Molding the future: Optimization of bioleaching of rare earth elements from electronic waste by *Penicillium expansum* and insights into its mechanism

Supplementary Material

Table S1. Composition of isolation and screening media

Media	Ingredients	Concentration (g/L)
Potato Dextrose Agar (PDA)	Potato extract	4.0
	Glucose	20.0
	Agar	15.0
Pikovskaya (PVK) agar Assay*	Glucose	10.0
	Yeast extract	0.5
	$Ca_3(PO_4)_2$	5.0
	$(NH_4)_2\ SO_4$	0.5
	$MgSO_4.7H_2O$	0.1
	KCl	0.2
	$FeSO_4.7H_2O$	0.002
	$MnSO_4.7H_2O$	0.002
	Agar	15.0
Chrome Azurol S (CAS) agar Assay**	*a. Growth medium*	
	KH_2PO_4	3.0
	Na_2HPO_4	12.0
	NaCl	0.5
	NH_4Cl	1.0
	Agar	15.0
	20% Glucose	20.0 (ml)
	b. CAS Indicator Solution:	
	Solution 1: 60 mg of CAS in 50 ml of ddH_2O.	
	Solution 2: 2.7 mg of $FeCl_3.6H_2O$ in 10 ml of 10 mM HCl.	
	Solution 3: 73 mg of HDTMA in 40 ml of ddH_2O.	
	Solutions were mixed and autoclaved.	
	Growth medium (a) was mixed with CAS Indicator Solution (b).	

*Positive result denoted by the formation of a clear halo zone around growth

**Positive result denoted by the formation of an orange halo zone around growth

Table S2. ANOVA for RSM regression models for the recovery of REE and base metals from WPCBs, and organic acid production. Model statistics and factor effects as the coefficient estimates. No significant effect (n.s), positive effect (+), and negative effect (-) (ANOVA, $p<0.05$).

Response	R^2	*p-value*	Factor effects						
			Factor A. Initial pH	Factor B. Phosphate concentration	Factor C. Buffering status	AB	AC	BC	B^2
La	0.81	0.0004	n.s.	(-)6.6	n.s.	n.s.	n.s.	(+)4.7	n.s
Pr	0.75	<0.0001	(+)2.9	(-)6.9	(+)3.0	n.s.	n.s.	n.s.	n.s.
Nd	0.75	0.0040	n.s.	(-)3.0	n.s.	n.s.	n.s.	(+)3.2	n.s.
Gd	0.80	0.0014	n.s.	(-)3.7	n.s	n.s.	n.s.	(+)3.5	n.s.
Tb	0.66	0.0236	n.s.	(-)1.8	n.s.	n.s.	(+)1.8	(+)2.3	n.s.
Dy	0.70	0.0005	n.s.	n.s.	(+)1.4	n.s.	n.s.	n.s.	n.s.
Er	0.71	0.0150	n.s.	(-)0.9	n.s.	n.s.	n.s.	(+)1.4	n.s.
Cu	0.66	0.0009	(-)5.3	(-)4.1	(-)6.3	n.s.	n.s.	n.s.	n.s.
Fe	0.82	0.0016	n.s.	n.s.	(+)4.3	n.s.	n.s.	(+)2.7	(-)4.9
Ni	0.75	<0.0001	(-)3.9	(+)4.2	(-)5.3	n.s.	n.s.	n.s.	n.s.
Gluconic	0.96	<0.0001	n.s.	(-)11.2	(+)51.7	n.s.	(+)17.1	(-)11.3	
Citric	0.72	0.0078	n.s.	(-)0.3	n.s.	n.s.	(-)0.3	n.s.	
Succinic*	0.94	<0.0001	n.s.	(+)1.1	(+)1.1	n.s.	n.s.	(+)1.0	(-)1.1
Malic	0.81	<0.0001	n.s.	(-)0.6	(-)1.3	n.s.	n.s.	n.s.	
Fumaric	0.68	0.0003	n.s.	(-)0.002	(+) 0.003	n.s.	n.s.	n.s.	
Total Org.	0.96	<0.0001	n.s.	(-)15.8	(+)54.6	n.s.	(+)12.6	(-)11.7	

Factor interactions are represented as AB, AC, and BC, with Initial pH as factor A; Phosphate concentration as factor B; and Buffering status as factor C. Response variable refers to recovery (%) of elements, and organic acids concentrations (mM) *Quadratic model also includes the term (-) 0.9 A^2

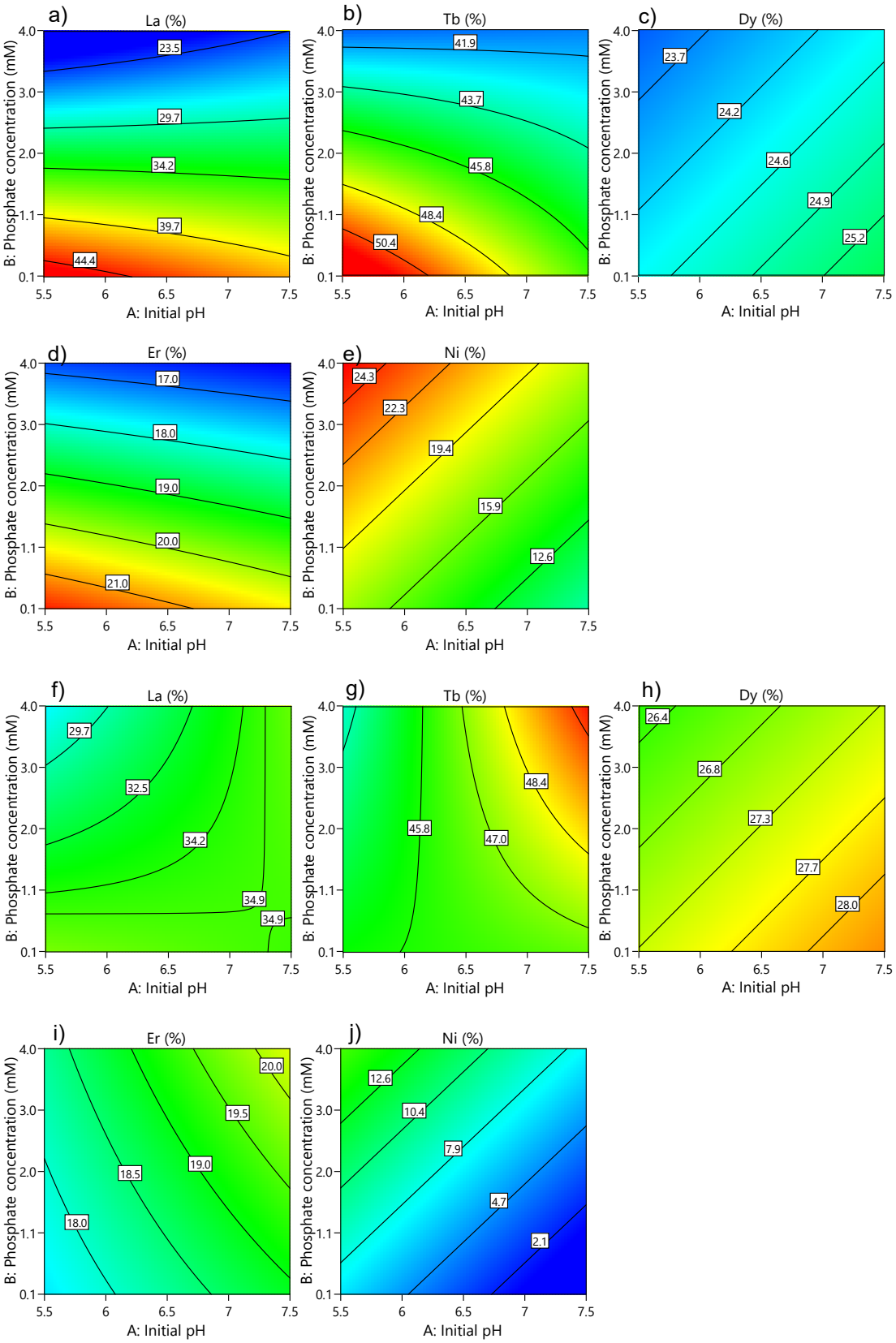


Figure S1. RSM contour plots of REE and base metals recovery from WPCBs by fungal bioleaching. Phosphate concentration in Y axis, initial pH in X axis, and recovery (%) in contour lines. Non-buffered medium (a-e) and buffered medium (f-j).

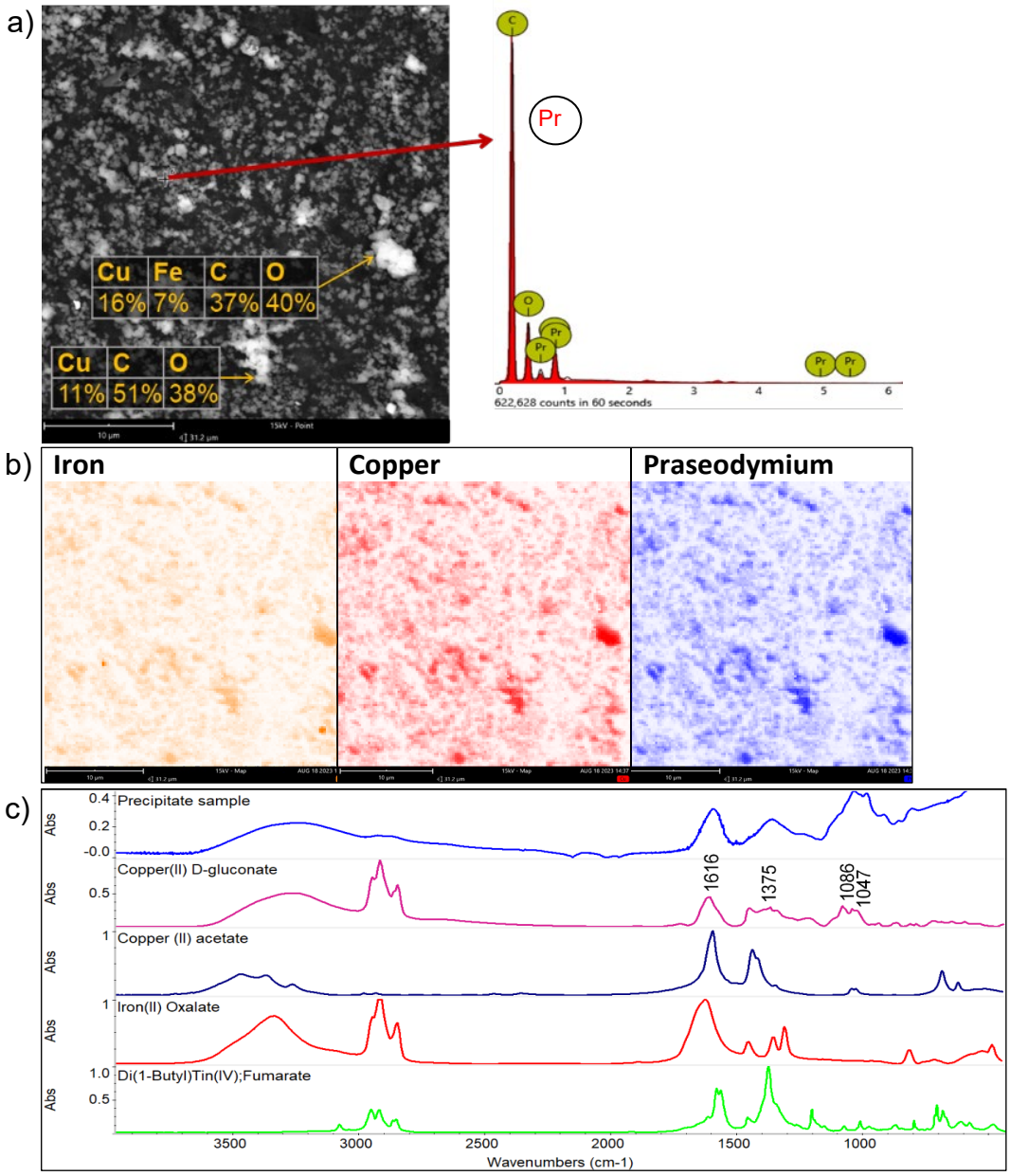
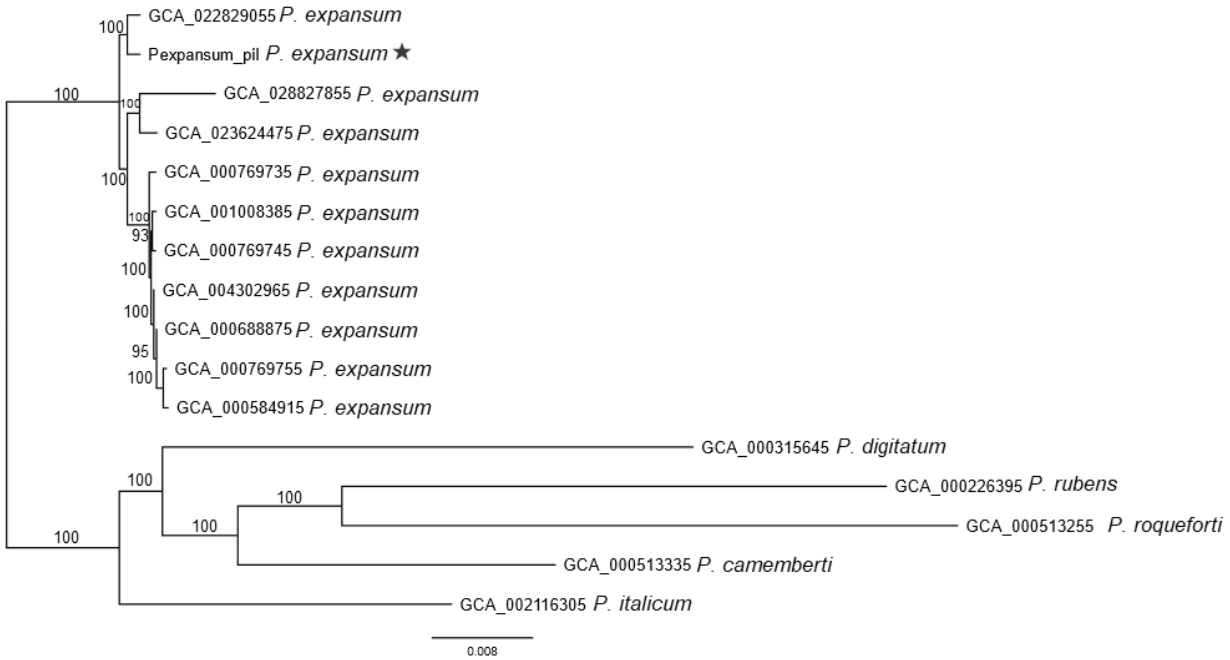


Figure S2. Analysis of precipitate samples from abiotic experiments. SEM-EDS single particle analysis (a) and elemental image mapping (b). FTIR spectra data of precipitate sample and known metal-organic complexes (c). Elemental maps display the distribution of each element within the analysed sample, areas with a more intense colour represent higher concentration of the elements.

a)



b)

Hit Table | Info

Name	Bit-Score	E Value	Grade	% Identical Sites	Query coverage	Sequence Length	Hit start	Hit end
contig_2_P. expansum isolate	815.453	0	70.5%	65.1%	75.93%	703	1,851,100	1,848,995

c)

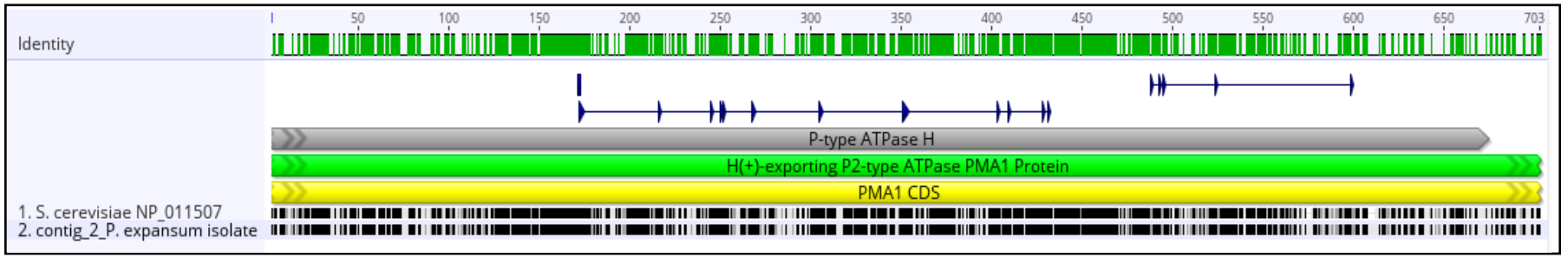


Figure S3. Phylogenetic tree representing the relationships between various *Penicillium expansum* strains and additional *Penicillium* species used as outgroups (a). The star symbol denotes the newly sequenced isolate strain. BLAST results indicating percentage of identity and other metrics in the genome search for H^+-ATPase protein using *Saccharomyces cerevisiae* PMA1 protein as a query (b). Comparison and alignment view of the fungal isolate sequence versus the reference *S. cerevisiae*, black colour indicates similarity, while grey and white spaces represent disagreements (c).