## **Bacteroidetes:**

# Potential for novel natural products

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INTEGRATE ANTIMICROBIAL RESISTANCE

0.82042

WARWICK

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	Background		Objectives
•	New antimicrobials are urgently needed against the increasing emergence of	1.	Investigate main phylogenetic drivers for NRPS and PKS diversity in soil
	bacteria resistant to multiple antibiotics.	2.	Investigate Bacteroidetes secondary metabolite potential for antimicrobial activity

- Nonribosomal peptide synthetase (NRPS) and polyketyde 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.

## 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 grampositive 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

Alg\_KI

Alg\_KII

Antarctic

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	n 16S - PKS	Mantel correlation 16S - N	Mantel correlation 16S - NRPS				
Phylum	R*	Phylum	R*				
Bacteroidetes	0.82899	Verrucomicrobia 0.4	8204				
Actinobacteria	0.80631	Bacteroidetes 0.8	1641				
🗙 Verrucomicrobia	0.79326	Actinobacteria 0.8	1329				
Proteobacteria	0.78704	Proteobacteria 0.7	7487				



**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: withe=16S rRNA, red=PKS/NRPS; Nodes=soil samples/location

### Flavobacterium johnsoniae SECONDARY METABOLITES POTENITAL

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

Probability Legend: over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%	ssion Number cular Weight	Grouping Ambiguity ange by Sample	10			<u>161809273</u> <u>161809274</u>	<u>8</u> 9	<u>2698586954</u> 2698586958	109253 468285	121183	11931	Predicted	
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FLAJ1 Aminotransferase OS=Flavobacterium johnsoniae (strain ATCC 170 A5FI3	137 50 kDa	18	7	123		161809280	6	2698586961	79083	85847	6765	Predicted	
8_FLAJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5FI3	138 387 k	★ INF		316		101000200	<u> </u>	200000001	10000	00047	0/00	Treatered	
39_FLAJ1 Beta-lactamase domain protein OS=Flavobacterium johnsoniae (str A5Fl3	139 62 kDa	INF		95		<u>161809281</u>	<u>15</u>	<u>2698586954</u>	360631	381806	21176	Predicted	
AJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5Fl4	140 229 K 141 92 kDa			90		161809282	9	2698586954	494544	512805	18262	Predicted	
$_{\rm L}$ FLAJI Tonb-dependent receptor OS=Flavobacterium johnsoniae (strain ATASFl4	141   92   kDa 142   27   kDa	INF		10			_					-	
146 FLAJ1 4'-nhosnhonantetheinvl transferase OS=Flavohacterium johnsoniaeA5Fl4	146 25 kDa	INF		3		<u>161809283</u>	<u>20</u>	<u>2698586954</u>	557786	581735	23950	Predicted	
7 FLAI1 L-threonine 3-dehvdrogenase OS=Flavobacterium johnsoniae (straiA5Fl4	147 37 kDa	INF		34		161809284	<u>7</u>	2698586954	1325092	1332120	7029	Predicted	
FI48_FLAJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5Fl4	148 329 k	INF		294		101000005	10	0000500055	501405	517105	15041	Due diete d	
149_FLAJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5Fl4	149 243 k	\star INF		118		161809285	<u>12</u>	2698586955	501485	517125	15641	Predicted	
FI50_FLAJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5FI5	150 182 k	\star INF		124		161809286	<u>5</u>	<u>2698586956</u>	366501	372979	6479	Predicted	
FI51_FLAJ1 Short-chain dehydrogenase/reductase SDR OS=Flavobacterium joh A5FI5	151 28 kDa	★ INF		28		161800287	39	2608586057	570537	621325	50789	Predicted	
onribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5Fl5	152 160 k	INF		65		101009207	<u>30</u>	2090300937	570537	021323	50769	Fredicied	
FI53_FLAJ1 Nonribosomal peptide synthetase OS=Flavobacterium johnsoniae (sA5FI5	153 277 k	48	4	208		<u>161809288</u>	<u>10</u>	<u>2698586958</u>	3200	24453	21254	Predicted	
FI58_FLAJ1 Response regulator receiver protein OS=Flavobacterium johnsoniaeA5FI5	158 25 kDa	INF	_	3	_								
FI89_FLAJ1 MscS Mechanosensitive ion channel OS=Flavobacterium johnsoniaeA5Fl8	189 88 KDa	0.3	2	2									
TA1_FLAJ1 Hypothetical lipoprotein OS=Flavobacterium Johnsoniae (strain ATCA5FlA	IAI 42 KDa	U.U INE	2	2									
A4_FLAJI Candidate beta-N-acetyigiucosaminidase Giycoside hydrolase familA5FIA	1A4 07 KDa		2	6									

#### **Figure 4** Genome analysis for secondary metabolite gene clusters

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

Genome\_ - Gene Cluster 1. Type = nrps. Location: 1 - 88078 nt Show pHMM detection rules used



#### Legend:

Biosynthetic Cluster Type

0.85 putative

0.96 putative

0.93 putative

0.94 putative

0.94 putative

0.00 terpen

0.99 nrps

0.78 putative

0.00 terpene

0.98 putative

0.00 siderophore

0.91 putative

0.97 t3pks

0.88 putative



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



CP000685_c3:	Flavobacterium johnsoniae UW101, complete genome. (83% of genes show similarity)
NZ_AMYW0100	0012_c1: Flavobacterium sp. WG21 WG21 contig00012, whole genome (50% of genes show simila
JPRN01000005_	_c1: Chryseobacterium sp. JM1 Contig05, whole genome shotgun se (19% of genes show similarity)

## 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.



