

Diversity and plasticity of simultaneously expressing extracellular protease inhibitors from four marine *Streptomyces* spp. isolated from West coast of India

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Supplementary Table 1:

Shortlisted PI producers for characterization and identification studies

Sr. No.	Putative species name	Abberv. used	Original code	GeneBank number	Location/ Source of isolation
1	<i>Streptomyces euryhalinus</i>	<i>S. eu</i>	AGC103	MN339845	Ade/Intertidal Sediment
2	<i>Streptomyces albogriseolus</i>	<i>S. al</i>	AGC145	MN339861	Harnai/Intertidal Sediment
3	<i>Streptomyces</i> sp. 13650 C	<i>S. sp</i>	AGC157	MN339864	Ade/Sponge
4	<i>Streptomyces longispororuber</i>	<i>S. lo</i>	AGC190	MN339882	Goa/Subtidal Sediment

Supplementary Table 2:

R_f values of stained and activity bands of subtilisin inhibition (SI), trypsin inhibition (TI) and chymotrypsin inhibition (CI) on Native-PAGE gels

Isolate	R _f values of Stained bands	R _f values of activity bands		
		SI	TI	CI
<i>S. eu</i>	0.192	0.189	0.14	0.26
<i>S. al</i>	0.500	0.491	-	-
<i>S. sp</i>	0.365	0.346	0.355	0.364
<i>S. lo</i>	0.156	0.160	0.162	0.171

Supplementary Table 3:

Enzymes used for *in-silico* analysis to check PI activities of *S. euryhalinus* (*S.eu*), *S. albogriseolus* (*S.al*), *S. sp. 13650 C* (*S.sp*) and *S. longispororuber* (*S.lo*) and their significance in the current study

Sr. No	Protease enzyme source	PDB ID	Significance in current study
1	HIV protease	4NKK	Commercial
2	<i>Helicoverpa</i> sp.	1JQG	Commercial
3	PlasmeprinII (from malarial parasite)	1LF4	Commercial
4	<i>Nocardioopsis</i> sp.	2OUA	Ecological
5	SARS-CoV 2 main protease	6W79	Commercial
6	<i>Pseudoalteromonas</i> sp.	1V6C	Ecological
7	ACE	1O86	Ecological and Commercial
8	<i>Pseudomonas</i> sp.	1G9K	Ecological and Commercial
9	<i>Vibrio</i> sp.	1SH7	Ecological and Commercial
10	<i>Bacillus</i> sp.	1P3E	Ecological and Commercial
11	Falcipain (from malarial parasite)	2GHU	Commercial

Supplementary Table 4:

Enzymes used for *in-vitro* assays to check PI activities of *S. euryhalinus* (*S.eu*), *S. albogriseolus* (*S.al*), *S. Sp. 13650 C* (*S.sp*) and *S. longispororuber* (*S.lo*)

Sr. No.	Organism	Source	Significance	Preparation used
1.	<i>Pseudomonas aeruginosa</i> (MN044759)	Oil spill near oil well refinery	Human as well as marine pathogen	Cell free supernatant
2.	<i>Pseudomonas aeruginosa</i> (MTCC 2295)	MTCC, India	Human as well as marine pathogen	Cell free supernatant
3.	<i>Vibrio harveyi</i> (MTCC 7779)	MTCC, India	Marine pathogen	Cell free supernatant

4.	<i>Vibrio alginolyticus</i> (KU612885)	Isolated from infected prawns	Human as well as marine pathogen	Cell free supernatant
5.	<i>Nocardiosis alba</i> (MN339742)	Sediments of Ade	Share same habitats as PI producers	Cell free supernatant
6.	<i>Nocardiosis alba</i> (MN339743)	Sediments of Harnai	Share same habitats as PI producers	Cell free supernatant
7.	<i>Helicoverpa armigera</i>	Reared insects	Potent crop pest	Gut extract

Supplementary Table 5:

Results of molecular docking of different proteases with protease inhibitors of *S. euryhalinus* (*S.eu*), *S. albogriseolus* (*S.al*), *S. sp. 13650 C* (*S.sp*) and *S. longispororuber* (*S.lo*)

Protease source	Measures	SSI inhibitor I16	Potato inhibitor I13	Kazal inhibitor I01	Serpin inhibitor I04
HIV	Residues in a cluster	81	195	127	64
	Weighted Score	-870.7	-866.9	-754	-852.3
<i>Bacillus</i> sp.	Residues in a cluster	NA	NA	242	192
	Weighted Score	NA	NA	-609.5	-798.9
<i>Plasmepsin II</i>	Residues in a cluster	89	211	116	139
	Weighted Score	-877	-1043.5	-748.4	-1004.1
<i>Helicoverpa</i> sp.	Residues in a cluster	61	NA	250	NA
	Weighted Score	-948.5	NA	-884.2	NA
<i>Pseudoalteromonas</i> sp.	Residues in a cluster	73	93	53	116
	Weighted Score	-838	-947.8	-711.6	-762.9
<i>Pseudomonas</i> sp.	Residues in a cluster	47	NA	98	86
	Weighted Score	-840.9	NA	-621	-702.9
<i>Vibrio</i> sp.	Residues in a cluster	62	NA	216	NA
	Weighted Score	-743.6	NA	-867.8	NA
<i>Nocardiosis</i> sp.	Residues in a cluster	228	NA	NA	NA
	Weighted Score	-865.6	NA	NA	NA

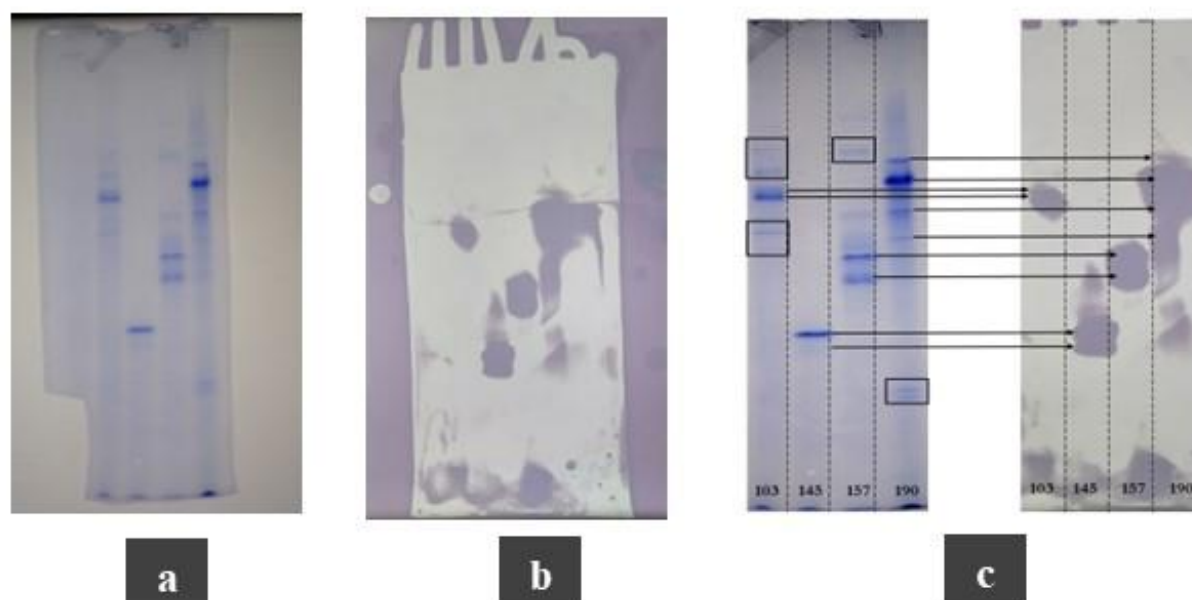
Supplementary Table 6:

Results of molecular docking of different SSI inhibitors obtained from *S. euryhalinus* (*S.eu*), *S. albogriseolus* (*S.al*), *S. sp. 13650 C* (*S.sp*) and *S. longispororuber* (*S.lo*) against *Nocardioopsis* serine protease

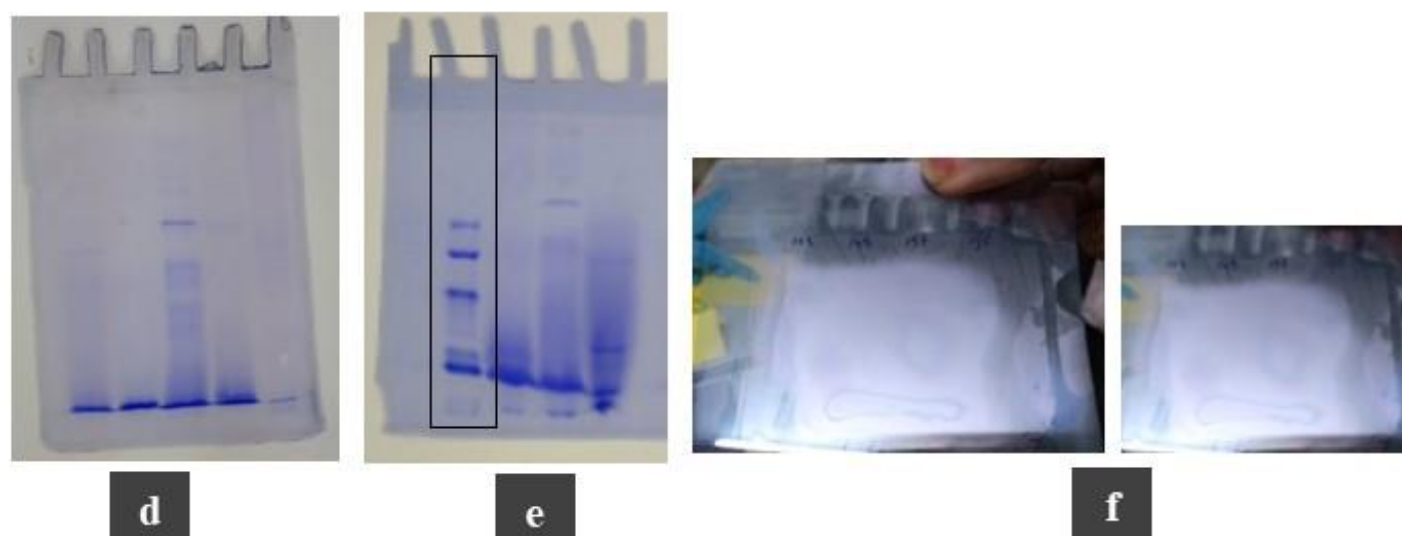
Sr. No.	Name	Mass (Da)	Members	Weighted Score	Organism
1	9ACTN Probable subtilase-type protease inhibitor	14,200	58	-708.2	<i>S.al</i>
2	SSI_STREU Protease inhibitor SIL-V3	11,378	84	-666.2	<i>S.lo</i>
3	SSI_STRFR subtilase-type protease inhibitor	14,712	112	-645.4	<i>S.lo</i>
4	SSI_STRGI Alkaline protease inhibitor	10900	121	-667.5	<i>S.sp</i>
5	SSI_STRLI Protease inhibitor	14,433	72	-788.4	<i>S.lo</i>
6	SSI_STRON Protease inhibitor SIL-V2	11,489	80	-604.7	<i>S.lo</i>
7	SSI1_STRCI Subtilisin inhibitor-like protein	11600	124	-686.6	<i>S.sp</i>
8	SSI2_STRLO Trypsin inhibitor STI2	14,548	75	-793.9	<i>S.lo</i>
9	SSI4_STRLA Subtilisin inhibitor-like protein 4	11,784	79	-689.3	<i>S.lo</i>
10	SSI5_STRFR Subtilisin inhibitor-like	11300	71	-677.6	<i>S.sp</i>
11	SSI8_STRVG Subtilisin inhibitor-like protein	11,436	156	-861.2	<i>S.eu, S.lo</i>
12	SSIX_STRAW Putative serine protease inhibitor SAV_2156	15,237	123	-829.8	<i>S.lo</i>

Supplementary Figure 1

For Figure 2 a: Original images of stained (a) and corresponding activity in Native gel (b) and comparison of inhibition bands with respect to stained bands (c)



For Figure 2 b: Original images of stained SDS-PAGE (a); resolved protein ladder (marked) (b) and untampered images of corresponding activity in SDS-PAGE gel (c)



For Figure 6 c: Original images of spot assay performed using PIs of *S. euryhalinus* (103), *S. albogriseolus* (145), *S. sp. 13650 C* (157) and *S. longisororuber* (190) against crude proteases of *V. Harvey* (VH), *V. alginolyticus* (VA), *P. aeruginosa* (PA) and *N. alba* (NA)

