Alternatives to antibiotics: Screening for safe probiotics

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INTRODUCTION

Gut health is a major factor in the optimal performance of production animals. It is becoming increasingly clear that the intestinal microbiota mediates key physiological processes thereby influencing the host. Probiotics can positively impact these processes and are thus seen as a promising tool in settings where antibiotic growth promoters are not used in animal production.

However, no two probiotic strains are the same and the importance of a thorough screening process to select strains which are both efficient and safe in use is crucial. Here, we report examples of applied screening showing how even closely related strains can differ and how complementary tools are of importance in the screening process.

METHODS

Several hundred strains of Bacillus have been screened with regards to performance, robustness and safety. To differentiate strains an initial screening applying a hemolysis assays was performed followed by EFSA recommended methods to study absence of toxicity and antimicrobial resistance as described in EFSA guideline: https://www.efsa.europa.eu.

Additional methods to support the safety profile of probiotic strains were also applied to support the analysis. These methods rely on whole genome sequencing for correct strain identification (Table 1 and Figure 1) and in silico analyses of the genetic potential of the strains (toxin- and antibiotic resistance gene profiling (Table 2) to aid further assessment of strain safety in use.

RESULTS

The screening of > 800 strains of Bacillus showed strain-specific differences in hemolysis and safety profile (MIC and resistance genes) and strain ID.

In silico tools assist in both correct ID and safety profiling

Species Identification

Risk assessment

Table 2: Whole genome sequence analysis can identify resistance genes: Example of in silico screen with ResFinder on two Bacillus strains and compared to phenotype (MIC thresholds as defined by EFSA)

Identification of resistance genes is not always obvious in phenotypic tests like MIC. By identifying genes through in silico analysis a more thorough assessment of the genotype vs phenotype can be performed to determine if the gene is expressed - and transferable to other organisms thereby posing a potential safety risk.

Table 1: 16S ID can result in faulty strain ID and correct taxonomy may require further analyses. Example with 5 Bacillus strains

Strain# Initial ID with 16S Correct ID with above tools
ZW B. subtilis B. subtilis
J6 B. subtilis B. subtilis
18 B. pumilus B. pumilus
R8 B. pumilus B. pumilus
HS B. subtilis B. rhabdodesa

Figure 1: Finding a novel subspecies: Phylogenetic tree for new B. subtilis strain.

Table 2: Whole genome sequence analysis can identify resistance genes: Example of in silico screen with ResFinder on two Bacillus strains and compared to phenotype (MIC thresholds as defined by EFSA)

<table>
<thead>
<tr>
<th>Positive Controls</th>
<th>Strain B4</th>
<th>Strain B4</th>
<th>Strain B5</th>
<th>Strain B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Chlorthiamphenicol</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Cindamycin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>98.77</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>97.82</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>99.88</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100</td>
<td>No hits</td>
<td>OK</td>
<td>No hits</td>
</tr>
</tbody>
</table>

Identification of resistance genes is not always obvious in phenotypic tests like MIC. By identifying genes through in silico analysis a more thorough assessment of the genotype vs phenotype can be performed to determine if the gene is expressed - and transferable to other organisms thereby posing a potential safety risk.

CONCLUSIONS

Probiotics possess a large potential to enhance animal performance in settings where antibiotic growth promoters are not used. However, no two strains are the same and finding the best combination of characteristic require testing in a range of assays. To perform in-depth and correct ID an safety analyses several complementary tests may need to be applied. Some strains may possess unwanted characteristics such as resistance to antibiotics and harboring of transferable antimicrobial resistance genes. Therefore thorough screening and further understanding of potential product candidates is essential to develop new, safe probiotic products.