Supplementary Materials

Simultaneous nitrogen and phosphorus removal by a novel cold-adapted *Acinetobacter bohemicus* strain FX-Q2: Performance, mechanism, and potential application

Text S1 Details of SEM analysis

Freshly harvested bacterial cells were prepared for SEM according to a previously reported method (Gu et al., 2024) with minor modifications. Cells were washed three times with 0.1 M phosphate-buffered saline (PBS) and fixed in 2.5% (v/v) glutaraldehyde at 4°C for 12 h. The fixed cells were collected by centrifugation (5000 rpm, 3 min), rinsed three times with PBS, and dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 100%). The dehydrated samples were dried using a critical point dryer, sputter-coated with gold, and examined by SEM at an acceleration voltage of 3.0 kV.

Text S2 Determination of enzyme activity

Bacterial culture was collected for centrifugation (10000 rpm, 4℃, 5 min). The cells were then washed for 3 times with 0.01 M PBS buffer (pH 7.4) for measuring ammonia monooxygenase (AMO), hydroxylamine oxidase (HAO) and Nitrate reductase (NR) activities or with 1.5 M NaCl buffer (containing 0.01 M EDTA and 1mM NaF, pH 7.4) for determining exopolyphosphatase (PPX) and polyphosphate kinase (PPK) activities (Zheng et al., 2011). The cells in suspension were lysed using an ultrasonic cell crusher (JY98-IIIDN, Scientz, China) in an ice bath. Subsequently, the debris was centrifuged again at 12,000 rpm for 5 min, and the crude enzyme extracts in the supernatant were obtained for the activity measurement. For the assay of nitrogen metabolism-related enzymes: AMO was measured by the concentration increase of NO_2^--N (Zheng et al., 2011). HAO activity was measured by the absorbance change at 400 nm (Otte et al., 1999). NR was measured by the formation of NO_2^--N from NO_3^--N (Li et al., 2021). One enzyme unit (U) of AMO, HAO, and NR was defined as the concentration of enzyme catalyzing the transformation of 1 μmol substrate to the product per min. For the assay of phosphorus metabolism-related enzymes: PPX activity was determined according to a previously published method (Zheng et al., 2011), with enzymatic reactions conducted at 30℃ and quantified spectrophotometrically at 405 nm. One enzyme unit (U) of PPX was defined as the production of 1μmol p-nitrophenol per min. The activity of PPK was determined using the modified enzyme-coupled assay with hexokinase (HK) and glucose-6-phosphate

dehydrogenase (G6PD) (Cui et al., 2020). One enzyme unit (U) of PPK was defined as the amount of enzyme that produced 1 μmol ATP per min.

Table S1 The main media used in this study

Medium	Composition and content of medium (g/L)							Trace element*
	CH_3COONa	$(NH_4)_2SO_4$	$NaNO_2$	$NaNO_3$	$K_2HPO_4·3H_2O$	NaCl	$MgSO_4·7H_2O$	
BM-1	5	0.5	-	-	0.3	0.12	0.05	2 mL
BM-2	5	0.25	-	0.25	0.3	0.12	0.05	2 mL
HNM	3.417	0.472	-	-	0.15	0.12	0.05	2 mL
ADM-1	3.417	-	0.493	-	0.15	0.12	0.05	2 mL
ADM-2	3.417	-	-	0.607	0.15	0.12	0.05	2 mL
SNDM	3.417	0.157	0.164	0.202	0.15	0.12	0.05	2 mL

* Trace element solution contained (per liter): 5 g EDTA-2Na, 5 g $FeSO_4·7H_2O$, 0.2 g $ZnSO_4·7H_2O$, 1.2 g $MnCl_2·4H_2O$, 0.5 g $CuSO_4·5H_2O$, 0.3 g $CoCl_2·6H_2O$, 0.2 g $Na_2MoO_4·2H_2O$, 0.1 g $CaCl_2$.

Table S2 Statistical information of the complete genome for strain FX-Q2.

Sample ID	FX-Q2
Gene No.	3,767
Genome size (bp)	3,887,361
Gene total length (bp)	3,274,671
Gene average length (bp)	869.30
Gene density (kb)	0.97
GC content in gene region (%)	40.52
Gene/Genome (%)	84.24
rRNAs	21
tRNAs	84

Table S3 Information of nitrogen and phosphorus metabolism-related genes.

Functional category	Gene_ID	Gene name	KEGG Description	NR Description	GOC Description
Nitrogen metabolism	gene0822	*gdhA*	glutamate dehydrogenase (NAD(P)+)	glutamate dehydrogenase (NAD(P)+)	glutamate dehydrogenase/leucine dehydrogenase
	gene1264	*gdhA*	glutamate dehydrogenase (NAD(P)+)	Glu/Leu/Phe/Val dehydrogenase	-
	gene2533	*gdhA*	glutamate dehydrogenase (NADP+)	NADP-specific glutamate dehydrogenase	glutamate dehydrogenase/leucine dehydrogenase
	gene2419	*glnA*	glutamine synthetase	MULTISPECIES: type I glutamate--ammonia ligase	Glutamine synthetase
	gene3226	*gltB*	glutamate synthase (NADPH) large chain	glutamate synthase	glutamate synthase domain 1
	gene3225	*gltD*	glutamate synthase (NADPH) small chain	FAD-dependent oxidoreductase	NADPH-dependent glutamate synthase beta chain or related oxidoreductase
	gene2240	*nirB*	nitrite reductase (NADH) large subunit	nitrite reductase large subunit	NAD(P)H-nitrite reductase, large subunit
	gene2241	*narK*	nitrate/nitrite transporter, MFS transporter, NNP family	MFS transporter	nitrate/nitrite transporter NarK
	gene2238	*nasC*	assimilatory nitrate reductase catalytic subunit	nitrate reductase	anaerobic selenocysteine-containing dehydrogenase
	gene2239	*nasD*	nitrite reductase [NAD(P)H] large subunit	nitrite reductase small subunit NirD	ferredoxin subunit of nitrite reductase or a ring-hydroxylating dioxygenase
	gene0287	*amt*	ammonium transporter, Amt family	ammonium transporter	ammonia channel protein AmtB
	gene2675	*amt*	ammonium transporter, Amt family	ammonium transporter	ammonia channel protein AmtB
	gene2989	*amt*	ammonium transporter, Amt family	ammonium transporter	ammonia channel protein AmtB
	gene1421	*npd*	nitronate monooxygenase	nitronate monooxygenase	NAD(P)H-dependent flavin oxidoreductase YrpB, nitropropane dioxygenase family
	gene0915	*cynT*	carbonic anhydrase	carbonic anhydrase	carbonic anhydrase
	gene1660	*NosD*	3-dehydroshikimate dehydratase	right-handed parallel beta-helix repeat-containing protein	nitrous oxide reductase accessory protein NosD, contains tandem CASH domains
	gene0679	-	sulfite reductase (NADPH) hemoprotein beta-component	nitrite/sulfite reductase	sulfite reductase, beta subunit (hemoprotein)

Functional category	Gene_ID	Gene name	KEGG Description	NR Description	GOC Description
Phosphorus metabolism	gene1128	*pstS*	phosphate transport system substrate-binding protein	substrate-binding domain-containing protein	ABC-type phosphate transport system, periplasmic component
	gene1129	*pstC*	phosphate transport system permease protein	phosphate ABC transporter permease subunit PstC	ABC-type phosphate transport system, permease component
	gene1130	*pstA*	phosphate transport system permease protein	phosphate ABC transporter permease PstA	ABC-type phosphate transport system, permease component
	gene1131	*pstB*	phosphate transport system ATP-binding protein [EC:7.3.2.1]	MULTISPECIES: phosphate ABC transporter ATP-binding protein PstB	ABC-type phosphate transport system, ATPase component
	gene0921	*ppk1*	polyphosphate kinase [EC:2.7.4.1]	polyphosphate kinase 1	Polyphosphate kinase
	pA_gene0158	*ppk1*	polyphosphate kinase [EC:2.7.4.1]	polyphosphate kinase 1	Polyphosphate kinase
	pB_gene0039	*ppk1*	polyphosphate kinase [EC:2.7.4.1]	polyphosphate kinase 1	Polyphosphate kinase
	gene0622	*ppx-gppA*	exopolyphosphatase / guanosine-5'-triphosphate,3'-diphosphate pyrophosphatase [EC:3.6.1.11 3.6.1.40]	exopolyphosphatase	Exopolyphosphatase/pppGpp-phosphohydrolase
	gene0369	*pap*	AMP-polyphosphate phosphotransferase [EC:2.7.4.33]	phosphate--AMP phosphotransferase	Polyphosphate kinase 2, PPK2 family
	gene2539	*adk*	adenylate kinase [EC:2.7.4.3]	adenylate kinase	Adenylate kinase or related kinase
	gene0279	*ppa*	inorganic pyrophosphatase [EC:3.6.1.1]	inorganic diphosphatase	Inorganic pyrophosphatase
	gene0191	*phoR*	two-component system, OmpR family, phosphate regulon sensor histidine kinase PhoR [EC:2.7.13.3]	phosphate regulon sensor histidine kinase PhoR	Sensor histidine kinase WalK
	gene0192	*phoB*	two-component system, OmpR family, phosphate regulon response regulator PhoB	MULTISPECIES: phosphate regulon transcriptional regulator PhoB	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain
	gene0323	*phoU*	phosphate transport system protein	phosphate signaling complex protein PhoU	Phosphate uptake regulator PhoU
	gene0956	*phoD*	alkaline phosphatase D [EC:3.1.3.1]	hypothetical protein	Phosphodiesterase/alkaline phosphatase D
	gene2635	*phoA*	alkaline phosphatase [EC:3.1.3.1]	MULTISPECIES: DedA family protein	Membrane integrity protein DedA, putative transporter, DedA/Tvp38 family

Functional category	Gene_ID	Gene name	KEGG Description	NR Description	GOC Description
	gene3064	*phoH*	phosphate starvation-inducible protein PhoH and related proteins	PhoH family protein	Phosphate starvation-inducible protein PhoH, predicted ATPase
	gene0224	*atpI*	ATP synthase protein I	ATP synthase subunit I	FoF1-type ATP synthase accessory protein AtpI
	gene0225	*atpB*	F-type H+-transporting ATPase subunit a	MULTISPECIES: F0F1 ATP synthase subunit A	FoF1-type ATP synthase, membrane subunit a
	gene0226	*atpE*	F-type H+-transporting ATPase subunit c	MULTISPECIES: F0F1 ATP synthase subunit C	FoF1-type ATP synthase, membrane subunit c/Archaeal/vacuolar-type H+-ATPase, subunit K
	gene0227	*atpF*	F-type H+-transporting ATPase subunit b	F0F1 ATP synthase subunit B	FoF1-type ATP synthase, membrane subunit b or b'
	gene0228	*atpH*	F-type H+-transporting ATPase subunit delta	MULTISPECIES: F0F1 ATP synthase subunit delta	FoF1-type ATP synthase, delta subunit
	gene0229	*atpA*	F-type H+/Na+-transporting ATPase subunit alpha [EC:7.1.2.2 7.2.2.1]	F0F1 ATP synthase subunit alpha	FoF1-type ATP synthase, alpha subunit
	gene0230	*atpG*	F-type H+-transporting ATPase subunit gamma	MULTISPECIES: F0F1 ATP synthase subunit gamma	FoF1-type ATP synthase, gamma subunit
	gene0231	*atpD*	F-type H+/Na+-transporting ATPase subunit beta [EC:7.1.2.2 7.2.2.1]	F0F1 ATP synthase subunit beta	FoF1-type ATP synthase, beta subunit
	gene0232	*atpC*	F-type H+-transporting ATPase subunit epsilon	MULTISPECIES: F0F1 ATP synthase subunit epsilon	FoF1-type ATP synthase, epsilon subunit
	gene3176	*ATP2C*	P-type Ca2+ transporter type 2C [EC:7.2.2.10]	hypothetical protein	Predicted PurR-regulated permease PerM

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