice the (propagated) analytical uncertainty (confidence level of 95%).

	Element (atomic %)			Elemental ratio	
	C1s	O1s	N1s	O/C	N/C
WT	73 ± 15	25.8 ± 5.3	1.3 ± 0.5	0.35 ± 0.10	0.018 ± 0.008
$\Delta phd1$	72 ± 14	26.0 ± 5.2	1.7 ± 0.4	0.36 ± 0.10	0.023 ± 0.007
$\Delta pks1$	74 ± 15	23.8 ± 5.0	2.1 ± 0.5	0.322 ± 0.093	0.028 ± 0.009
$\Delta pks1/\Delta phd1$	77 ± 15	21.6 ± 4.3	1.3 ± 0.4	0.281 ± 0.080	0.017 ± 0.006
$\Delta sdh1$	73 ± 15	23.9 ± 5.8	3.1 ± 0.8	0.33 ± 0.10	0.042 ± 0.014

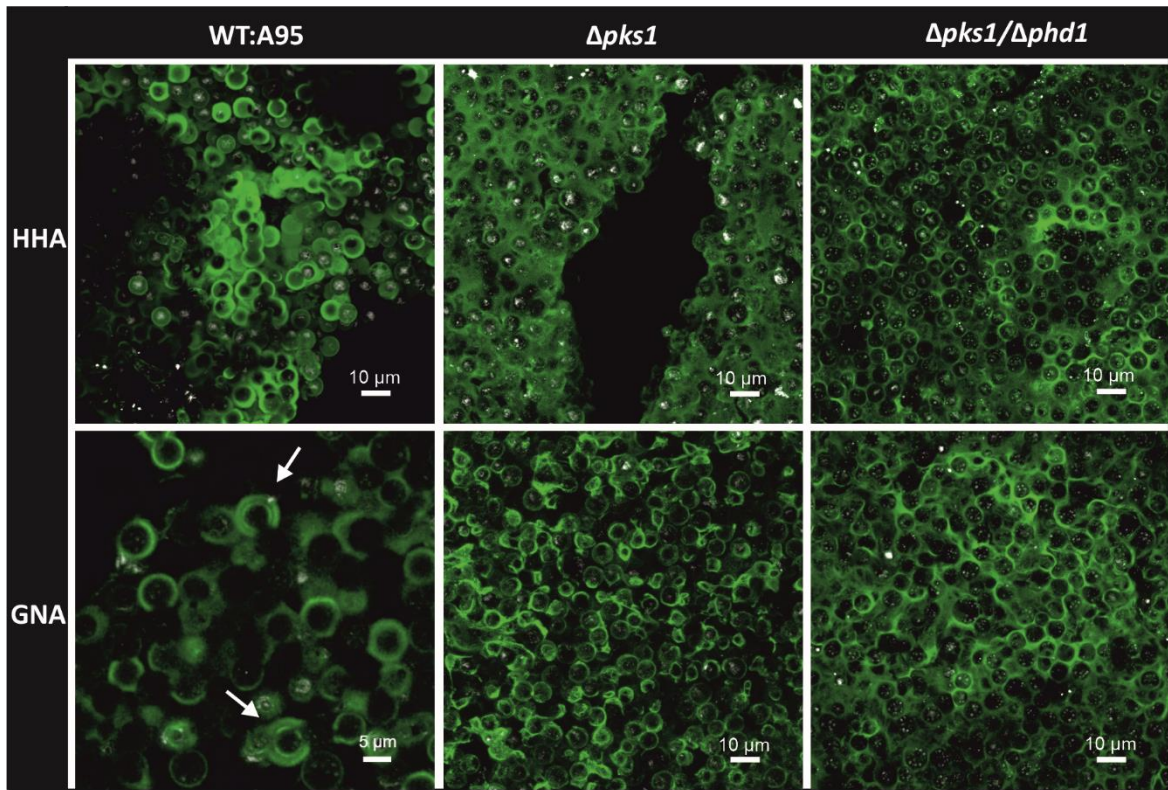
Supplementary Table 5: Fungal biofilms in interaction with olivine as tested in batch dissolution experiments - overview of all the results partially presented in Figures 3 and 4. Experiments were run abiotically, with the WT of *K. petricola*, or with a pigment mutant of *K. petricola* ($\Delta pks1$, $\Delta sdh1$, $\Delta phd1$, $\Delta pks1/\Delta phd1$). All experiments were conducted at 25°C and pH 6, reactors were shaken at 150 rpm and incubated under continuous light (90 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). The dissolution rates of day 55 are shown as it is impossible to calculate them at the end of the experiment due to the low difference in the aqueous Mg concentration (see Methods). Shown are the means of the three biological replicates together with uncertainties (in parenthesis). Uncertainties of the biomass values represent once the standard error of the biological replicates. Uncertainties of the pH, dissolution rate and grade of attachment represent twice the (propagated) analytical uncertainties, or twice the standard error of the biological replicates (whichever was higher). The final olivine dissolution rates are calculated based on the difference between the aqueous Mg concentration at day 69 and day 6. n.a. stands for not analysed.

Experiment	Biomass (mg ml ⁻¹)		pH	Dissolution rate (mol s ⁻¹ cm ⁻²) x 10 ⁻¹⁶	Grade of attachment (%)
	final	fresh, unreacted medium	final, reacted medium	day 55	final
Abiotic	n.a.	5.92 (±0.02)	6.02 (±0.04)	9.7 (±4.1)	n.a.
WT	1.21 (±0.39)	5.92 (±0.02)	5.99 (±0.03)	38 (±13)	28.7 (±8.4)
$\Delta phd1$	1.22 (±0.29)	5.92 (±0.02)	5.97 (±0.04)	34 (±12)	47 (±12)
$\Delta pks1$	1.04 (±0.31)	5.91 (±0.02)	5.90 (±0.02)	17.6 (±6.5)	6.8 (±3.2)
$\Delta pks1/\Delta phd1$	1.33 (±0.27)	5.91 (±0.02)	5.90 (±0.02)	18.8 (±6.9)	5.4 (±2.6)
$\Delta sdh1$	1.64 (±0.56)	5.91 (±0.02)	6.02 (±0.02)	59 (±19)	19.3 (±3.4)

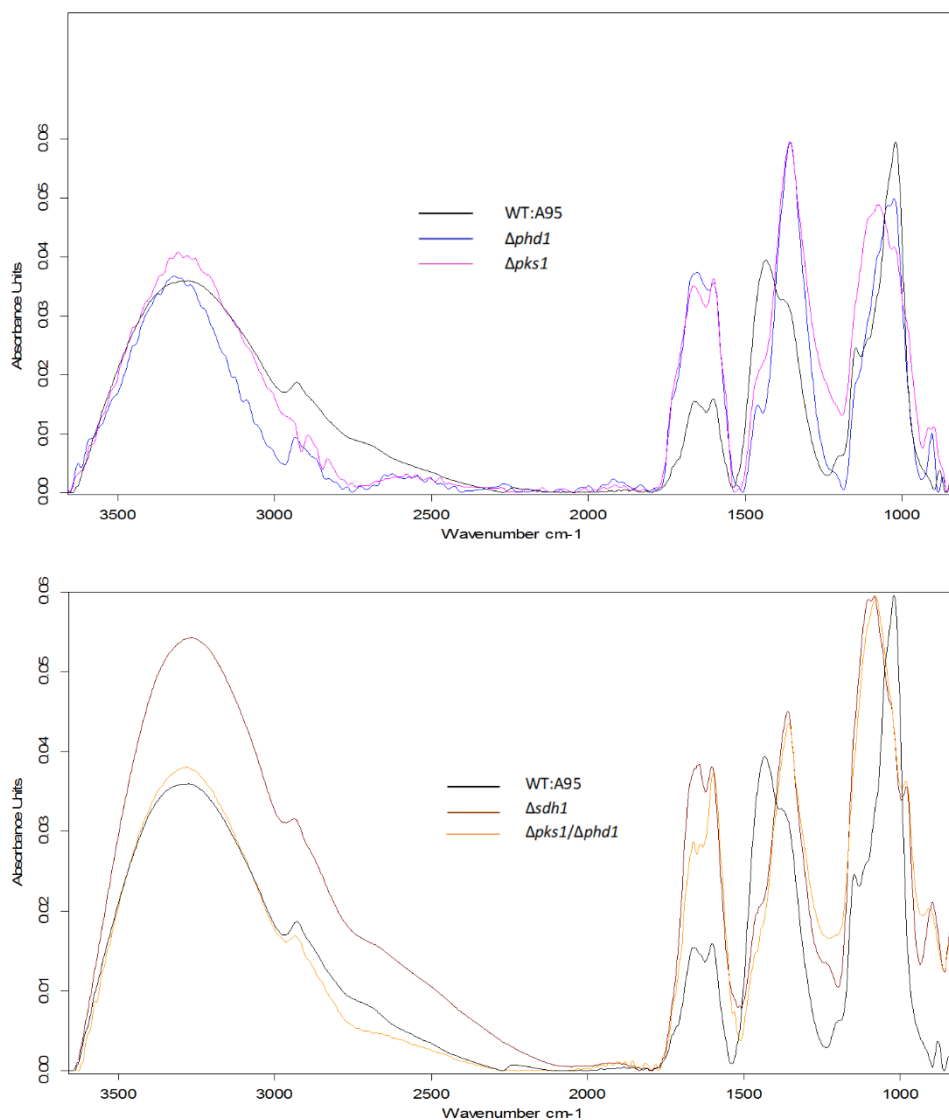
Supplementary Table 6: Pairwise correlation among key variables. Given are the Pearson correlation coefficient (r), 95% confidence interval (CI), test statistic, t , with three degrees of freedom ($t(3)$), and the p value (* stands for $p \leq 0.05$, ** stands for $p \leq 0.01$).

Variable pair		Pearson r	95% CI	$t(3)$	p
EPS amount (mg/g)	α -glucan-related Glcp linkages (Mol%)	-0.98	[-1.00, -0.75]	-9.01	0.003**
EPS amount (mg/g)	Grade of attachment (%)	-0.9	[-0.99, -0.07]	-3.51	0.039*
EPS amount (mg/g)	Dissolution rate (mol $s^{-1} m^{-2}$)	-0.63	[-0.97, 0.57]	-1.4	0.256
α -glucan-related Glcp linkages (Mol%)	Grade of attachment (%)	0.94	[0.35, 1.00]	4.86	0.017*
α -glucan-related Glcp linkages (Mol%)	Dissolution rate (mol $s^{-1} m^{-2}$)	0.65	[-0.54, 0.97]	1.5	0.231
Grade of attachment (%)	Dissolution rate (mol $s^{-1} m^{-2}$)	0.4	[-0.74, 0.95]	0.76	0.503

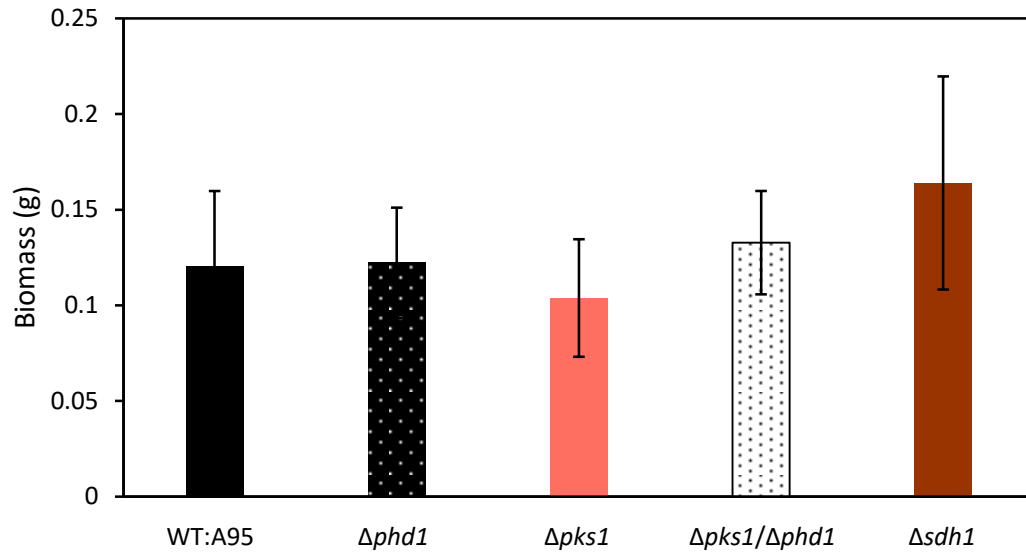
3. Supplementary Figures



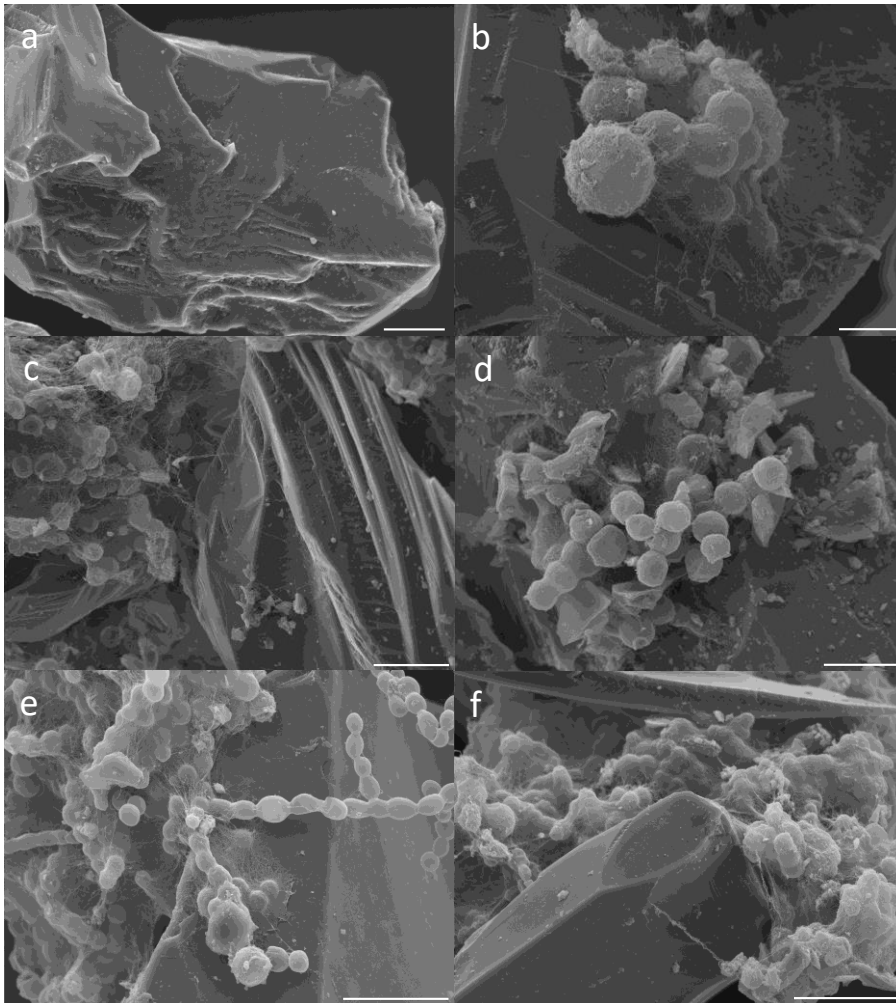
Supplementary Figure 1: Confocal laser scanning microscopy images of EPS matrices of 7-day old biofilms as produced by *K. petricola* wildtype and the mutants $\Delta pks1$ and $\Delta pks1/\Delta phd1$: binding of two selected lectins (fluorescent dye-labelled) HHA and GNA was tested. The lectin HHA only bound to WT cells on the outer rims of the biofilm due to its reduced diffusion throughout this biofilm. This is not the case for the melanin-deficient mutants. Upon application of the lectin GNA that specifically visualises structures containing $(\alpha-1,3)$ mannose residues, double rings became visible around WT cells (indicated by arrows): the inner ring likely show the cell wall, the outer ring the EPS layer.



Supplementary Figure 2: Fourier transformed infrared (FTIR) spectrum of EPS extracted from the WT and pigment mutants of *K. petricola*. Each spectrum represents the mean of five recorded spectra, is base line corrected and relieved of the CO₂ peak. The wild type spectrum from Breitenbach et al.,⁵ is shown for comparison.



Supplementary Figure 3: Fungal biofilms of WT and tested mutants: estimation of the final biomass of the olivine dissolution experiments. Growth was not significantly different for any strain. Error bars represent once the standard error of the three biological replicates.



Supplementary Figure 4: Scanning electron microscope images of olivine-biofilm samples from the dissolution experiment. Shown are images of samples from the a) the abiotic control, b) the WT set-up, c) the $\Delta pks1$ mutant set-up, d) the $\Delta sdh1$ mutant set-up, e) the $\Delta phd1$ mutant set-up and f) the $\Delta pks1/\Delta phd1$ mutant set-up. The scale bars of panel a has a length of 20 μm , panel b a length of 5 μm , panel c a length of 20 μm , panel d a length of 10 μm , panel e a length off 20 μm and panel f a length of 20 μm .

4. References

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