

Genomic and metabonomic insights into the lignin-degrading potential of a novel halophilic bacterial strain *Salinicoccus* sp. HZC-1

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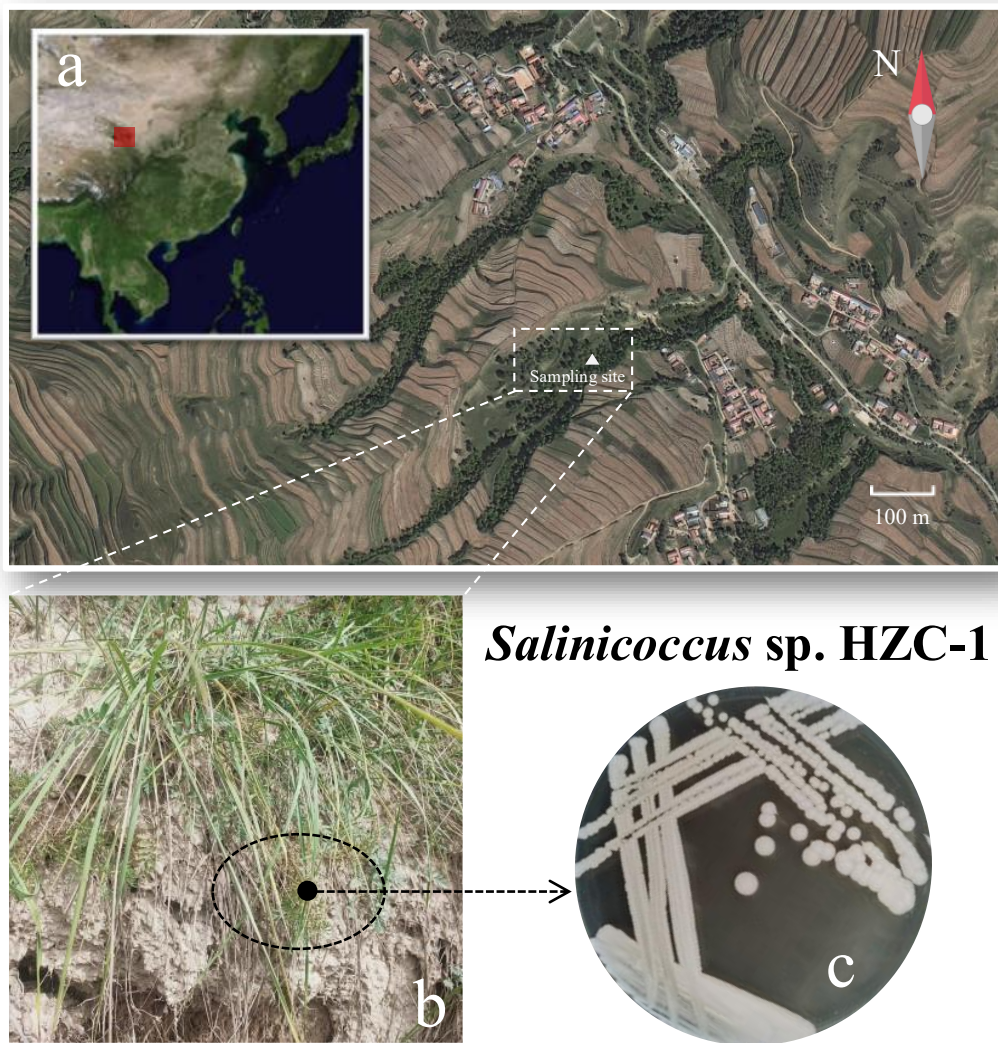


Fig. S1. Study site of sample collection map showing the sampling sites [background layers was obtained from Ovital] (a), A section of Haidong City showing the isolation site (b), Colony morphology of the isolated bacterial strain HZC-1 (c)

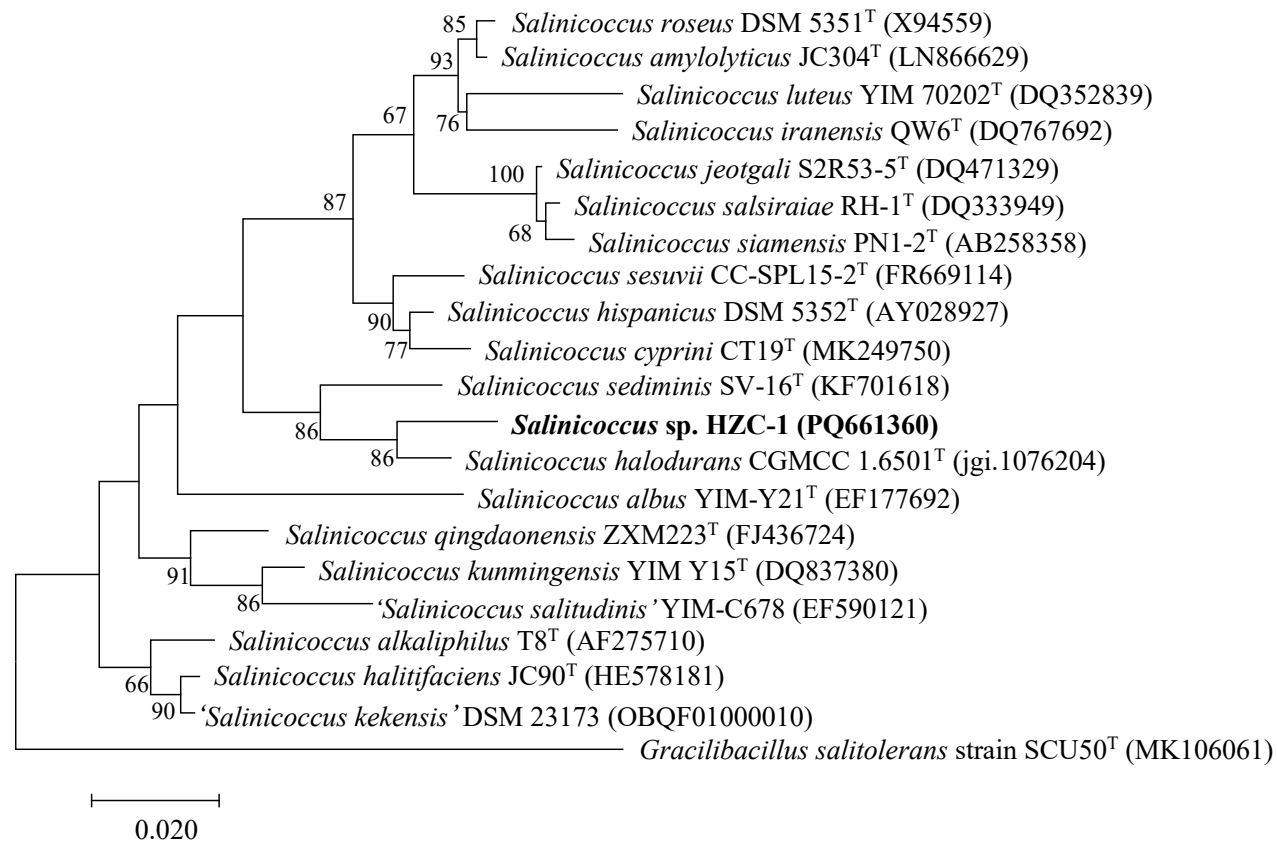


Fig. S2. Maximum likelihood tree based on 16S rRNA gene sequences showing the position of strain HZC-1 compared with other related taxa.

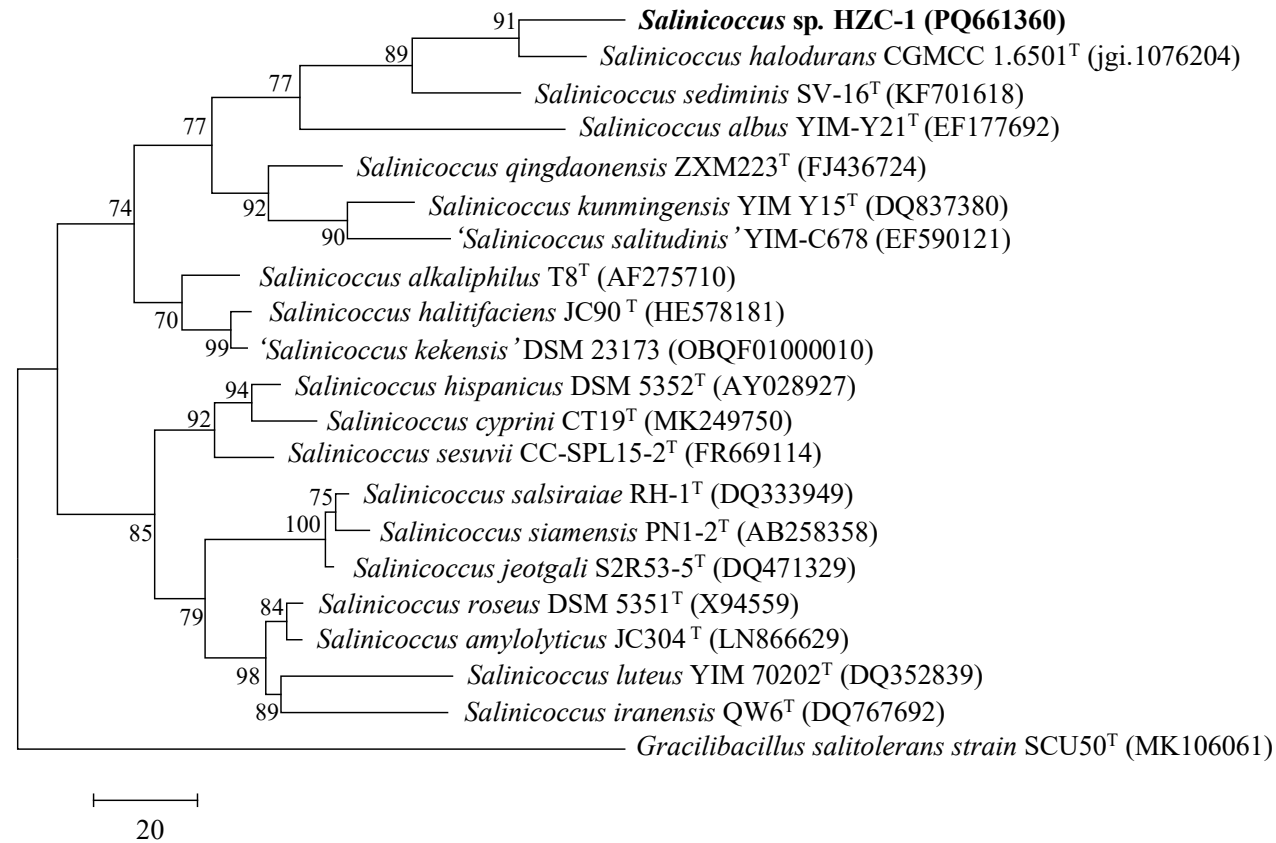


Fig. S3. Maximum-parsimony tree based on 16S rRNA gene sequences showing the position of strain HZC-1 compared with other related taxa.

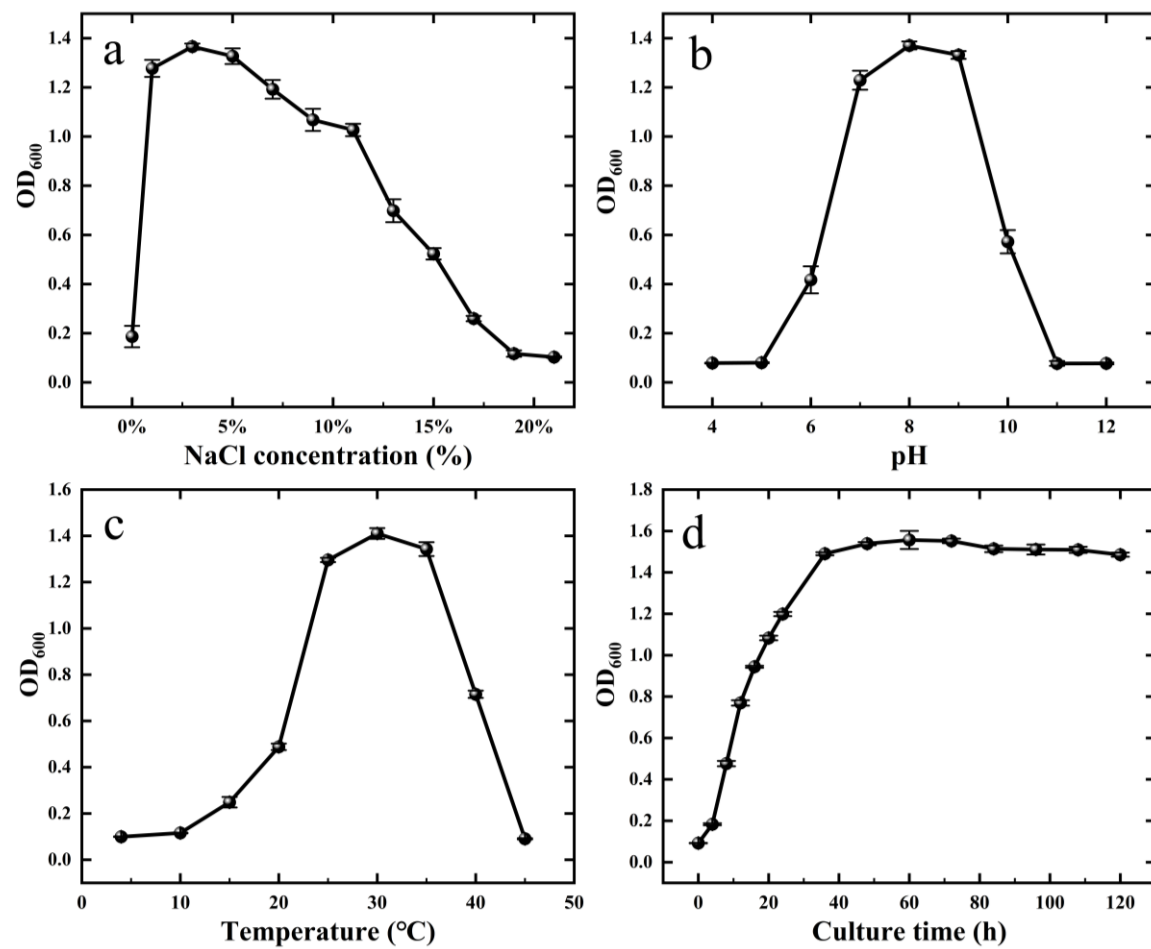


Fig. S4. Growth characteristics of strain HZC-1 under different NaCl concentrations (a), initial pH values (b) and temperatures (c), as well as optimal culture conditions (d)

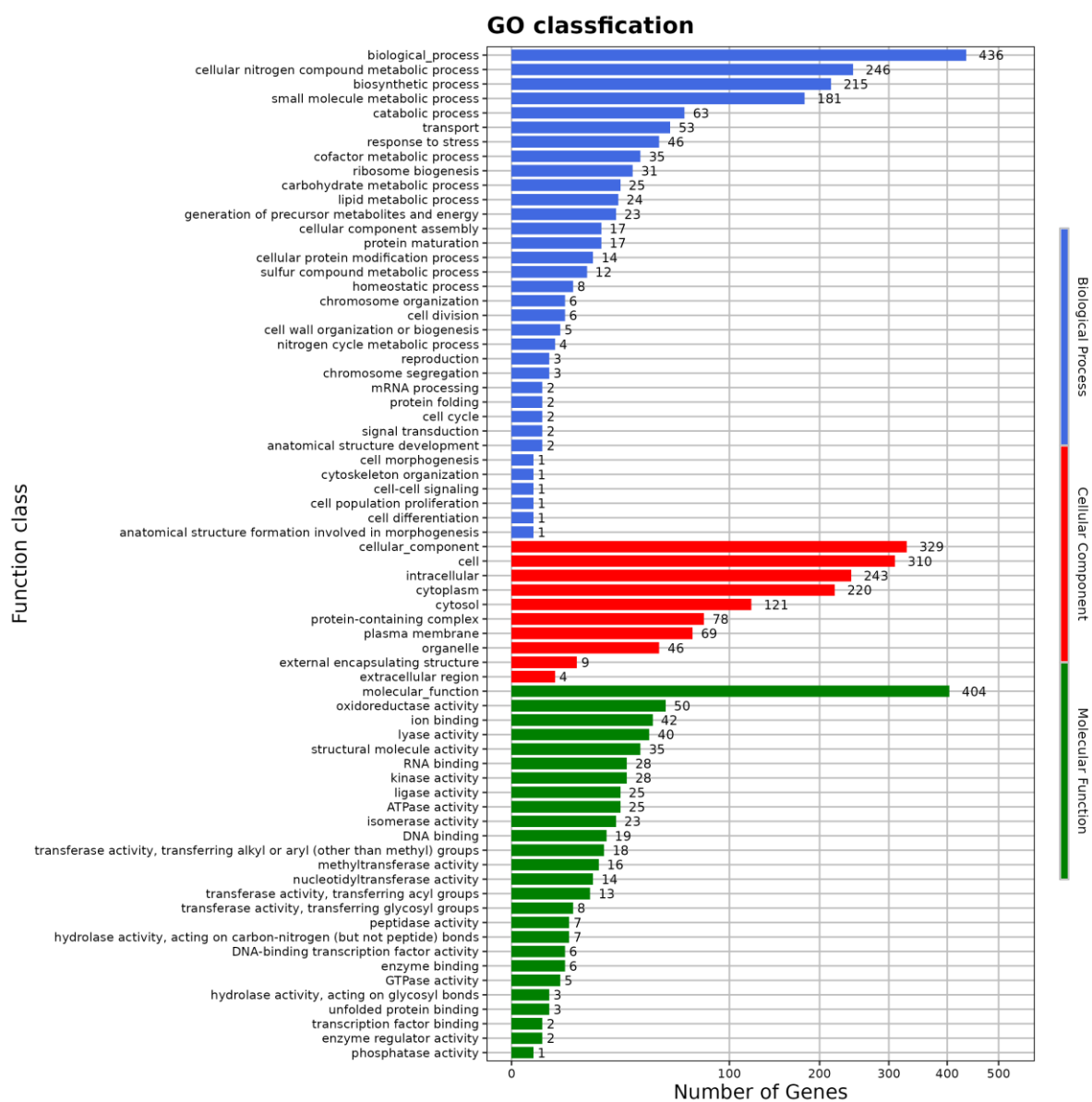


Fig. S5. Functional classification of all predicted CDS in the *Salinicoccus* sp. HZC-1 genome based on the GO database

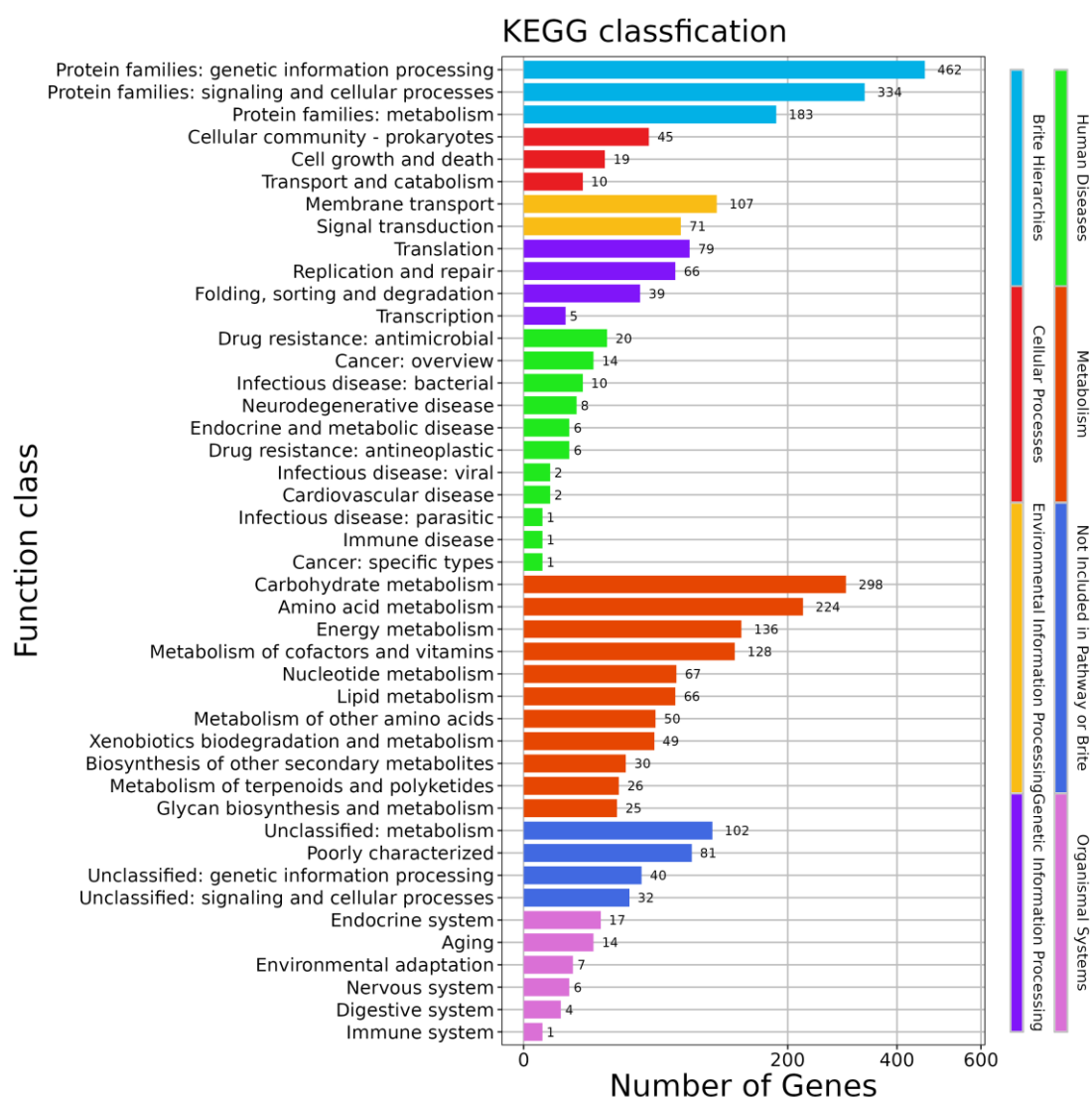


Fig. S6. Functional classification based on all predicted CDS in the genome of strain HZC-1 in the KEGG database

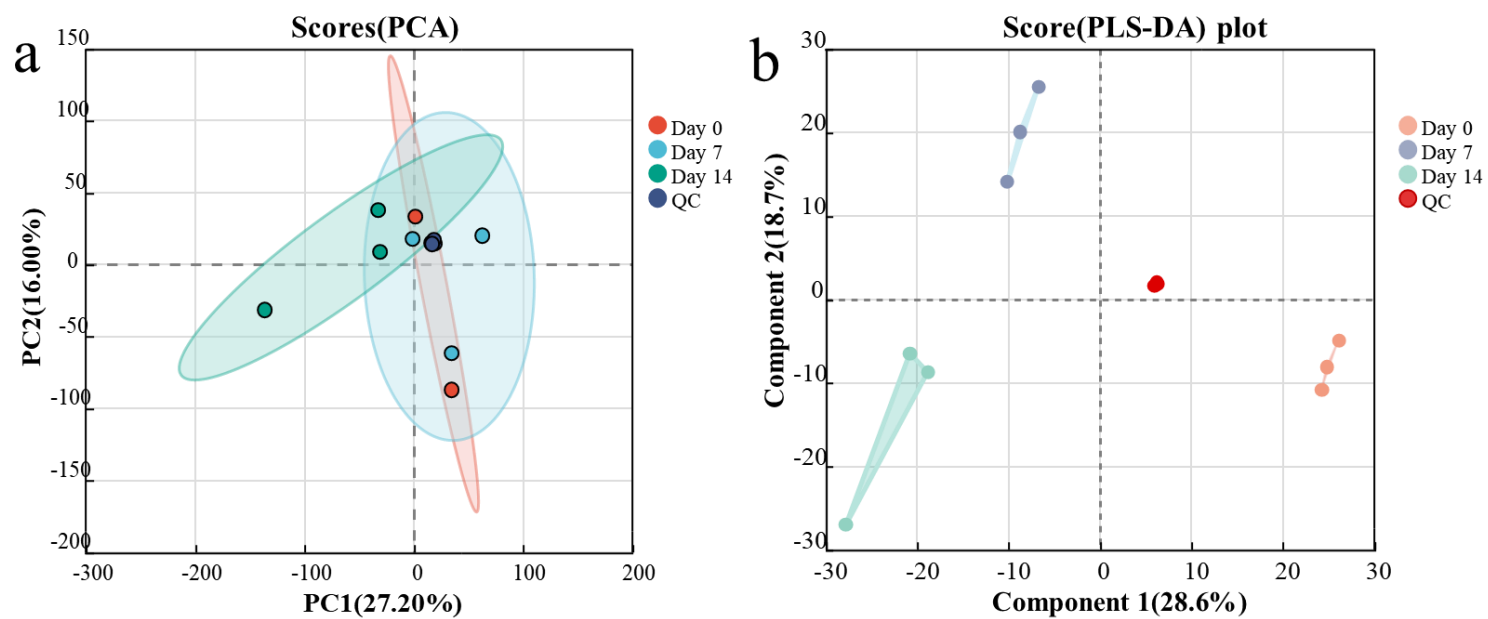


Fig. S7. Analyses of differential metabolites in fermented broth of strain HZC-1 during the incubation period. PCA on days 0, 7 and 14 (a); PLS-DA (b).

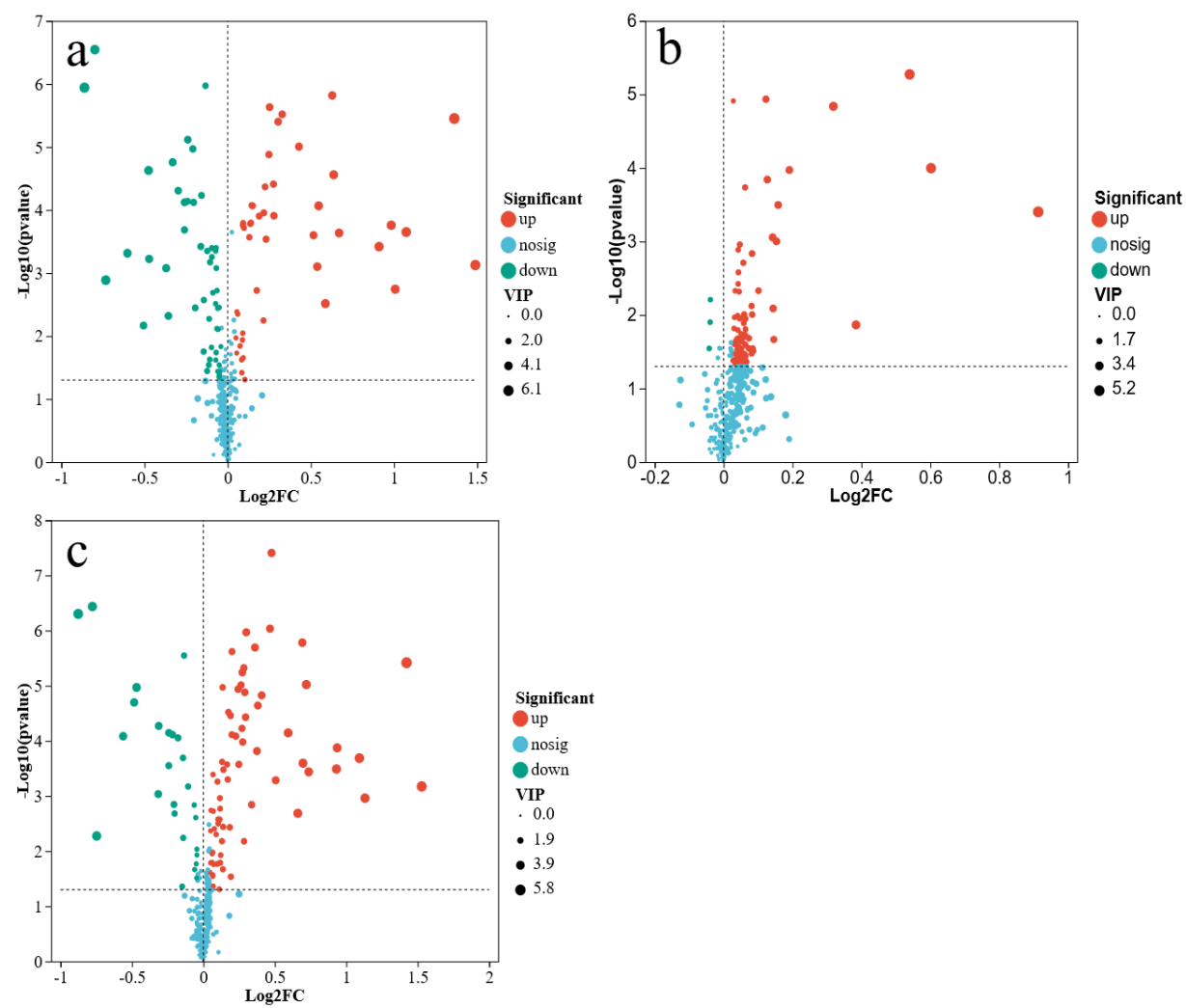


Fig. S8. Volcanic diagram of metabolite differences at different fermentation stages. Day 7 vs Day 0 (a); Day 14 vs Day 7 (b); Day 14 vs Day 0 (c).

Table S1 Rapid genome-wide annotation of strain HZC-1 and three neighboring relatives using the RAST server

Annotation Category	1	2	3	4
Respiration	33	34	31	36
Regulation and Cell signaling	14	14	14	14
DNA Metabolism	62	62	54	55
Nitrogen Metabolism	24	24	6	17
Cell Wall and Capsule	34	34	44	43
Cofactors, Vitamins, Prosthetic Groups, Pigments	105	116	118	113
Carbohydrates	147	163	182	172
Metabolism of Aromatic Compounds	7	7	21	5
Secondary Metabolism	4	4	4	4
Protein Metabolism	153	159	100	109
Fatty Acids, Lipids, and Isoprenoids	28	27	44	40
Disease and Defense	40	44	39	51
Amino Acids and Derivatives	233	253	274	252
Iron acquisition and metabolism	12	11	10	17
Sporulation	7	7	7	7
Phosphorus, Prophages, Transposable elements, Plasmids	11	6	4	0

Xenobiotics Transport	36	34	41	37
Cell Division and Cell Cycle	5	5	5	5
Potassium metabolism	2	2	2	2
Stress Response	35	38	33	33
RNA Metabolism	40	39	39	39
Sulfur Metabolism	6	6	6	6
Nucleotides and Nucleosides	77	81	84	81
Phosphorus Metabolism	2	13	2	2
Dormancy and Sporulation	7	7	7	7

Taxa: 1, *Salinicoccus* sp. HZC-1; 2, *S. halodurans* CGMCC 1.6501^T; 3, *S. carniancri* Crm^T; 4, *S. sediminis* SV-16^T.

Table S2 Salt tolerance-related genes of strain HZC-1 based on GO functional annotation

GO function	GO ID	GO ontologies	Number
Sodium ion transmembrane transport	GO:0035725	Biological process	1
Sodium ion transmembrane transporter activity	GO:0015081	Molecular function	1
Sodium ion transport	GO:0006814	Biological process	1
Sodium:proton antiporter activity	GO:0015385	Molecular function	1
Glycine betaine transport	GO:0031460	Biological process	5
Glycine C-acetyltransferase activity	GO:0008890	Molecular function	1
Glycine-tRNA ligase activity	GO:0004820	Molecular function	1
Quaternary ammonium group transmembrane transporter activity	GO:0015651	Molecular function	3
Quaternary ammonium group transport	GO:0015697	Biological process	5
Response to osmotic stress	GO:0006970	Biological process	3
Proton-transporting ATP synthase activity, rotational mechanism	GO:0046933	Molecular function	1
Proton-transporting ATP synthase complex	GO:0045259	Cellular component	3
Proton-transporting ATP synthase complex, catalytic core F(1)	GO:0045261	Cellular component	1
Proton-transporting ATP synthase complex, coupling factor F(o)	GO:0045263	Cellular component	2
Proton-transporting two-sector ATPase complex	GO:0016469	Cellular component	3
Proton-transporting two-sector ATPase complex, catalytic domain	GO:0033178	Cellular component	1
Proton-transporting two-sector ATPase complex, proton-transporting domain	GO:0033177	Cellular component	1
Protein-phosphocysteine-trehalose phosphotransferase system transporter activity	GO:0090589	Molecular function	1

Trehalose transport	GO:0015771	Biological process	1
Peptidyl-proline modification	GO:0018208	Biological process	1
Cell wall biogenesis	GO:0042546	Biological process	5
Cell wall organization or biogenesis	GO:0071554	Biological process	5
Peptidoglycan-based cell wall biogenesis	GO:0009273	Biological process	5
Cellular response to oxygen radical	GO:0071450	Biological process	5
Cellular response to oxidative stress	GO:0034599	Biological process	4
Cellular response to reactive oxygen species	GO:0034614	Biological process	1
Superoxide dismutase activity	GO:0004784	Molecular function	1
Energy coupled proton transmembrane transport, against electrochemical gradient	GO:0015988	Biological process	1
Proton motive force dependent protein transmembrane transporter activity	GO:0009977	Molecular function	1
Proton transmembrane transport	GO:1902600	Biological process	8
Proton transmembrane transporter activity	GO:0015078	Molecular function	5
Intracellular monoatomic cation homeostasis	GO:0030003	Biological process	3
Monoatomic cation homeostasis	GO:0055080	Biological process	3

Table S3 Salt tolerance-related genes of strain HZC-1 based on KEGG database

Subsystem	Gene	Function	EC	KEGG ko	KEGG TC
Ion Transport and Homeostasis	<i>ktrA</i>	potassium uptake protein TrkA	-	ko:K03499	2.A.38.1, 2.A.38.4
	<i>ktrD</i>	transport protein	-	-	-
	<i>cadA</i>	E1-E2 ATPase	3.6.3.3, 3.6.3.5	ko:K01534	3.A.3.6
	<i>czcD</i>	Co Zn Cd efflux system component	-	ko:K16264	2.A.4.1
	<i>mntH</i>	H-stimulated, divalent metal cation uptake system	-	ko:K03322	2.A.55.2.6, 2.A.55.3
	<i>nhaC_1</i>	Na H antiporter	-	-	-
	<i>nhaC_2</i>	Na H antiporter	-	ko:K03315	2.A.35
	<i>nhaC2</i>	Na ⁺ /H ⁺ antiporter family	-	ko:K18218	-
	<i>mnhA</i>	-antiporter subunit	-	ko:K05565	2.A.63.1, 2.A.63.2
Compatible Solute Synthesis and Transport	<i>opuD</i>	BCCT, betaine/carnitine/choline family transporter	-	ko:K05020	2.A.15.1.1, 2.A.15.1.11
	<i>proA</i>	Catalyzes the NADPH-dependent reduction of L-glutamate 5-phosphate into L-glutamate 5-semialdehyde and phosphate.	1.2.1.41	ko:K00147	-

	<i>proB</i>	Catalyzes the transfer of a phosphate group to glutamate to form L-glutamate 5-phosphate	2.7.2.11	ko:K00931	-
	<i>proC</i>	Catalyzes the reduction of 1-pyrroline-5-carboxylate (PCA) to L-proline	1.5.1.2	ko:K00286	-
	<i>proP</i>	proline betaine transporter	-	ko:K03762	2.A.1.6.4
	<i>betL</i>	Belongs to the BCCT transporter (TC 2.A.15) family	-	ko:K05020	2.A.15.1.1, 2.A.15.1.11
	<i>glnA</i>	Glutamine synthetase	6.3.1.2	ko:K01915	-
	<i>glnR</i>	transcriptional	-	ko:K03713, ko:K15580	3.A.1.5.1, 3.A.1.5.18, 3.A.1.5.19, 3.A.1.5.25
	<i>glnQ</i>	ABC transporter, ATP-binding protein	3.6.3.21	ko:K02028, ko:K16960, ko:K16963	3.A.1.3, 3.A.1.3.13
	<i>putP</i>	Belongs to the sodium solute symporter (SSF) (TC 2.A.21) family	-	ko:K03307, ko:K11928	2.A.21, 2.A.21.2
Oxidative Stress Response	<i>dps</i>	Belongs to the Dps family	-	ko:K04047	-
	<i>katA</i>	serves to protect cells from the toxic effects of hydrogen peroxide	1.11.1.6	ko:K03781	-
	<i>katE</i>	serves to protect cells from the toxic effects of hydrogen peroxide	1.11.1.6	ko:K03781	-

Molecular Chaperones and Proteases	<i>groL</i>	Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions	-	ko:K04077	-
	<i>groS</i>	Binds to Cpn60 in the presence of Mg-ATP and suppresses the ATPase activity of the latter	-	ko:K04078	-
	<i>clpP</i>	Cleaves peptides in various proteins in a process that requires ATP hydrolysis.	3.4.21.92	ko:K01358	-
	<i>clpB</i>	Part of a stress-induced multi-chaperone system in cooperation with DnaK, DnaJ and GrpE	-	ko:K03695	-
Proton Pumps and ATP Synthases	<i>atpA</i>	Produces ATP from ADP in the presence of a proton gradient across the membrane. The alpha chain is a regulatory subunit	3.6.3.14	ko:K02111	3.A.2.1
	<i>atpB</i>	it plays a direct role in the translocation of protons across the membrane	-	ko:K02108	3.A.2.1
	<i>atpG</i>	Produces ATP from ADP in the presence of a proton gradient across the membrane.	-	ko:K02115	3.A.2.1
	<i>atpH</i>	F(1)F(0) ATP synthase produces ATP from ADP in the presence of a proton or sodium gradient.	-	ko:K02113	3.A.2.1
Efflux Pumps and Membrane Proteins	<i>phoR</i>	Histidine kinase	2.7.13.3	ko:K07636	-
Other Relevant Genes	<i>phoP</i>	Alkaline phosphatase synthesis transcriptional regulatory protein	-	ko:K07658	-

Table S4 Lignin-degrading enzyme-related genes of strain HZC-1 based on GO functional annotation

GO function	GO ID	GO ontologies	Number
Benzene-containing compound metabolic process	GO:0042537	Biological process	2
Carbohydrate metabolic process	GO:0005975	Biological process	22
Carbon-carbon lyase activity	GO:0016830	Molecular function	12
Carbon-oxygen lyase activity	GO:0016835	Molecular function	10
Catalase activity	GO:0004096	Molecular function	1
Copper ion binding	GO:0005507	Molecular function	2
Dioxygenase activity	GO:0051213	Molecular function	2
Heme binding	GO:0020037	Molecular function	2
Lyase activity	GO:0016829	Molecular function	30
Manganese ion binding	GO:0030145	Molecular function	2
Oxidoreductase activity	GO:0016491	Molecular function	43
Peroxidase activity	GO:0004601	Molecular function	1
Reactive oxygen species metabolic process	GO:0072593	Biological process	3
Xenobiotic metabolic process	GO:0017144	Biological process	34
Xenobiotic transport	GO:0015893	Biological process	7
Prephenate dehydrogenase activity	GO:0008977	Molecular function	1

Table S5 Lignin-degrading pathways of strain HZC-1 based on the KEGG database

EC	KEGG ko	KEGG Pathway	Description
2.5.1.54, 5.4.99.5	ko:K03856, ko:K13853	ko00400, ko01100, ko01110, ko01130, ko01230, map00400, map01100, map01110, map01130, map01230	Chorismate mutase
4.2.1.51	ko:K04518	ko00400, ko01100, ko01110, ko01130, ko01230, map00400, map01100, map01110, map01130, map01230	Prephenate dehydratase
-	-	-	Flavin oxidoreductase
1.5.1.36, 1.7.1.6	ko:K03206, ko:K19784, ko:K22393	ko00740, ko01100, map00740, map01100	Catalyzes the reductive cleavage of azo bond in aromatic azo compounds to the corresponding amines. Requires NADPH, but not NADH, as an electron donor for its activity (By similarity)

-	ko:K01118	-	Catalyzes the reductive cleavage of azo bond in aromatic azo compounds to the corresponding amines. Requires NADH, but not NADPH, as an electron donor for its activity
4.1.3.39	ko:K01666	ko00360, ko00362, ko00621, ko00622, ko01100, ko01120, ko01220, map00360, map00362, map00621, map00622, map01100, map01120, map01220	Catalyzes the retro-aldol cleavage of 4-hydroxy-2- oxopentanoate to pyruvate and acetaldehyde. Is involved in the meta-Cleavage pathway for the degradation of aromatic compounds
1.2.1.10	ko:K04073	ko00360, ko00362, ko00620, ko00621, ko00622, ko00650, ko01100, ko01120, ko01220, map00360, map00362, map00620, map00621, map00622, map00650, map01100, map01120, map01220	Catalyzes the conversion of acetaldehyde to acetyl-CoA, using NAD and coenzyme A. Is the final enzyme in the meta-cleavage pathway for the degradation of aromatic compounds

-	ko:K04750	-	Glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily
1.13.11.11	ko:K00453	ko00380, ko01100, map00380, map01100	Heme-dependent dioxygenase that catalyzes the oxidative cleavage of the L-tryptophan (L-Trp) pyrrole ring and converts L- tryptophan to N-formyl-L-kynurenine. Catalyzes the oxidative cleavage of the indole moiety
1.13.11.2	ko:K07104	ko00361, ko00362, ko00622, ko00643, ko01100, ko01120, ko01220, map00361, map00362, map00622, map00643, map01100, map01120, map01220	Glyoxalase bleomycin resistance protein dioxygenase superfamily protein

Table S6 Differential metabolites of lignin degradation by strain HZC-1

ID	Metabolite	Formula	KEGG Pathway ID	Regulate
pos_3189	Anthranilic Acid	C ₇ H ₇ NO ₂	map01100;map01220;map00996; map00997;map01120;map00627; map00998;map00999;map01230; map00621;map01110;map00400; map00380;map00405	Up
pos_3333	<i>L</i> -Tyrosine	C ₉ H ₁₁ NO ₃	map01100;map01110;map00350; map01240;map00730;map00130; map01210;map00998;map00999; map01230;map00680;map00460; map00997;map00261;map00360; map00970;map01055;map00400; map00401;map01059	Up
neg_652	<i>L</i> -Malic Acid	C ₄ H ₆ O ₅	map01100;map00630;map01110; map01120;map01200;map00710; map00680;map00020;map02020; map00620;map00720	Up
neg_1367	2-Isopropylmalic Acid	C ₇ H ₁₂ O ₅	map01100;map01110;map00290; map01210;map00620;map01230	Up

neg_1410	Benzoquinoneacetic Acid	C ₈ H ₆ O ₄	-	Up
neg_1464	Ethyl Syringate	C ₁₁ H ₁₄ O ₅	-	Up
neg_1578	3-Hydroxysebacic Acid	C ₁₀ H ₁₈ O ₅	-	Up
neg_5012	4-Hydroxybenzoic Acid	C ₇ H ₆ O ₃	map01100;map01220;map01110; map01120;map01240;map00627; map00130;map00623;map00363; map00362;map00790	Up
neg_5049	2-Hydroxysebacic Acid	C ₁₀ H ₁₈ O ₅	-	Up
neg_5071	Vanillic Acid	C ₈ H ₈ O ₄	map00627;map01120	Up
neg_5096	Ferulic Acid	C ₁₀ H ₁₀ O ₄	map01100;map00996;map01110	Up
neg_5110	Phenylpyruvic Acid	C ₉ H ₈ O ₃	map01100;map01110;map01210; map00470;map01230;map00360;map00400	Up
neg_5548	2-Methylsuccinic Acid	C ₅ H ₈ O ₄	-	Up

neg_5802	Fumaric Acid	$C_4H_4O_4$	map01100;map01220;map00220; map01110;map01120;map00650; map00190;map01200;map02020; map00760;map00250;map00620; map00350;map00020;map00360; map00643;map00720	Up
neg_5763	Succinic Acid	$C_4H_6O_4$	map01100;map01220;map00310; map01110;map00640;map00920; map01120;map00630;map01200; map00620;map00760;map00361; map00250;map02020;map00360; map00350;map00020;map00650; map00190;map00720	Up