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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. 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. These attributes are largely determined by phylogeny.
- Some antibiotic producers also possess the ability to withstand nutritional stress.
- Here we use these attributes to identify potential antibiotic producers.

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 Hewson Medium). Four representative colonies with different morphological characteristics were selected and purified.
- Isolates were cultured on various solid and liquid media with different nutrient levels and at different incubation temperatures to establish nutritional versatility.
- The antiSMASH database 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 genus were obtained from literature and the NCBI-database.
- Genomic DNA was extracted from isolates, followed by 16S rRNA gene amplification in PCR reactions.
- AmpF Bard fragments were sequenced using Applied Biosystem Big Dye technology.
- Metabolic profiling of fermentation broths will be carried out using suitable producers.

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.

Conclusions:
- Nutritional versatility 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 analyses to predict optimal 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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