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Cold- and salt-tolerant hydrolase-producing bacteria from the high-altitude cold desert of Ladakh, India: Characterization and comparative *in silico* analysis

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SUPPLEMENTARY FILE

Table of Contents

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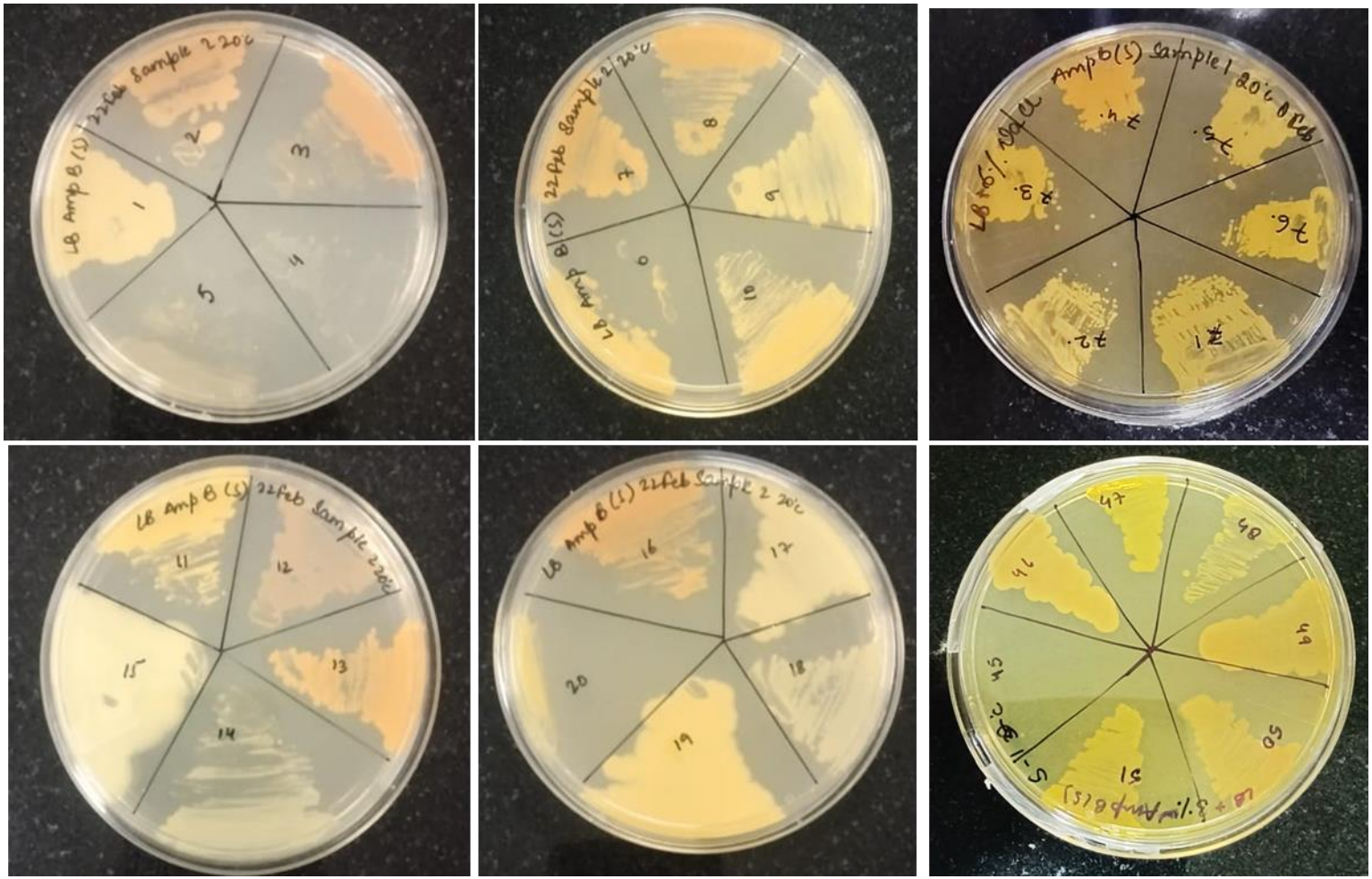

Supplementary Figure S1: Sector-plate representing bacterial isolates recovered from Pangong Lake and Indus River samples. The plates were divided into sectors to evaluate multiple isolates simultaneously.

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Supplementary Table S1: Ecological source and macroscopic phenotypes of primary high-altitude isolates

Temp	Salinity	Colony No.	Size	Colour	Shape	Elevation	Texture	Margin
Indus River								
37°C	0.90%	1	moderate	transparent	round	raised	glistening	entire
		2	moderate	cream	round	raised	glistening	entire
		3	small	cream	irregular	raised	matte	undulated
		4	big	white	round	raised	slimy	entire
		5	moderate	yellow	round	raised	slimy	entire
		6	big	red	irregular	raised	slimy	undulated
		7	moderate	yellow	round	raised	slimy	entire
		8	small	yellow	round	raised	matte	entire
		9	very small	yellow	round	raised	slimy	entire
		10	very small	yellow	round	raised	glistening	entire
		11	big	transparent	round	raised	slimy	entire
		12	very small	white	round	raised	slimy	entire
		13	very small	transparent	round	raised	slimy	entire
		14	small	transparent	round	raised	slimy	entire
		15	small	yellow	round	raised	glistening	entire
		16	moderate	white	round	raised	slimy	irregular
		17	small	yellow	round	raised	matte	entire
		18	mall	cream	round	convex	matte	entire
		19	moderate	transparent	irregular	raised	slimy	irregular
		20	small	orange	punctiform	raised	matte	undulated
		21	very small	yellow	round	raised	matte	entire
		22	big	white	round	raised	slimy	entire
		23	big	red	irregular	raised	slimy	undulated
		24	small	cream	round	raised	matte	entire
		25	small	salmon orange	round	raised	glistening	entire

		26	big	transparent	irregular	raised	slimy	undulated
		27	big	white	round	raised	slimy	entire
	3%	57	small	pink	round	raised	slimy	entire
		58	small	transparent	round	raised	slimy	entire
		59	very small	white	round	raised	slimy	entire
		107	moderate	pink	round	raised	slimy	entire
		108	moderate	cream	round	raised	slimy	entire
	6%	67	big	off-white	round	raised	slimy	entire
		68	moderate	yellow	irregular	raised	slimy	undulated
		69	moderate	brown	round	raised	slimy	entire
		70	moderate	yellow	round	raised	slimy	entire
	9%	80	small	white	round	raised	slimy	entire
		81	small	white	round	raised	glistening	entire
		82	moderate	orange	round	raised	glistening	entire
20°C	0.90%	1	moderate	yellow	round	raised	slimy	entire
		2	small	salmon orange	round	raised	slimy	entire
		3	small	orange	round	raised	slimy	entire
		4	small	transparent	round	raised	slimy	entire
		5	small	transparent	round	raised	slimy	entire
		6	moderate	yellow	round	raised	slimy	entire
		7	moderate	salmon orange	round	raised	slimy	entire
		8	very small	salmon orange	round	raised	slimy	entire
		9	very small	yellow	round	raised	slimy	entire
		10	moderate	yellow	round	raised	slimy	entire
		11	moderate	yellow	round	raised	slimy	entire
		12	big	red	round	raised	slimy	entire
		13	moderate	salmon orange	round	raised	slimy	entire
		14	moderate	cream	round	raised	slimy	entire
		15	big	white	round	raised	slimy	entire
		16	very small	red	round	raised	slimy	entire
		17	moderate	yellow	round	raised	slimy	entire

		18	very small	cream	round	raised	slimy	entire
		19	moderate	white	round	raised	slimy	entire
		20	moderate	yellow	round	raised	slimy	entire
	3%	60	moderate	white	round	raised	slimy	entire
		109	big	yellow	round	raised	matte	entire
	6%	110	big	white	round	raised	slimy	entire
		111	moderate	yellow	round	raised	slimy	entire
	9%	83	small	off-white	round	raised	glistening	entire
		84	small	white	round	raised	slimy	entire
Pangong Lake								
37°C	0.90%	1	moderate	white	round	raised	slimy	entire
		2	moderate	yellow	round	raised	slimy	entire
		3	big	white	round	raised	matte	entire
		4	small	yellow	round	raised	slimy	entire
		5	small	white	round	raised	slimy	entire
		6	small	light red	round	raised	slimy	entire
		7	small	yellow	irregular	raised	glistening	undulated
		8	moderate	yellow	irregular	raised	slimy	undulated
		9	small	white	round	raised	slimy	undulated
		10	big	yellow	round	raised	slimy	entire
		11	small	yellow	round	raised	slimy	entire
	3%	45	moderate	transparent	irregular	raised	slimy	entire
		46	moderate	white	round	raised	slimy	entire
		47	small	yellow	round	raised	slimy	entire
		48	very small	cream	round	raised	slimy	entire
		49	big	yellow	round	raised	slimy	entire
		50	small	salmon orange	round	raised	slimy	entire
		51	very small	yellow	round	raised	slimy	entire
	6%	61	small	off-white	round	raised	slimy	entire
		62	small	yellow	round	raised	slimy	entire
		63	very small	off-white	round	raised	slimy	entire

		64	very small	yellow	round	raised	slimy	entire
		65	moderate	orange	round	raised	slimy	entire
		66	very small	yellow	round	raised	slimy	entire
	9%	77	very small	off-white	round	raised	slimy	entire
		78	very small	white	round	raised	glistening	entire
	12%	85	small	off-white	round	raised	slimy	entire
		113	small	yellow	round	raised	slimy	entire
20°C	0.90%	39	very small	yellow	round	raised	slimy	entire
		40	moderate	cream	round	raised	matte	entire
		41	small	cream	round	raised	matte	entire
		42	very small	yellow	round	raised	matte	entire
		43	small	cream	round	raised	matte	entire
		44	moderate	yellow	round	raised	slimy	entire
	3%	52	small	cream	round	raised	slimy	entire
		53	small	yellow	round	raised	slimy	entire
		54	small	yellow	round	raised	slimy	entire
		55	very small	off-white	round	raised	slimy	entire
		56	moderate	yellow	round	raised	slimy	entire
	6%	71	moderate	white	round	raised	slimy	entire
		72	small	white	round	raised	slimy	entire
		73	moderate	yellow	round	raised	slimy	entire
		74	small	peach	round	raised	slimy	entire
		75	moderate	white	round	raised	slimy	entire
		76	moderate	yellow	round	raised	slimy	entire
	9%	79	very small	off-white	round	raised	slimy	entire
	12%	86	small	creamy	round	raised	slimy	entire

Supplementary Table S2: Semi-quantitative extracellular enzymatic profiling of primary isolates (zone of clearance in mm)

Temp (°C)	Colony No.	Amylase	Protease	Endoglucanase	Xylanase
Indus River					
20	1	8	7	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	11	-	-
	7	-	-	-	-
	8	-	-	-	-
	9	-	-	-	-
	10	-	-	-	-
	11	-	-	-	-
	12	-	-	-	-
	13	-	12.5	-	-
	14	-	-	-	-
	15	13	16	-	-
	16	9	13	-	-
	17	-	-	10	-
	18	-	-	-	-
	19	5	8	-	-
	20	-	-	-	-
	57	-	-	-	-
	67	-	15.5	-	-
	109	-	17	-	-
37	1	-	-	-	5.5
	2	5	-	11	5
	3	-	-	-	-
	4	-	15	-	6

	5	11	-	-	-
	6	5.5	10	9	12
	7	-	13	-	10
	8	12	-	-	15
	9	6	16.5	-	-
	10	-	-	6	8
	11	-	-	-	-
	12	12	12	-	8.5
	13	-	-	-	-
	14	-	-	-	-
	15	14	14	-	15.5
	16	12	12	-	11
	17	-	-	-	-
	18	5	-	7	-
	19	-	-	-	6
	20	-	-	-	-
	21	-	14.5	7.5	13
	22	13	11.5	-	9.5
	23	-	10	-	-
	24	-	-	-	-
	25	10	-	-	11
	26	-	12.5	-	-
	27	-	14	-	-
	58	8.5	-	-	-
	59	-	-	-	-
	60	6.5	-	-	7
	68	5	-	-	14
	69	5	-	-	8
	70	6	-	-	6.5
	80	-	-	-	-
	81	-	-	-	-

	82	6	16	-	8
	107	6	-	-	-
	108	-	-	-	-
Pangong Lake					
20	39	-	-	-	-
	40	-	-	-	-
	41	-	-	-	-
	42	5	-	-	-
	43	6	-	+	-
	44	15	-	5.5	-
	52	10	12	-	-
	53	-	10	-	-
	54	8	-	6	-
	55	-	12.5	-	-
	56	10	-	-	-
	71	-	-	-	-
	72	-	-	-	-
	73	-	-	-	-
	74	-	-	7	-
	75	5	-	-	-
	76	5	-	-	-
	79	-	-	-	-
	86	-	-	-	-
37	1	-	-	-	-
	2	5	17	-	-
	3	-	-	-	10
	4	6	-	-	12
	5	5.4	-	-	-
	6	-	-	-	-
	7	5.8	-	12	-
	8	-	-	-	11.5

	9	13	-	-	13
	10	8.5	-	-	14
	11	7	-	-	-
	45	-	-	-	-
	46	-	-	-	-
	47	-	11.5	-	-
	48	-	-	-	-
	49	16	-	5	5
	50	-	7.5	-	-
	51	-	-	-	-
	61	-	-	-	-
	62	-	-	-	-
	63	11	-	-	-
	64	8.5	-	-	7.5
	65	-	-	-	-
	66	6.5	-	-	-
	77	9	-	-	-
	78	-	-	-	-
	85	-	-	-	-
	113	-	-	-	-

Halo-zone diameters were measured in triplicate and reported as mean values, used as a comparative semi-quantitative descriptor for ranking and prioritizing isolates for downstream characterization. Error was found in the range of 5%.

Supplementary Table S3: Enzymatic activity of the isolates at different parameters

Source	**Indus River**			**Pangong Lake**		
Strains	**XBL43**	**XBL45**	**XBL48**	**XBL47**	**XBL49**	**XBL50**
Amylase						
Salinity range (% w/v)	5-15	2-10	-	5-15	3-12	-
Optimum salinity (% w/v)	6	5	-	8	7	-
pH range	6.0-9.0	5.0-8.0	-	4.0-7.0	7.0-11.0	-
Optimum pH	8.0	6.5	-	6.0	9.0	-
Temperature range (ºC)	10-50	15-55	-	10-40	30-55	-
Optimum temperature (ºC)	30	30	-	25	40	-
Protease						
Salinity range (% w/v)	4-12	-	1-9	2-10	-	4-14
Optimum salinity (% w/v)	6	-	5	6	-	8
pH range	6.0-9.0	-	5.0-9.0	7.0-11.0		6.0-9.0
Optimum pH	8.0	-	7.5	9.0	-	7.0
Temperature range (ºC)	10-55	-	20-55	10-50	-	15-40
Optimum temperature (ºC)	30	-	40	30	-	30
Endoglucanase						
Salinity range (% w/v)	-	-	-	-	4-12	-
Optimum salinity (% w/v)	-	-	-	-	7	-
pH range	-	-	-	-	5.0-8.0	-
Optimum pH	-	-	-	-	6.5	-
Temperature range (ºC)	-	-	-	-	25-55	-
Optimum temperature (ºC)	-	-	-	-	45	-
Xylanase						
Salinity range (% w/v)	4-12	1-8	2-10	0.9-7	3-11	-
Optimum salinity (% w/v)	6	4	6	3	7	-
pH range	6.0-10.0	5.0-8.0	6.0-10.0	4.0-7.0	7.0-11.0	-
Optimum pH	8.0	6.5	8.0	5.5	9.0	-
Temperature range (ºC)	10-50	15-50	20-55	10-40	30-50	-
Optimum temperature (ºC)	35	35	45	25	35	-

All enzyme assays were performed in triplicate and the mean values were reported.