

Bacteroidetes: Potential for novel natural products

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Background

- New antimicrobials are urgently needed against the increasing emergence of bacteria resistant to multiple antibiotics.
- Nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) are two of the major biosynthetic systems for natural products (antimicrobial) biosynthesis.
- Previous studies on biosynthetic diversity of antimicrobial compounds gene clusters on different soil environments showed a high correlation between NRPS or PKS and Bacteroidetes, in particular in Antarctic soil (Fig.1 and 2, Table 1).
- Flavobacterium johnsoniae* DSM 2064, a member of the Bacteroidetes phylum, expressed a NRPS cluster in response to phosphate depletion.

Objectives

- Investigate main phylogenetic drivers for NRPS and PKS diversity in soil
- Investigate Bacteroidetes secondary metabolite potential for antimicrobial activity

Methods

- Amplicon sequencing was performed for 16S rRNA gene, PKS and NRPS biosynthetic genes on eDNA from different soils.
- Microbial inhibitory assays of *F. johnsoniae* in the presence of different gram-positive and multi-resistant gram-negative bacterial strains.
- Proteomic analysis and molecular cloning for detection of the gene cluster.
- LC-MS analysis for compounds identification in different growth conditions.

Results

MICROBIAL COMMUNITIES AND SECONDARY METABOLITE GENE DIVERSITY

The co-occurrence of NRPS and PKS OTUs between soils was tested using OTU networks (Fig.1). A significant correlation ($P < 0.001$) between key phyla and PKS and NRPS diversity was found using Mantel tests (Table 1) and Procrustes transformation (Fig. 2).

Table 1 Mantel correlation between main phyla 16S rRNA and functional genes PKS and NRPS diversity based on Bray-Curtis dissimilarity matrices. * $p < 0.001$

Mantel correlation 16S - PKS		Mantel correlation 16S - NRPS	
Phylum	R*	Phylum	R*
★ Bacteroidetes	0.82899	★ Verrucomicrobia	0.82042
Actinobacteria	0.80631	★ Bacteroidetes	0.81641
★ Verrucomicrobia	0.79326	Actinobacteria	0.81329
Proteobacteria	0.78704	Proteobacteria	0.77487

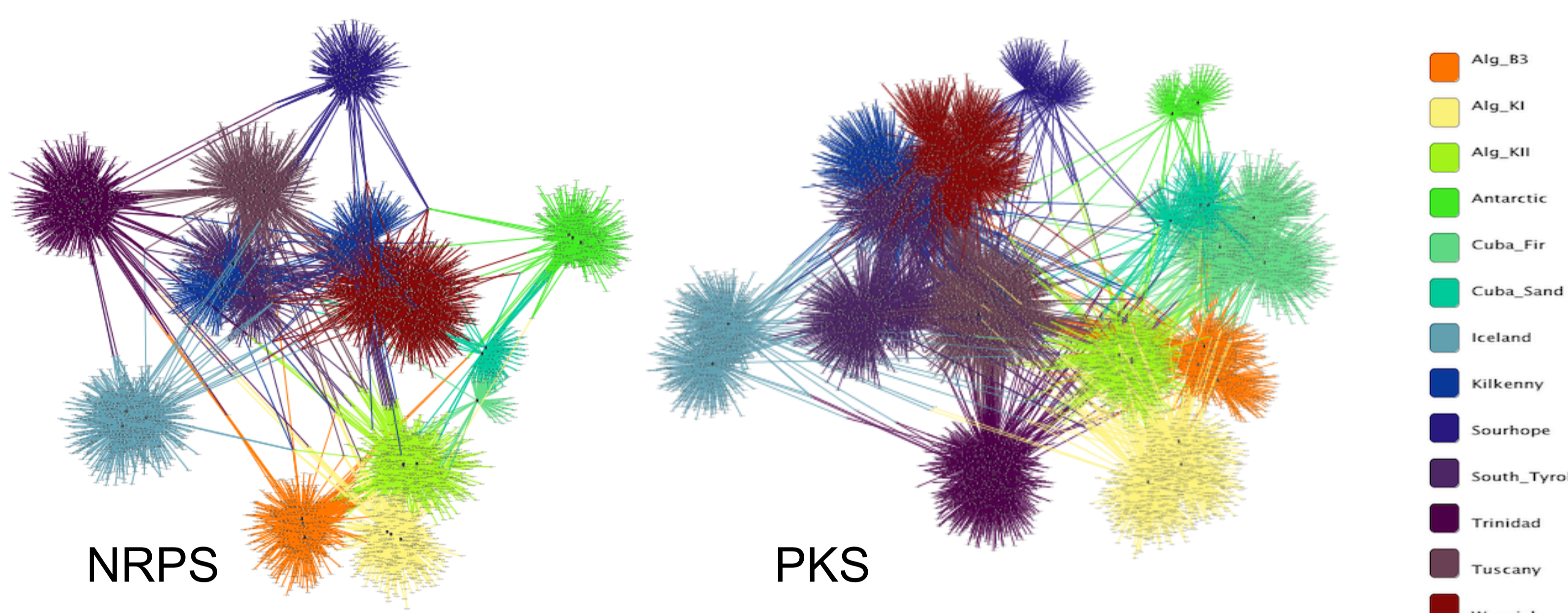


Figure 1 OTU networks of NRPS and PKS gene diversity. Main nodes represent samples while edges represent single shared/unique OTUs.

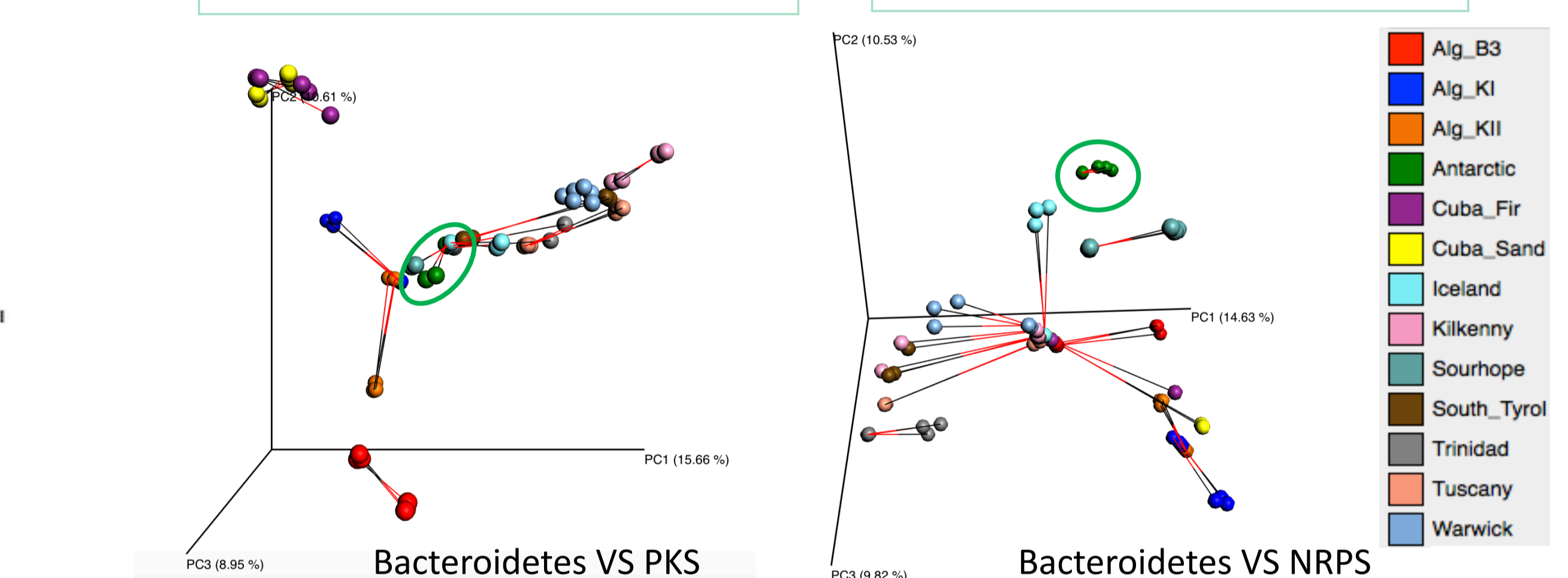


Figure 2 Procrustes transformations representing the correlation between Bacteroidetes 16S rRNA and PKS/NRPS genes according to soil sample location. Edges: with=16S rRNA, red=PKS/NRPS; Nodes=soil samples/location

Flavobacterium johnsoniae SECONDARY METABOLITES POTENTIAL

Figure 3 Proteome of *F. johnsoniae* in Pi depletion conditions – NRPS cluster expression

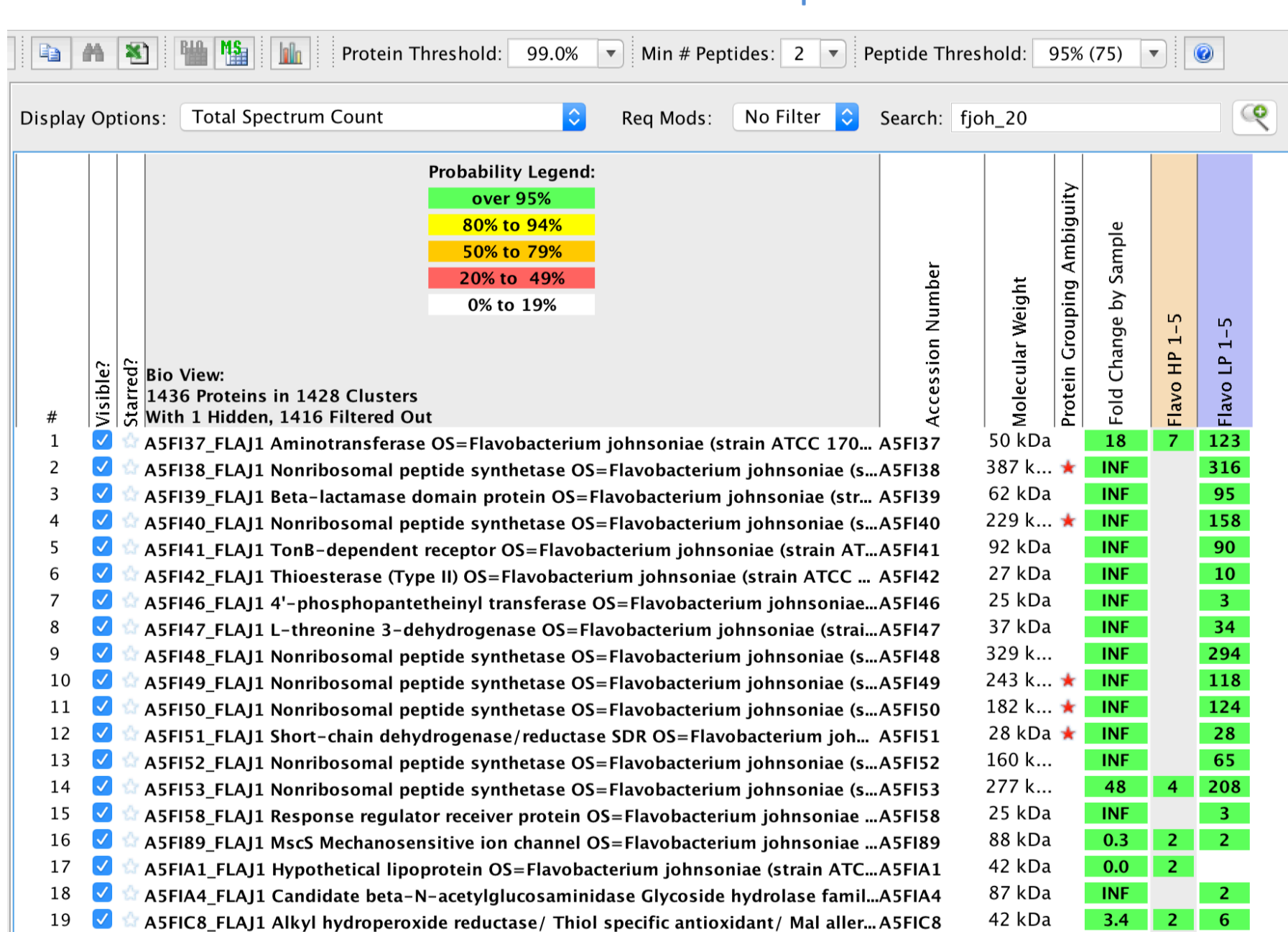


Figure 4 Genome analysis for secondary metabolite gene clusters

Cluster ID	Gene Count	Scaffold	Start Coord	End Coord	BC Length	Genbank ID	Evidence Type	Prediction Probability	Biosynthetic Cluster Type
161809273	8	2698586954	109253	121183	11931		Predicted	0.85	putative
161809274	9	2698586958	468285	477672	9388		Predicted	0.96	putative
161809275	12	2698586958	556110	576427	20318		Predicted	0.93	putative
161809276	11	2698586958	25273	36550	10778		Predicted	0.94	putative
161809277	56	2698586959	121444	189930	68487		Predicted	0.94	putative
161809278	19	2698586959	426010	446849	20840		Predicted	0.00	terpene
161809279	25	2698586960	147402	235479	88078		Predicted	0.99	nrps
161809280	6	2698586961	79083	85847	6765		Predicted	0.78	putative
161809281	15	2698586964	380631	381806	21176		Predicted	0.00	terpene
161809282	9	2698586964	494544	512805	18262		Predicted	0.98	putative
161809283	20	2698586964	557768	581735	23560		Predicted	0.98	putative
161809284	7	2698586964	1325092	1332120	7029		Predicted	0.95	putative
161809285	12	2698586965	501485	517125	15641		Predicted	0.00	siderophore
161809286	5	2698586966	386501	372979	6479		Predicted	0.91	putative
161809287	38	2698586967	570537	621325	50789		Predicted	0.97	l3pks
161809288	10	2698586968	3200	24453	21254		Predicted	0.88	putative

Figure 5 Gene cluster antiSMASH analysis: cluster organization, predicted structure and similarity with sequenced genomes

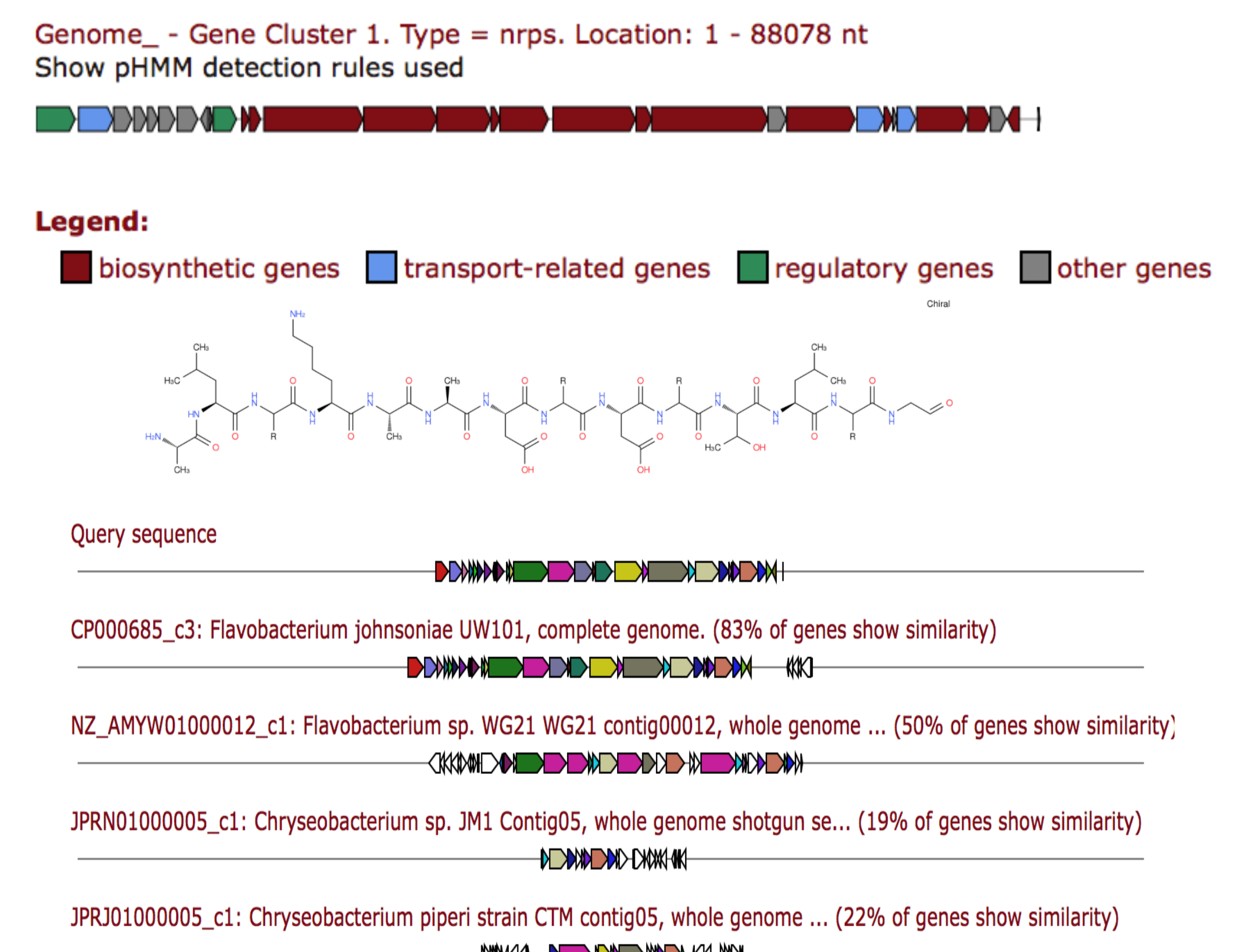
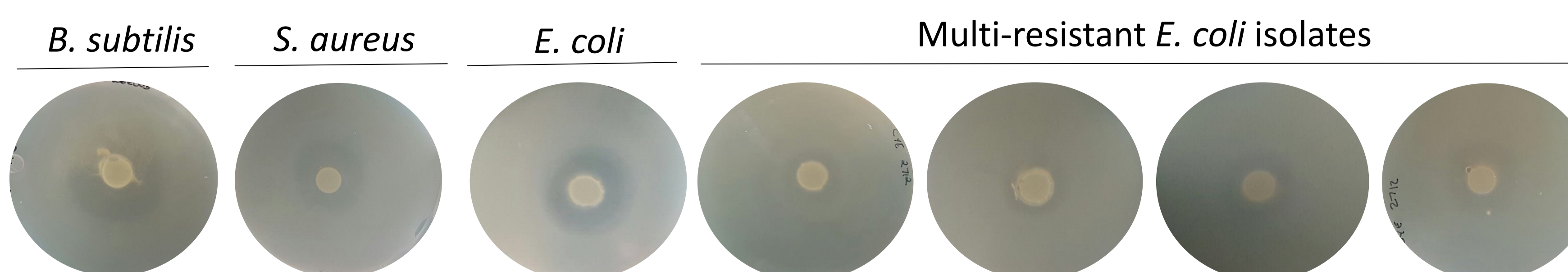


Figure 6 Microbial inhibitory assays against gram-positive and gram-negative strains



Conclusions

A significant diversity of NRPS and PKS in soils can be linked to Bacteroidetes; soils with a diverse population of this phylum such as Antarctica represent potential hotspots for novel bioactive secondary metabolites. The Bacteroidetes *F. johnsoniae* showed antimicrobial activity against gram-positive and gram-negative strains. Identification of the compound related to this activity and other potentially novel compounds is in progress.