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 (1:10) (slant 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. 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 genera were obtained from literature and the NCBI-database. Metabolic profiling of fermentation broths will be carried out using suitable analytical techniques.

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.

<table>
<thead>
<tr>
<th>Pseudomonas</th>
<th>Hafnia</th>
<th>Obesumbacterium</th>
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<tbody>
<tr>
<td>Typical genome size of species (Mb)</td>
<td>6.2</td>
<td>4.7</td>
</tr>
<tr>
<td>log of total strains per isolates</td>
<td>1,236</td>
<td>14</td>
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<tr>
<td>Average no. of BGCs per strain</td>
<td>11</td>
<td>3</td>
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<tr>
<td>Average no. of antibiotic-encoding BGCs</td>
<td>7</td>
<td>2</td>
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Table 1: BGC distribution and typical genome size of species in identified genera.

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.

References:


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