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Genome-guided Screening of Bacterial Isolates to Identify Potential Antibiotic Producers.

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Background:

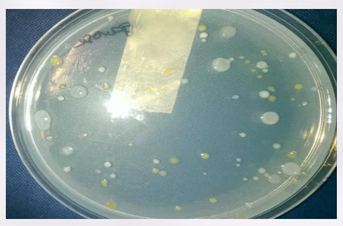
- Multidrug resistant infections could reduce global economic output by $100 trillion by 2050.[1] Therefore, the need for new antibiotics cannot be overemphasized.
- Bacterial secondary metabolites remain a relatively untapped source of new therapies. However, the ability to produce these compounds is not universal.
- Key attributes of producing strains include a large genome (>3Mb), and the presence of antibiotic-encoding biosynthetic gene clusters (BGCs) within the genome.[2] These attributes are largely determined by phylogeny.
- Some antibiotic producers also possess the ability to withstand nutritional stress.[3]
- Here we use these attributes to identify potential antibiotic producers.

Objectives:

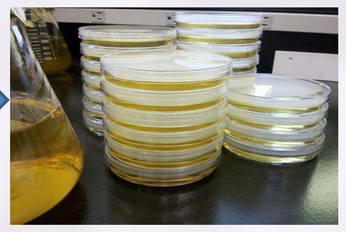
- Use ultra minimal substrate media to isolate nutritionally versatile bacterial strains from nutrient-rich soil sample collected from the rhizosphere.
- Identify isolates by 16S rRNA gene sequence comparison.
- Use genomic data i.e. typical genome size and BGC distribution, to identify potential antibiotic producers.
- Obtain whole genome sequence data of potential antibiotic-producing strains.
- Scan genomes for antibiotic-encoding BGCs, and prioritise novel, silent or cryptic genes for *in silico* analyses to predict optimal fermentation conditions for gene expression.

Methods:

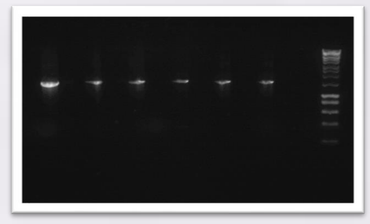
A topsoil sample was collected from the rhizosphere approximately 3cm beneath the soil surface.



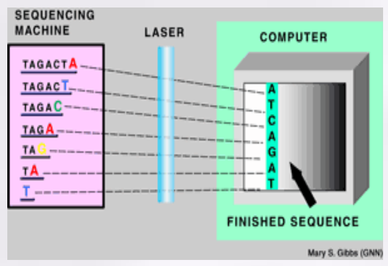
Bacterial strains were isolated on ultra minimal substrate media (1:100 Ravan Media). Four representative colonies with different morphological characteristics were selected and purified.



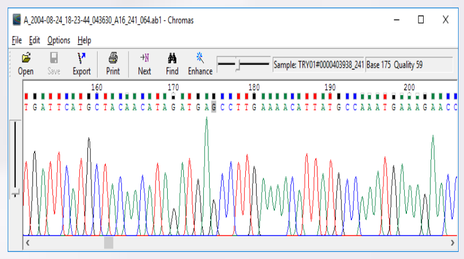
Isolates were cultivated on various solid and liquid media with different nutrient levels and at different incubation temperatures to establish nutritional versatility.



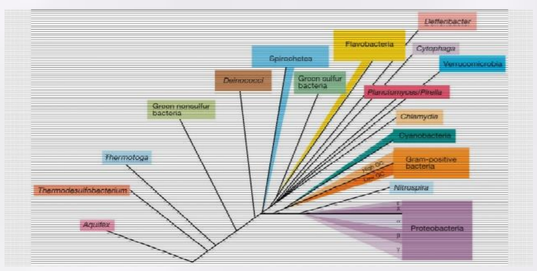
Genomic DNA was extracted from isolates, followed by 16S rRNA gene amplification in PCR reactions



Amplified DNA fragments were sequenced using Applied BioSystem Big-Dye technology



16S rRNA gene sequence data was analysed by CHROMAS and entered into BLAST for comparison. Identity was set at ≥99% sequence alignment.



The antiSMASH database[4] was browsed by phylogeny to locate identified genera. The BGC distribution in these genera were noted. The typical genome size associated with species within the genera were obtained from literature and the NCBI database.

Results:

- Up to 65 distinct colonies were recovered on ultra minimal substrate media as described above.
- Initially, four representative colony types (i.e. different morphological characteristics) were selected for purification.
- All four isolates were found to be nutritionally versatile.
- Isolates were identified as *Pseudomonas (1)*, *Hafnia* (2) and *Obesumbacterium (1) species.*
- BGC distribution in these species, and typical genome size are outlined in Table 1.
- Figure 1 shows the classes of secondary metabolites with antibiotic properties that could be encoded by BGCs in these species.

	Pseudomonas	*Hafnia*	*Obesumbacterium*
Typical genome size of species (Mb)	6.2	4.7	5.0
No of listed strains (antiSMASH DB)	1,236	14	1
Average no of BGCs per strain	11	3	2
Average no of antibiotic-encoding BGCs	7	2	0

Table 1: BGC distribution and typical genome size of species in identified genera.

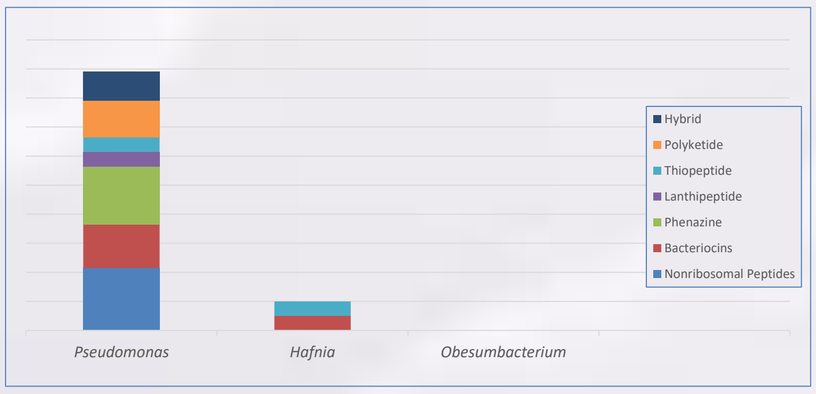


Figure 1: Classes of secondary metabolites with antibiotic properties encoded by BGCs in identified species.

Conclusions:

- Nutritionally versatile bacterial strains have been recovered by the isolation protocol described here.
- To date isolates analysed belong to genera of secondary metabolite producers. The *Pseudomonas sp.* may have the largest genome with more antibiotic-encoding BGCs compared to other isolates, making it the most promising strain for genome mining.
- The potentials of the *Hafnia* and the *Obesumbacterium sp.* as secondary metabolite producers may be understated given the typical genome size associated with these species. These isolates are also expected to be antibiotic producers given their ecological origin. They are therefore also suitable for genome mining.
- The genome-guided screening exercise described here is being explored as a tool to facilitate and expedite rational drug discovery initiatives.

Future work plan:

- Whole genome sequence data will be obtained for all four isolates.
- The data will be submitted to the antiSMASH database for identification, annotation and analysis of potential antibiotic-encoding BGCs.
- Novel, cryptic or silent BGCs will be prioritised for *in silico* analysis to predict favourable fermentation conditions, and chemical structures of compounds of interest.
- Metabolic profiling of fermentation broths will be carried out using suitable analytical techniques.
- Novel compounds with properties consistent with those of antibiotics i.e. low molecular weight, low lipophilicity, and carrying a net charged, will be selected for further bioactivity assays.

References

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