

**Screening of Neutrophil Activating Factors from a Metagenome
Library of Sponge-Associated Bacteria (TENTATIVE)**

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Supplemental Figures and Tables

Supplemental Table S1. List of selected clones by *in silico* screening

Clone #	Register #	Originated library	annotation						
1	201-B10	Styllisa massa Fosmid	Ubiquitin-protein ligase						
2	204-H07	Styllisa massa Fosmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
3	96-E02	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
4	314-A11	Styllisa massa Fosmid	Ubiquitin-protein ligase						
5	165-G12	Styllisa massa Fosmid	PPE-repeat proteins; Predicted ATPase (AAA+ superfamily)						
6	167-H02	Styllisa massa Fosmid	Predicted ATPase (AAA+ superfamily)						
7	168-F05	Styllisa massa Fosmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
8	101-F08	Styllisa massa Fosmid	UDP-N-acetylmuramyl pentapeptide synthase						
9	101-A09	Styllisa massa Fosmid	UDP-N-acetylmuramyl pentapeptide synthase						
10	224-H06	Styllisa massa Fosmid	UDP-N-acetylmuramyl pentapeptide synthase						
11	97-D01	Styllisa massa Fosmid	MoxR-like ATPases; AAA ATPase containing von Willebrand factor type A (vWA) domain						
12	97-G03	Styllisa massa Fosmid	Phosphoribosylaminoimidazole (AIR) synthetase						
13	372-F11	Styllisa massa Fosmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
14	40-F03	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
15	62-E09	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
16	63-E06	Hytios erecta plasmid	Folypolyglutamate synthase						
17	65-F04	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
18	66-D10	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
19	67-B03	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
20	24-H07	Hytios erecta plasmid	Folypolyglutamate synthase						
21	27-G11	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
22	28-F03	Hytios erecta plasmid	Predicted unusual protein kinase						
23	30-B01	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
24	31-F12	Hytios erecta plasmid	UDP-N-acetylmuramate-alanine ligase						
25	201-F06	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
26	22-D07	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
27	23-D12	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
28	81-G09	Hytios erecta plasmid	UDP-N-acetylmuramate-alanine ligase						
29	85-B10	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
30	85-D06	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase; UDP-N-acetylmuramylalanine-D-glutamate ligase						
31	89-B02	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
32	91-C11	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
33	93-D03	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
34	179-F03	Styllisa massa Fosmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
35	181-F01	Styllisa massa Fosmid	Folypolyglutamate synthase						
36	181-G02	Styllisa massa Fosmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
37	189-B09	Hytios erecta plasmid	UDP-N-acetylmuramate-alanine ligase						
38	192-B03	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
39	193-C12	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
40	204-G09	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
41	206-H01	Hytios erecta plasmid	Folypolyglutamate synthase						
42	210-D04	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
43	210-D11	Hytios erecta plasmid	Folypolyglutamate synthase						
44	212-G09	Hytios erecta plasmid	Folypolyglutamate synthase						
45	213-G03	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase; UDP-N-acetylmuramyl pentapeptide synthase						
46	214-H01	Hytios erecta plasmid	Folypolyglutamate synthase						
47	2-B09	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
48	4-C03	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
49	4-D10	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
50	6-H02	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
51	12-C03	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
52	13-A04	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
53	33-C05	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
54	38-F05	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
55	52-C04	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
56	54-D09	Hytios erecta plasmid	Folypolyglutamate synthase						
57	54-F05	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
58	58-C04	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
59	69-B06	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
60	72-E08	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
61	73-B12	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
62	73-H09	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
63	78-C05	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
64	96-D08	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
65	96-E02	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
66	97-B04	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins; Peptide arylamidases						
67	100-G12	Hytios erecta plasmid	5-formyltetrahydrofolate cycle-ligase						
68	139-F08	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
69	141-C04	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
70	143-C01	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
71	143-G03	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase; UDP-N-acetylmuramyl tripeptide synthase						
72	186-F06	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
73	103-C09	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
74	104-D10	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
75	104-F11	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
76	105-F10	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
77	112-A08	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
78	114-G03	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
79	114-C10	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
80	120-F09	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
81	121-F11	Hytios erecta plasmid	Folypolyglutamate synthase						
82	123-H09	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins;						
83	125-D02	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
84	129-A07	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
85	137-B09	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase; UDP-N-acetylmuramyl pentapeptide synthase						
86	140-B04	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
87	18-C04	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
88	146-C03	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
89	147-E02	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
90	147-F11	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
91	150-F01	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
92	152-E10	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
93	161-B04	Hytios erecta plasmid	UDP-N-acetylmuramate-alanine ligase						
94	164-H10	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
95	165-H12	Hytios erecta plasmid	Folypolyglutamate synthase						
96	169-H12	Hytios erecta plasmid	UDP-N-acetylmuramylalanine-D-glutamate ligase						
97	174-A03	Hytios erecta plasmid	Folypolyglutamate synthase						
98	183-A02	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins;						
99	178-A01	Hytios erecta plasmid	Predicted ATPase (AAA+ superfamily)						
100	175-G11	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
101	181-H05	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
102	184-F03	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
103	180-A04	Hytios erecta plasmid	FOG: Arkyrin repeat						
104	180-B10	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins;						
105	171-F08	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
106	22-F10	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
107	20-D11	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
108	19-A04	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						
109	45-H06	Hytios erecta plasmid	Predicted unusual protein kinase						
110	53-F11	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
111	61-G06	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
112	59-G11	Hytios erecta plasmid	Folate-dependent phosphoribosylglycinamide formyltransferase PurN						
113	57-H05	Hytios erecta plasmid	UDP-N-acetylmuramate-alanine ligase						
114	05-H04	Hytios erecta plasmid	D-alanine-D-alanine ligase and related ATP-grasp enzymes						
115	32-F04	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
116	34-D08	Hytios erecta plasmid	Non-ribosomal peptide synthetase modules and related proteins						
117	39-H09	Hytios erecta plasmid	UDP-N-acetylmuramyl pentapeptide synthase						
118	40-D11	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase						
119	41-B06	Hytios erecta plasmid	UDP-N-acetylmuramyl tripeptide synthase; UDP-N-acetylmuramyl pentapeptide synthase						
120	42-H04	Hytios erecta plasmid	Formyltetrahydrofolate synthetase						

Supplemental Table S2. List of primers designed for gene expression

No.	Name	sequence
1	EcoRI_ORF1_-2F	GAG AGA <u>GAA TTC</u> TCA GTG CTC GAG GCC TCG TCC CAT GGC
2	EcoRI_ORF2_1F	GAG AGA <u>GAA TTC</u> ATG CAC TCC TGG TTG CCG GCA AGG GGC
3	EcoRI_ORF3_1F	GAG AGA <u>GAA TTC</u> ATG CCG GCC GCC ACT TCA GCG GCC GTA TTC
4	EcoRI_ORF4_1F	GAG AGA <u>GAA TTC</u> ATG CTG TTC CAT TTC CTG GTT CCG CTC
5	NotI_ORF1_754R	GAG AGA <u>GCG GCC GCT</u> CAG GCG CTC CCC GCA GCA CCG GCA CG
5	SbfI(PstI)_ORF1_754R	GAG AGA <u>CCT GCA GGT</u> CAG GCG CTC CCC GCA GCA CCG GCA CG
6	XhoI_ORF2_456R	GAG AGA <u>CTC GAG</u> TCA GCT CCT TGG TGC CGG TCT TGC CGA C
7	NotI_ORF3_936R	GAG AGA <u>GCG GCC GCT</u> CAG TGC GCC TTC GAC CCG GTG ACC CG
7	SbfI(PstI)_ORF3_936R	GAG AGA <u>CCT GCA GGT</u> CAG TGC GCC TTC GAC CCG GTG ACC CG
8	NotI-PstI_ORF4_693R	GAG AGA <u>GCG GCC GCC</u> TGC AGA TAG TCC GCA AAG AAC GCG TTG
2	EcoRI_ORF2_pHSG398	GAG AGA <u>GAA TTC</u> AAT GCA CTC CTG GTT GCC GGC AAG GGG C
3	EcoRI_ORF3_pHSG398	GAG AGA <u>GAA TTC</u> AAT GCC GGC CGC CAC TTC AGC GGC CGT ATT C
4	EcoRI_ORF4_pHSG398	GAG AGA <u>GAA TTC</u> AAT GCT GTT CCA TTT CCT GGT TCC GCT C
9	M13-P8	AGC GGA TAA CAA TTT CAC ACA GGA AAC
10	M13-P7	CGC CAG GGT TTT CCC AGT CAC GAC

The restriction enzyme recognition sequence was indicated by underline.

M13-P8 and M13-P7 are primers derived from the vector sequence, and the restriction enzyme digestion site used is the sequence in the multi-cloning site of the vector.

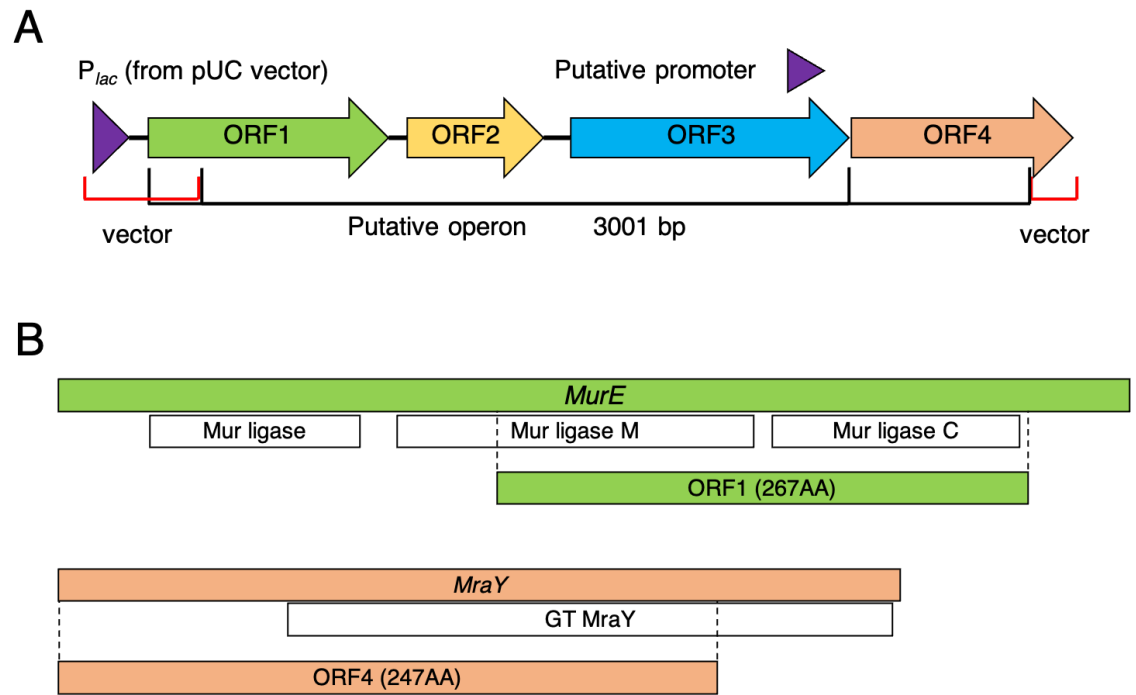


Figure S1. Arrangement of the open reading frames in the metagenomic fragment of clone #115 (A) and the truncated regions of homologous proteins in ORF1 and ORF4 (B).

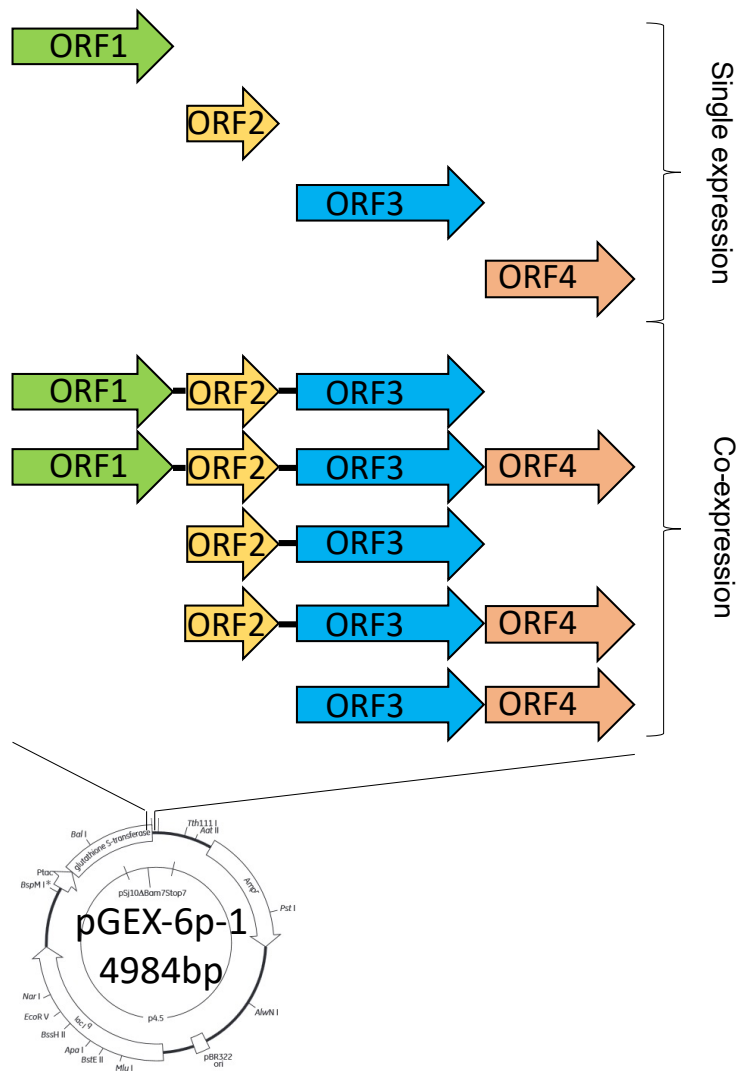


Figure S2. Expression vector constructs for single ORF expression or co-expression. The inserts were amplified by PCR and cloned into MCS of oGEX-6p-1.