



Supplementary Material

Genomic and metabolomic investigation of a rhizosphere isolate *Streptomyces netropsis* WLXQSS-4 associated with a traditional Chinese medicine

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Supplementary Tables and Figures

Table 1. Secondary metabolites gene clusters identified in S. netropsis WLXQSS-4.

No	Region	BGC Type	From	То	Most similar	Similarity
1	Region 1.1	indole	51 478	71 825	KIIOWII CIUSIEI	
2	Region 1.2	terpene	603 609	623 506		
3	Region 1.3	lanthipeptide	644.254	666.487	alvcinocin A	9%
4	Region 1.4	terpene	672.388	692.712	<u> </u>	
5	Region 1.5	siderophore	700,276	710,224	desferrioxamine E	100%
6	Region 1.6	other	745,160	786,533	A-503083 A	7%
7	Region 1.7	siderophore	1,581,615	1,593,564		
8	Region 1.8	terpene	1,924,713	1,945,053		
9	Region 1.9	T3PKS	2,120,118	2,160,713	chloropentostati n	9%
10	Region 1.10	linaridin	2,474,856	2,495,008	legonaridin	50%
11	Region 1.11	ladderane	2,643,237	2,700,700	vazabitide A	28%
12	Region 1.12	butyrolacton e	3,091,492	3,102,556	griseoviridin	8%
13	Region 1.13	T1PKS	3,449,880	3,498,595	berninamycin A	33%
14	Region 1.14	melanin	4,227,817	4,236,356	melanin	28%
15	Region 1.15	melanin	4,450,489	4,460,905	melanin	28%
16	Region 1.16	linaridin	4,533,015	4,553,593	pentostatine / vidarabine	12%
17	Region 1.17	lanthipeptide	4,572,452	4,595,100	SapB	100%
18	Region 1.18	ectoine	5,025,826	5,036,224	ectoine	100%
19	Region 1.19	transAT-PKS	5,437,035	5,551,637	calyculin A	32%
20	Region 1.20	T2PKS	5,671,575	5,756,808	JBIR-76 / JBIR- 77	68%
21	Region 1.21	terpene	5,884,803	5,904,975	guadinomine	7%
22	Region 1.22	T1PKS	6,059,563	6,133,399	borrelidin	52%
23	Region 1.23	terpene	6,153,042	6,174,256		
24	Region 1.24	NRPS	6,176,361	6,228,931	streptobactin	47%
25	Region 1.25	T3PKS	6,332,926	6,368,849	violapyrone B	28%
26	Region 2.1	terpene	64,971	84,230		
27	Region 2.2	lassopeptide	139,695	162,389	lagmysin	80%
28	Region 2.3	NRPS	213,201	293,484	vazabitide A	17%
29	Region 2.4	NRPS	301,963	351,870	enduracidin	4%

30	Region 2.5	NRPS	398,765	467,523	salinosporamide A	45%
31	Region 2.6	T1PKS	534,821	664,616	aureothin	100%
32	Region 2.7	T1PKS	665,727	833,898	nystatin A1	72%
33	Region 2.8	NRPS	866,511	918,299	deimino-antipain	66%
34	Region 2.9	NRPS	981,848	1,101,191	netropsin	100%
35	Region 2.10	siderophore	1,441,513	1,455,189		
36	Region 2.11	terpene	1,488,508	1,515,160	hopene	76%
37	Region 2.12	indole	1,542,279	1,565,584	AT2433-A1	14%
38	Region 2.13	butyrolacton e	1,603,139	1,613,909		
39	Region 2.14	NRPS	1,638,931	1,716,293	bacillibactin	46%
40	Region 2.15	NRPS	1,853,481	1,947,344	atratumycin	55%

Table 2. Deduced functions of ORFs in the alloaureothin BGC from S. netropsis WLXQSS-4.

Gene	Size (a.a)	Protein homologue (accession number)	Identity (%)	Proposed Function
Orf(+2)	500	WP_184731891.1	98.9	carboxylesterase family protein
Orf(+1)	297	WP_184731894.1	97.6	polysaccharide deacetylase family protein
aluD	273	aurD (CAE02599.1)	90	AurD protein /transcriptional regulator
aluE	505	aurE (CAE02600.1)	91	p-nitrobenzoate CoA ligase
aluF	338	aurF(CAE02601.1)	83	p-aminobenzoate N-oxygenase
aluA	1920	aurA (CAE02602.1)	79	polyketide synthase type I
aluG	701	aurG (CAE02603.1)	80	PABA synthase
aluH	410	aurH (CAE02604.1)	81	bifunctional cytochrome P450 monooxygenase
aluB	2183	aurB (CAE02605.1)	77	polyketide synthase type I
aluC	2282	aurC (CAE02606.1)	75	polyketide synthase type I
alul	229	aurl (CAE02607.1)	77	O-methyl transferase
Orf(-1)	432	WP_184731913.1	97.4	siderophore 2,3- dihydroxybenzoate-glycine- threonine trimeric ester bacillibactin synthetase
Orf(-2)	339	WP_184732233.1	98.7	LuxR family transcriptional regulator
Orf(-3)	449	WP_184732235.1	99.5	MFS transporter



Figure 1. The complete genome of rhizosphere Streptomyces Isolates *S. netropsis* WLXQSS-4. The five circles (outer to inner) represent forward strand CDSs, reverse strand CDSs, nomenclature, and locations of predictive secondary metabolites generated using antiSMASH 3.0 software, GC content and GC skew. Putative alloaurethin cluster herein referred to cluster *alu*.











Figure 4. ¹H-NMR spectrum of 1.



Figure 5. ¹³C-NMR spectrum of 1.



Figure 6. ¹H-NMR spectrum of 2.



Figure 7. ¹³C-NMR spectrum of 2.



Figure 8. MS spectrum of 3.



Figure 9. ¹H-NMR spectrum of 3.



Figure 10. ¹³C-NMR spectrum of 3.



Figure 11. ¹H-NMR spectrum of 4.



Figure 12. ¹³C-NMR spectrum of 4.



Figure 13. ¹H-NMR spectrum of 5.



Figure 14. ¹³C-NMR spectrum of 5.



Figure 15. ¹H-NMR spectrum of 6.



Figure 16. ¹³C-NMR spectrum of 6.



Figure 17. HR-MS spectrum of 7.



Figure 18. ¹H-NMR spectrum of 7.



Figure 19. ¹³C-NMR spectrum of 7.



Figure 20. ¹H-NMR spectrum of 8.



Figure 21. ¹³C-NMR spectrum of 8.



Figure 22. MS spectrum of 9.



Figure 23. ¹H-NMR spectrum of 9.



Figure 24. ¹³C-NMR spectrum of 9.



Figure 25. MS spectrum of 10.



Figure 26. ¹H-NMR spectrum of 10.



Figure 27. ¹³C-NMR spectrum of 10.



Figure 28. ¹H-NMR spectrum of 11.



Figure 29. ¹³C-NMR spectrum of 11.



Figure 30. HR-MS spectrum of 12.



Figure 31. ¹H-NMR spectrum of 12.



Figure 32. ¹³C-NMR spectrum of 12.

33-No22-Amedium CI-Trp-200ul #6300 RT: 12.40 AV: 1 NL: 2.85E7 T: FTMS - c ESI Full ms [50.0000-750.0000] 100- 231.0328



Figure 33. HR-MS spectrum of compound 6'-chloropimprinine.







Figure 35. HR-MS spectrum of compound 6'-chloropimprinethine.

Relative Abundance

60-

106.0401



10-116.9276 421.1675 476.1556 174.9554 239.1285 326.9728 578.9855 744.4460 0 100 200 300 400 500 600 700 m/z

Figure 36. HR-MS spectrum of compound 6'-bromopimprinethine.



Figure 37. The possible biosynthesis pathway of pimprinine derivatives.



Figure 38. Two postulated pathways for the biosynthesis of β-carboline compound flazin.



Figure 39. MS spectrum of 13.



Figure 40. HR-MS spectrum of compound 13.



Figure 41. ¹H-NMR spectrum of 13.



Figure 42. ¹³C-NMR spectrum of 13.



Figure 43. The antibacterial assay against *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*.



Figure 44. MTT assay to determine compound (**1-12**) cytotoxicity against hela, lung cancer (A-549), and PC-3. Three sample concentrations (25µm, 50µm and 100µm) types of cells were treated with ethanol for 48 h. The cell viability was measured by MTT assay. Values are expressed as mean and SD. *p<0.05 compared with the control group.



Figure 45. KEGG pathway annotation statistics of S. netropsis WLXQSS-4.



Figure 46. KEGG pathway related with the tryptophan metabolism in *S. netropsis* WLXQSS-4. The red labelled genes were found in the genome.