Appl. Environ. Microbiol.

A Highly Expressed High Molecular Weight S-Layer Complex of *Pelosinus* Strain UFO1 Binds Uranium

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Supplementary Material

Tables S1 – S6

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Supplementary Table 1: Purification of UBC from a cell membrane and cytoplasmic fraction. Cell extract was prepared (as described in the Methods) from 20 g cells. Values for the DEAE FF column are taken from the flow through fraction that was concentrated and loaded onto the Superose 6 column. Values for the Superose 6 column are taken from the peak uranium fractions (6 and 7).

Purification Step	Protein (mg)	Uranium (μmol)	% Yield	Fold Purification	Uranium/ Protein Dimer
Cell Extract	145.0	0.87	100	1.0	0.6
DEAE FF	32.0	0.47	54.0	2.5	1.4
Superose 6	0.4	0.01	2.6	2.4	3.3
Final			1.1	5.8	

Supplementary Table 2: Expression levels and conserved domains of *Pelosinus sp.* strain UFO1 genes with homology to the uranium binding complex genes UFO1_4202 and UFO1_4203.

Gene	Expression	Fold change with U addition	p_{adj}	UFO1_4202 % Identity (% Coverage)	UFO1_4203 % Identity (% Coverage)	Length (Amino Acids)	S-layer domain	OprB superfamily	OM_channels superfamily	DUF3373
UFO1_4206	8.9×10^0	1.71	0.69	41 (47)	47 (52)	376	Yes	No	No	No
UFO1_0809	1.7×10^1	0.86	0.93	36 (93)	32 (99)	487	Yes	No	No	Yes
UFO1_1298	1.8×10^1	1.29	0.78	55 (26)	49 (30)	446	Yes	No	No	No
UFO1_4762	4.7×10^1	0.89	0.8	60 (29)	39 (100)	449	Yes	No	No	No
UFO1_1594	4.7×10^1	1.48	0.4	23 (85)	23 (93)	397	No	No	No	Yes
UFO1_0175	1.7×10^2	0.92	0.9	33 (100)	35 (100)	420	Yes	No	No	No
UFO1_3545	4.3×10^2	1.03	0.98	33 (100)	30 (100)	439	No	No	No	No
UFO1_3576	6.3×10^2	1.04	1	28 (100)	28 (100)	463	Yes	No	No	No
UFO1_4203	5.0×10^4	0.96	0.87	100 (100)	39 (100)	457	Yes	No	Yes	Yes
UFO1_4202	1.1×10^5	0.68	0.1	39 (100)	100 (100)	414	Yes	Yes	No	No

Supplementary Table 3: Purification of UBC from extracellular spent medium. Extracellular spent medium (3L) was prepared as described in the Methods. Values for the DEAE FF column are taken from peak fractions 11-15 which were concentrated and loaded onto the Superose 6 column. Values for the Superose 6 column are taken from the peak uranium fraction (6).

Purification Step	Protein (mg)	Uranium (μmol)	% Yield	Fold Purification	Uranium/ Protein Dimer
Cell Extract	435.5	1.9	100	1.0	0.4
DEAE FF	363.3	0.3	13.8	0.2	0.1
Superose 6	0.1	0.0004	0.2	4.7	0.3
Final			0.02	0.8	

Supplementary Table 4: Metal analysis of peak fraction from extracellular UBC size exclusion column.

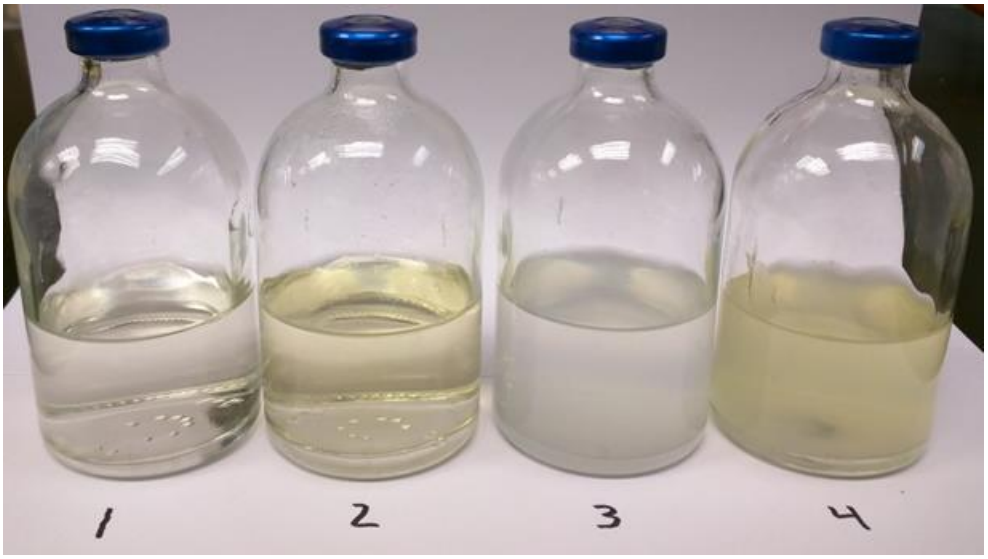
Metal	Concentration (μM)	Overlap with U peak
Ti	0.14	Yes
V	0.02	Yes
Cr	0.04	No
Mn	0.00	No
Fe	0.10	No
Co	0.08	Yes
Ni	2.09	No
Cu	0.02	No
Zn	0.88	No
Mo	0.00	No
Cd	0.00	No
W	0.02	Yes
Pb	0.00	No
U	0.88	No

Supplementary Table 5: Most highly transcribed genes of *Pelosinus sp.* strain UFO1.

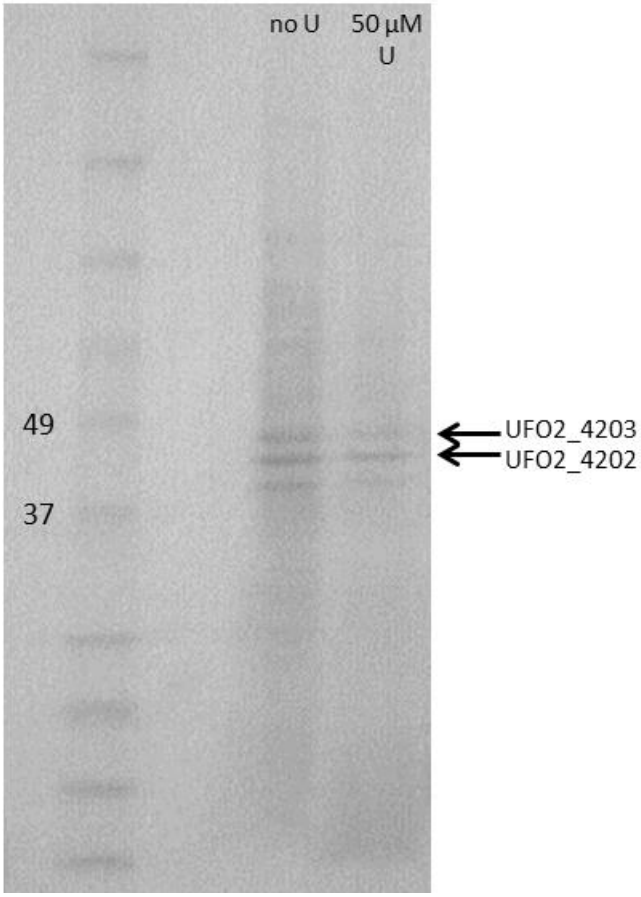
Gene	Expression level (Counts)	Fold change with U addition	P_{adj}	Gene Description
UFO1_4202	1.1E+05	0.7	0.10	S-layer domain-containing protein
UFO1_2336	8.1E+04	1.2	0.57	Manganese/iron superoxide dismutase
UFO1_4203	5.0E+04	1.0	0.87	S-layer domain-containing protein
UFO1_3815	3.9E+04	1.3	0.34	Tetratricopeptide repeat-containing protein
UFO1_0431	3.9E+04	1.2	0.39	Formate acetyltransferase
UFO1_4131	3.8E+04	1.4	0.42	Linocin M18 bacteriocin protein
UFO1_4086	3.5E+04	0.5	0.00	Sigma 54 protein/ribosomal protein S30EA
UFO1_4132	3.5E+04	1.1	0.92	Rubrerythrin
UFO1_3888	3.3E+04	0.6	0.01	5-carboxymethyl-2-hydroxymuconate isomerase
UFO1_1284	3.3E+04	1.0	0.98	Chaperonin (60 kDa)
UFO1_4112	3.3E+04	1.1	0.86	Flagellin domain protein
UFO1_3290	2.6E+04	1.2	0.67	Nitroreductase
UFO1_0294	2.5E+04	1.3	0.57	Peptidase M10A and M12B
UFO1_0970	2.3E+04	0.8	0.29	Pyridoxamine 5-phosphate oxidase
UFO1_3291	2.2E+04	0.8	0.57	Hypothetical protein

Supplementary Table 6: *Pelosinus sp.* strain UFO1 genes with over 8-fold increased expression in cells grown in the presence of 50 µM uranium.

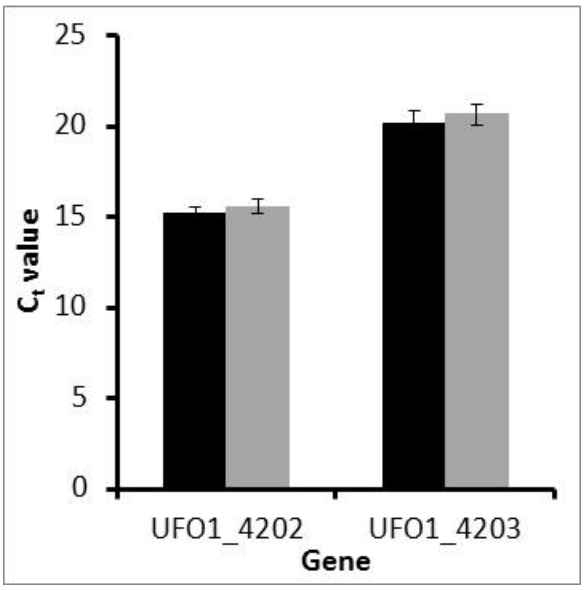
Gene	Expression (no U)	Expression (50 µM U)	Fold Change	p_{adj}	Gene Description
UFO1_3682	87	2357	27.2	0.30	Hypothetical protein
UFO1_1409	38	802	21.4	0.33	Hypothetical protein
UFO1_3260	195	3074	15.7	0.17	Hypothetical protein
UFO1_2742	243	3025	12.5	0.20	Hypothetical protein
UFO1_2808	698	8560	12.3	0.14	Hypothetical protein
UFO1_3253	428	3667	8.6	0.30	Hypothetical protein
UFO1_1596	615	4780	7.8	0.20	Hypothetical protein



Supplementary Figure 1: *Pelosinus* strain UFO1 reduces anthraquinone-2,6-disulfonate (AQDS) to AH_2DS during growth. When reduced to AH_2DS, AQDS changes color to a deeper yellow/orange as seen in bottles 2 and 4 (1). 1) Uninoculated medium containing 100 µM AQDS. 2) Uninoculated medium containing 100 µM AH_2DS. 3) Strain UFO1 culture with no AQDS added to the growth medium. 4) Strain UFO1 culture with 100 µM AQDS added to the growth medium.



Supplementary Figure 2: The uranium binding complex (UBC) is present in the spent medium of cultures of *Pelosinus sp.* strain UFO1. Strain UFO1 was grown in modified R2 broth with and without 50 µM uranyl-acetate present in the growth medium. Spent medium was concentrated 50 fold using a 3 kDa filter and was run on a denaturing SDS-PAGE gel. Labeled bands were identified using in gel tryptic digest MALDI.



Supplementary Figure 3: Reverse transcription-PCR of UBC genes. Strain UFO1 was grown in modified R2 broth without (black) and with (grey) 50 µM uranyl-acetate present in the growth medium, before RNA isolation and reverse transcription-PCR analysis.

References

1. **Liu C, Zachara JM, Foster NS, Strickland J.** 2007. Kinetics of reductive dissolution of hematite by bioreduced anthraquinone-2, 6-disulfonate. Envir Sci Tech **41:**7730-7735.