STRAINS MATTER

Combinations of selected strains are key to a successful solution

Christophe Bostvironnois, *DVM – Chr. Hansen, Hoersholm, Denmark*

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Bacteria were first observed and described by Antonie van Leeuwenhoek in 1676 when he published his observations in a series of letters to the Royal Society.

Today, it is known that bacteria outnumber every other type of living organism. Researchers estimate that the total number of bacteria at any moment in time ranges in the millions of trillions of trillions (that's 30 zeros).

Most bacteria never encounter animals of any kind, but many find themselves in close proximity and have adapted to live on, in, and with us and the animals in our care.

Although potentially pathogenic bacteria certainly exist, most bacteria pose no threat and a relatively few provide remarkable benefits.

The challenge is finding those unique strains of bacteria that, when consumed in adequate amounts, confer a demonstrable benefit to their host, that is to say, are probiotic in nature.

Table 1. Classification steps for bacteria and humans

Scientific classification	Example of Bacillus subtilis	Example of Homo sapiens
Domain	Bacteria	Animalia
Phylum	Firmicutes	Chordata
Class	Bacillus	Mammalia
Order	Baciliales	Primates
Family	Bacillaceae	Hominidae
Genus	Bacillus	Homo
Species	subtilis	sapiens

Bacteria, like all other living organisms, can be classified in zoologic terms of Domain, Phylum, Class, Order, Family, Genus, and Species. In Table 1, the classifications are shown for bacteria and for humans. We don't classify groups of humans beyond species.

However, for bacteria we continue to separate genetic variation to the level of strain. Bacteria strains are subtypes of a species that have unique genetic identities and distinctive morphological, biochemical, and behavioral features.

For instance, the single bacterial species, *Bacillus subtilis*, consists of more than 1,000 strains that are recorded in international strain banks.

In contrast, there are only 250 breeds of Bos taurus (cattle), 340 breeds of Canis lupus (dog), and 65 breeds of Gallus gallus domesticus (chicken) recognized throughout the world.

All combined, the identified breeds of three species of animals barely comes close to the diversity observed within one species of bacteria.

Below are a few examples that illustrate how the genetic diversity among strains of *Bacilli* translates into meaningful differences in observable characteristics.

EXAMPLE 1
ENZYME PRODUCTION OF DIFFERENT STRAINS OF *BACILLI*

Cellulase and xylanase are two fiber degrading enzymes that break down cellulose and hemicellulose, respectively. The production and secretion of these important enzymes varies exponentially among strains of *Bacilli* (see figures 1a and 1b).

Fig. 1a. Cellulase and xylanase level produced by four *Bacillus subtilis* strains as identified by their strain code (modified from Larsen *et al.* 2014).

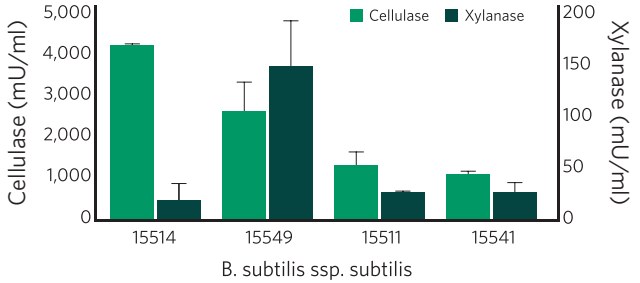
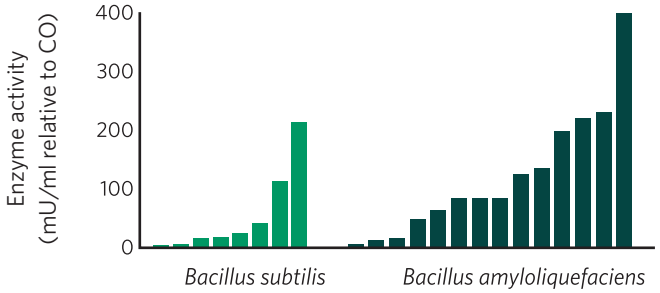


Fig. 1b. *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains with their xylanase activity.



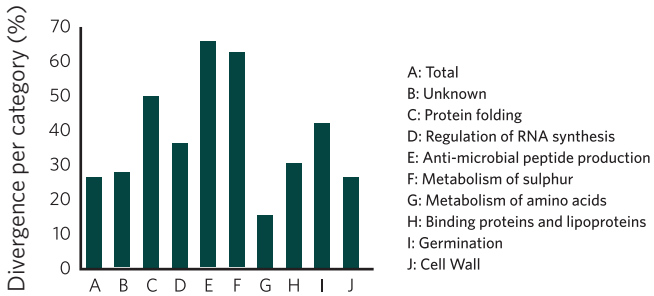
EXAMPLE 2
HIGH GENETIC DIVERSITY WITHIN *BACILLUS SUBTILIS*

In the same category or function, genes of the same strain or species can be identified as divergent if their expression would lead to a different outcome.

The number of genes associated with different category groups and the levels of total divergence among a certain strain identify diversity can be evaluated by genotyping.

In Fig. 2, 17 different *Bacillus subtilis* were genotyped (Earl *et al*. (2017) in order to understand the level of genetic similarity for a certain function. Traits crucial for the survival of the strain were identified such as: cell wall production, metabolism, germination, etc.

Fig. 2. Level of genetic divergence between 17 *Bacillus subtilis* strains (modified from Earl *et al.*, 2017).



We can see a very high diversity of genetic divergence for most of the function, from 16% for metabolism of amino acids to even 66% of divergence for anti-microbial peptide production.

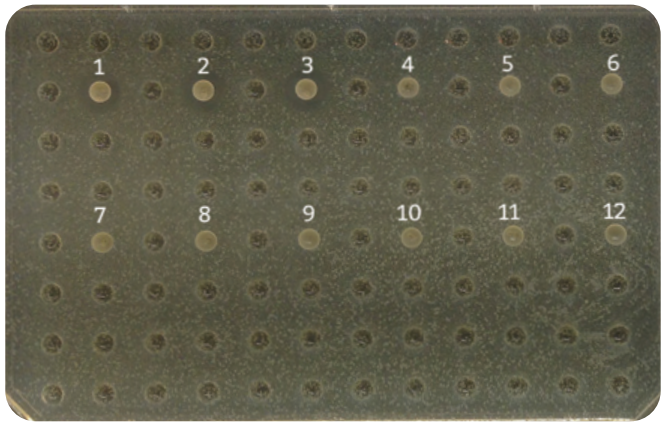
EXAMPLE 3
PATHOGEN INHIBITION ACTIVITY

In Fig. 3, we can visualize the pathogen inhibition effect of some *Bacillus* strains from Chr. Hansen.

To test inhibitory effects against *Salmonella* Heidelberg, an in vitro pathogen inhibition test was conducted in Denmark (EXP-18- AH5439).

Salmonella Heidelberg was plated in agar and *Bacillus* strains were inoculated to see their inhibitory effects to this pathogen. Inhibition zones were measured. The inhibitory activity of different *Bacillus subtilis* strains against the pathogenic bacteria *Salmonella* Heidelberg varies greatly. Some *Bacillus* strains have no effect on *Salmonella* inhibition, while others do.

Fig. 3. *Salmonella* heidelberg inhibition by different *Bacillus* (Chr. Hansen Animal Health Innovation). Products were diluted in peptone saline diluent. Product suspensions were added to 'hedgehog' plate. 1,2,3 = inhibition, 4-12 = no inhibition.



CONCLUSION

Assessing bacterial genetic diversity is critical for strain identification. Selecting and differentiating strains is the basis for success as different strains all have unique properties. When the right strains are selected for the right target solution, they can be an effective solution to counter major food and health challenges. There is not one strain that is effective against each problem. It is a matter of selecting the right strains or combination of strains for the right objective.

Chr. Hansen recently selected three *Bacillus* strains in a new product for the poultry market: **GALLIPRO® Fit**. The foundation was the selection and combination of strains that were most effective for the poultry industry. The strains were selected due to their strong pathogen inhibition and enzyme production abilities.

STRAINS MATTER. Combinations of selected strains are key to a successful solution from which poultry farmers can benefit.

References are available from the author on request.