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.

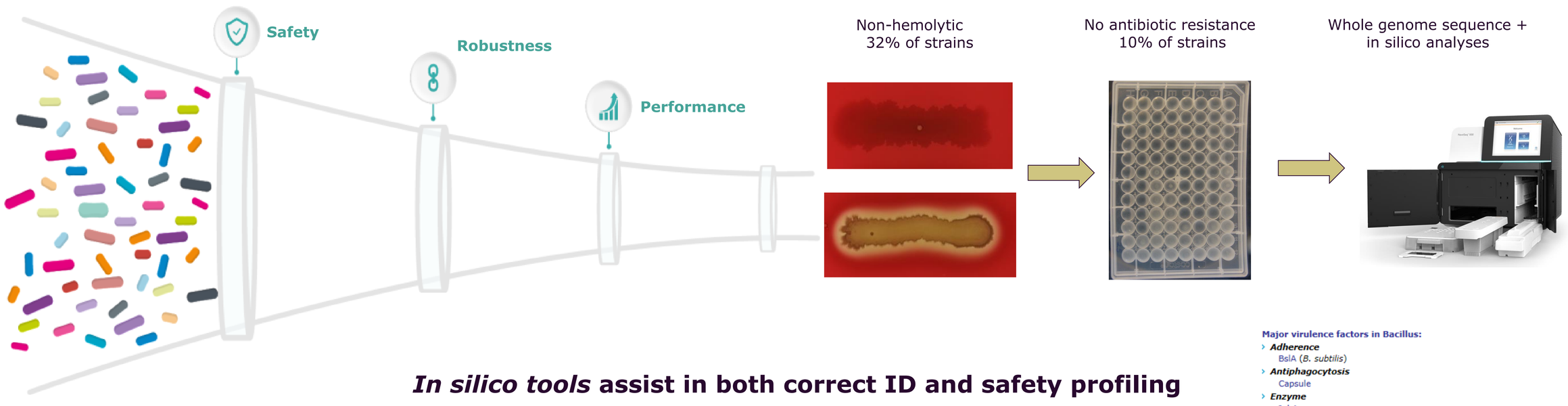
However, no two probiotic strains are the same and the importance of a thorough screening process to select strains which are both efficient and safe in use is crucial. Here, we report examples of applied screening showing how even closely related strains can differ and how complementary tools are of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

Preliminary taxonomic call — Kraken Taxonomic Sequence Classification System
Phylogenetics — PhyloPhlAn; Dendroscope 3 by Daniel H. Huson with contributions from Benjamin Albrecht, Philippe Gambette, Leo van Iersel, Celine Scornavacca and others.
Species confirmation — ANI Average Nucleotide Identity

Risk assessment

Virulence factors/toxins — VFDB Virulence factors of Pa
Antibiotic resistance — ResFinder 2.1
https://cge.cbs.dtu.dk/services/ResFinder/

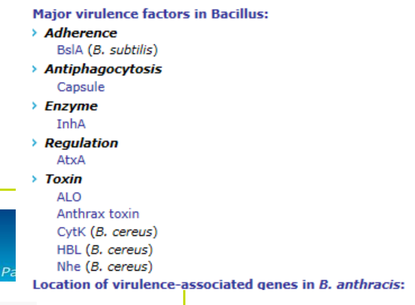


Table 1: 16S ID can result in faulty strain ID and correct taxonomy may require further analyses. Example with 5 Bacillus strains

Strain#	Initial ID with 16S	Correct ID with above tools
ZW	*B. subtilis*	*B. subtilis*
J6	*B. subtilis*	*B. subtilis*
1S	*B. pumilus*	*B. pumilus*
RG	*B. pumilus*	*B. safensis*
H5	*B. subtilis*	*B. aryabhattai*

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 i*n silico* screen with ResFinder on two Bacillus strains and compared to phenotype (MIC thresholds as defined by EFSA)

	Positive Controls	Strain 84 ResFinder	Strain 84 MIC	Strain 85 ResFinder	Strain 85 MIC
Ampicillin	100	No hits	OK	No hits	OK
Chloramphenicol	100	No hits	OK	No hits	OK
Clindamycin	100	No hits	OK	No hits	OK
Erythromycin	98.77	No hits	OK	No hits	OK
Gentamycin	100	No hits	OK	No hits	OK
Kanamycin	100	No hits	OK	No hits	OK
Streptomycin	100	No hits	OK	97.89	OK
Tetracycline	100	No hits	OK	99.35	Above
Vancomycin	100	No hits	OK	No hits	OK

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.

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