

Supporting Information for:

An Indonesian marine bacterium, *Pseudoalteromonas rubra*, produces antimicrobial prodiginine pigments

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Supporting materials and figures

- 1) **Table S1:** *P. rubra* strains that have been deposited in NCBI
- 2) **Figure S1:** The distribution map from 45 strains of *P. rubra* that have been found until now
- 3) **Figure S2:** The number of isolated *P. rubra* found from different sources
- 4) **Figure S3:** The plot of $\log k'$ value of 2-methyl-3-propyl-prodiginine ($k' = -0.605$), 2-methyl-3-butyl-prodiginine ($k' = -0.339$), prodigiosin ($k' = -0.048$), 2-methyl-3-hexyl-prodiginine ($k' = 0.099$), and 2-methyl-3-heptyl-prodiginine ($k' = 0.249$) versus number of the carbon atoms of the alkyl side chains of the pigment molecule showed a linear line with $R^2 = 0.9755$
- 5) **Figure S4:** The comparison mass spectra of standard prodigiosin hydrochloride and purified pigment peak #4 from *P. rubra* strain PS1
- 6) **Figure S5:** ESI-MS/MS analysis of the purified compounds
- 7) **Table S2:** Prodigiosin-producing marine bacteria

1) Table S1

No	<i>P. rubra</i> Strain	Accession Number	Sampling Location	No	<i>P. rubra</i> Strain	Accession Number	Sampling Location
1	ATCC 29570	NR026223	Mediterranean Sea near Nice	24	OCN096	NZLFZX01000000	Kaneohe Bay, Hawaii, USA
2	PS1	LC476556	Sika Island, Alor, Indonesia	25	AN34	JQ409378	Havelock Island, Andaman Sea
3	SB14	LC487904	Sebanjar Beach, Alor, Indonesia	26	B1M	HQ439549	Hawaii, Oahu, Kaneohe Bay, USA
4	SW1	LC435074	Kondang Merak Coast, Malang	27	PC13b	HQ439548	Hawaii, Oahu, Kaneohe Bay, USA
5	SCSIO 6842	CP013611	Bay of Bengal, Indian Ocean	28	G31a	HQ439547	Hawaii, Oahu, Kaneohe Bay, USA
6	1020R	AB373122	Cape Muroto coast, Kochi, Japan	29	PC13d	HQ439541	Hawaii, Oahu, Kaneohe Bay, USA
7	W3	NZRHHZ01000000	Lamma Island, Hongkong	30	PC13c	HQ439540	Hawaii, Oahu, Kaneohe Bay, USA
8	T7	NZRHHY01000000	Lamma Island, Hongkong	31	BRA007	HQ840708	Taiba Beach, Brazile
9	W5	NZRHIA01000000	Lamma Island, Hongkong	32	KJN19_02	LC328972	Karimun Jawa Island, Indonesia
10	Wn5	LT604915	Arabian Sea, West Coast	33	Bu15_31	KY671183	North Sulawesi, Indonesia
11	B32	LC325178	Mediterranean Sea	34	SDP12	MK672867	Japan
12	IGB9	MF356667	Igang, Guimaras, Philippines	35	W240	GU826580	Grand Bahama Island, Bahamas
13	MS01	MF179501	Mexico: Sonora	36	F75064	JF281761	Kavaratti Island, Lakshadweep, India
14	YA-13M-2	KY366346	Micronesia	37	N8AM91	KF193911	Korea
15	GC3	LC435077	Kondang Merak Coast, Malang	38	L-21	HG315019	Goa, Dona Paula Bay, India
16	BF1A IBRL	KP330490	Malaysia	39	S2722	FJ457192	Ghozo Island
17	S4059	FJ457237	Pacific Ocean	40	S2599	FJ457186	Solomon Sea
18	S2678	FJ457189	New Georgia Island	41	S2472	FJ457185	Coral Sea
19	S2676	FJ457188	New Georgia Island	42	S2471	FJ457184	Coral Sea
20	TKA09_02	LC275063	Karimun Jawa Island, Indonesia	43	S1946	FJ457169	Indian Ocean near west Australia
21	UMTBB216	MG896160	Bidong Island, Terengganu, Malaysia	44	S1944	FJ457168	Indian Ocean near west Australia
22	UMTBB120	MG896142	Bidong Island, Terengganu, Malaysia	45	S1943	FJ457167	Indian Ocean near west Australia
23	TKJD22	LC385643	Karimun Jawa Island, Indonesia				

Table S1. *P. rubra* strains that have been deposited in National Center for Biotechnology Information (NCBI) ⁶¹

2) Figure S1

According to data in **Table 1**. The distribution map of *P. rubra* was created using QGIS version 2.18.19 and the ESRI continent base map. As shown as in **Figure S1** below, *P. rubra* has been discovered throughout the region spanning the Indian Pacific to Atlantic Oceans. However, most strains have been found in the Indo-Pacific area.

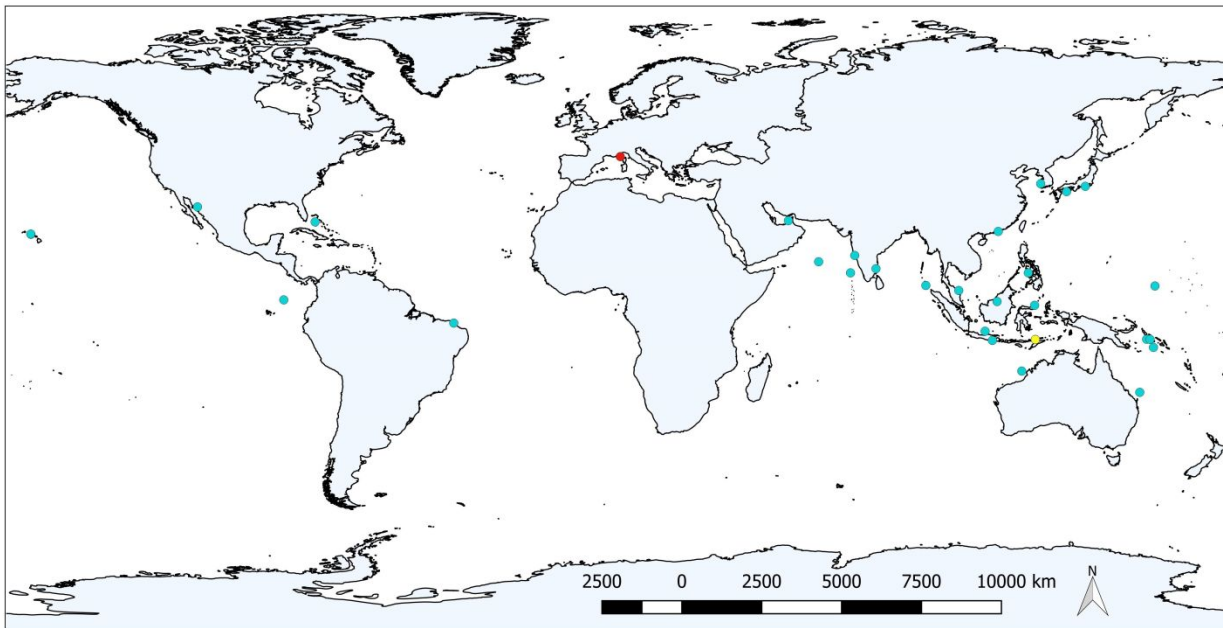


Figure S1. The distribution map from 45 strains of *P. rubra* that have been found until now. Red spot is the first location where *P. rubra* was found. Yellow spot is the sampling location in this study. Whilst blue spots are other locations for the isolated *P. rubra*. The data was obtained from NCBI ⁶¹

3) Figure S2

Based on our literature study in National Center for Biotechnology Information we found that the known 45 strains of *P. rubra* were isolated from various habitats. The graphic is shown in **Figure S2** below.

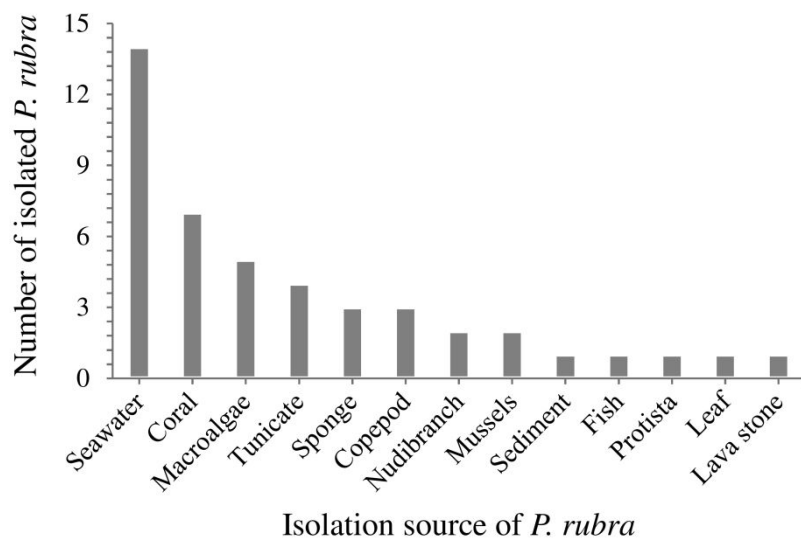


Figure S2. The number of isolated *P. rubra* found from different sources. The data were obtained from NCBI ⁶¹

4) Figure S3

The identification of prodigiosin and four derivatives was confirmed by the linear relationship between the log capacity factor (k') of the pigments and the number of the carbon atoms in the alkyl side chain of the pigment molecule. The capacity factor (k') was measured by $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the retained and unretained solute in the given system, respectively. 1. 2-methyl-3-propyl-prodiginine (C of the alkyl side chains = 3); 2. 2-methyl-3-butyl-prodiginine (C = 4); 3. prodigiosin (C = 5); 4. 2-methyl-3-hexyl-prodiginine (C = 6); 5. 2-methyl-3-heptyl-prodiginine (C = 7)

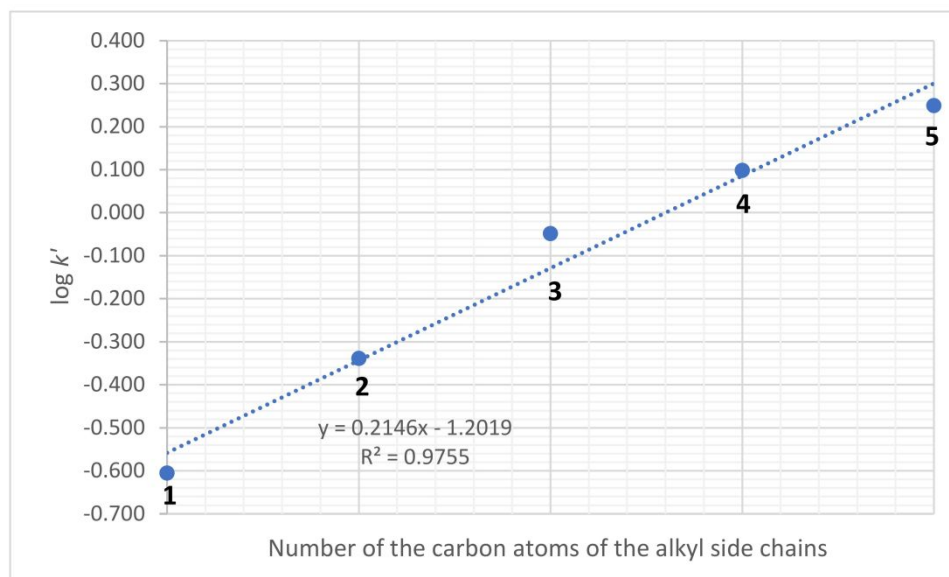


Figure S3. The plot of $\log k'$ value of 2-methyl-3-propyl-prodiginine ($k' = -0.605$), 2-methyl-3-butyl-prodiginine ($k' = -0.339$), prodigiosin ($k' = -0.048$), 2-methyl-3-hexyl-prodiginine ($k' = 0.099$), and 2-methyl-3-heptyl-prodiginine ($k' = 0.249$)

versus number of the carbon atoms of the alkyl side chains of the pigment molecule showed a linear line with $R^2 = 0.9755$

5) **Figure S4**

A prodigiosin standard was purchased from Sigma-Aldrich, MERCK. Determination of the pigments was based on their MS data and spectral properties. The simple MS method was used with isocratic HPLC elution by 0.1% formic acid in a mixture of methanol (90%) and water (10%) for 2 min at a flow rate of 0.3 mL/min without a column. The others parameter same in section Experimental Section: MS/MS Analysis. The precursor ions were fragmented in product ion scan mode by using CE -35, -20, and -10.

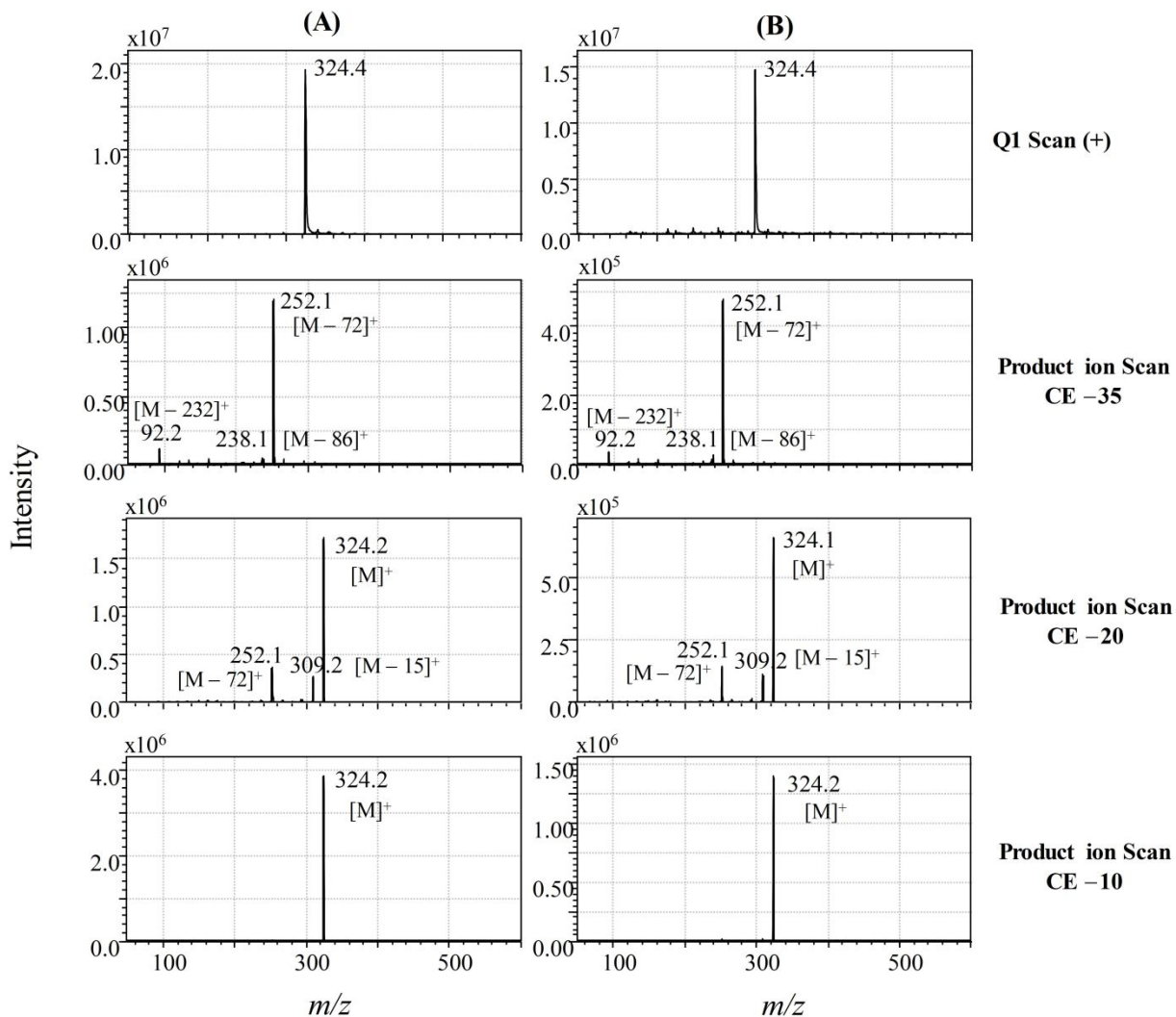


Figure S4. The comparison mass spectra of standard prodigiosin hydrochloride (A) and purified pigment peak #4 from *P. rubra* strain PS1 (B). Their same Q1 (+) and product ion scan (+) indicates that peak #4 in chromatogram HPLC is prodigiosin

6) Figure S5

Two minor compound peak #1 and #2 were determined using MS/MS according to method same with prodigiosin. Peak #1 was identified as 2-methyl-3-propyl-prodiginine and #2 as 2-methyl-3-butyl-prodiginine.

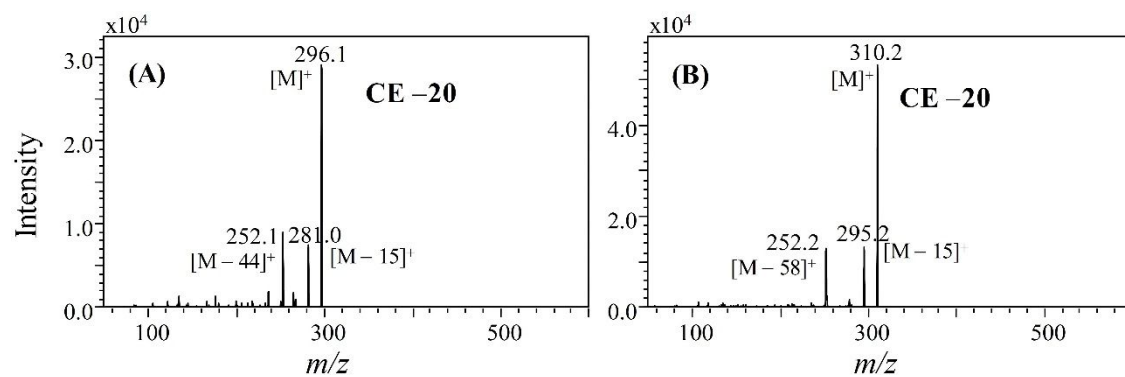


Figure S5. ESI-MS/MS analysis of the purified compounds. Product ion scan MS spectra of 2-methyl-3-propyl-prodiginine **(A)**, and 2-methyl-3-butyl-prodiginine **(B)**

7) Table S2

Bacteria strains	Isolation sources	Location found	Compounds													Ref.	
			A	B	C	D	E	F	G	H	I	J	K	L	M		
<i>P. rubra</i> strain PS1	Seawater	Alor Island, Indonesia	-	-	+	+	+	+	+	+	+	-	-	-	-	-	Present study
<i>P. rubra</i> ATCC 29570	Seawater	Mediterranean Sea, France	-	-	+	-	-	+	-	-	-	-	-	-	-	-	21,25
<i>P. denitrificans</i>	Seawater	Japan Sea, Japan	-	-	+	-	-	-	-	-	-	-	-	-	-	-	47
<i>P. bacteriolytica japonica</i>	<i>Laminaria</i>	Japan	-	-	-	-	-	+	-	-	-	-	-	-	-	-	23
<i>P. sp.</i> 1020R	Seawater	Cape Muroto, Japan	-	-	-	-	+	+	+	+	-	-	-	-	-	-	26
<i>Zooshikella rubidus</i> S1-1	Sediment	Yellow Sea, Korea	-	-	+	-	+	+	+	+	+	+	-	-	-	-	33

<i>Zooshikella marina</i>	Beach sand	Kachigad Beach, India	-	-	+	-	-	+	-	-	-	-	-	-	-	50
<i>Zooshikella ganghwensis</i>	Sediment	Ganghwa Island, Korea	-	-	-	-	-	+	-	-	-	-	-	-	-	51
<i>Hahella chejuensis</i>	Sediment	Cheju Island, Korea	-	-	-	-	+	+	+	+	-	-	-	-	-	48,58
<i>Hahella</i> sp. strain MS- 02-063	Sediment	Omura Bay, Japan	-	-	-	-	-	+	-	-	-	-	-	-	-	49
<i>Vibrio psychroerythrus</i>	Flounder eggs	Trondheim, Norway	-	-	-	-	-	+	-	-	-	-	-	-	-	40,59
<i>Vibrio ruber</i>	Seawater	Keelung, Taiwan	-	-	-	-	-	+	-	-	-	-	-	-	-	41
<i>Vibrio</i> sp. DSM 14379	Seawater	Northern Adriatic Sea	-	-	-	-	-	+	-	-	-	-	-	-	-	42
<i>Beneckea gazogenes</i>	Saltwater marsh	Woods Hole, United States	-	-	-	-	-	+	-	-	-	-	-	-	-	38
<i>Pseudomonas magnesorubra</i>	<i>Caulerpa peltata</i>	Bombay, India	-	-	-	-	-	+	+	+	-	-	-	-	-	46
<i>Pseudovibrio denitrificans</i> strain Z143-1	Tunicate	Zamboanga, Philippines	-	-	-	-	-	-	-	+	-	-	-	-	-	39
<i>Streptomyces</i> sp. SCSIO 11594	Sediment	South China Sea	+	+	-	-	-	-	-	-	-	-	-	-	-	43
<i>Streptomyces griseoviridis</i>	Soil	Texas, United States	-	-	-	-	-	-	-	-	-	-	+	+	-	44,60

Streptomyces coelicolor Soil Not informed + - - - - - - - - - - + 5,45

A3(2)

Table S2. Prodigiosin-producing marine bacteria. Compound: A, undecylprodigiosin; B, metacycloprodigiosin; C, cycloprodigiosin; D, 2-methyl-3-propyl prodiginine; E, 2-methyl-3-butyl prodiginine; F, prodigiosin; G, 2-methyl-3-hexyl prodiginine; H, 2-methyl-3-heptyl prodiginine; I, 2-methyl-3-octyl prodiginine; J, 2-methyl-3-nonyl prodiginine; K, prodigiosin R1; L, prodigiosin R2; M, butyl-metacycloheptylprodiginine. +, detected and -, not detected