Visualising germination and activity of Bacillus subtilis in broilers

The use of probiotics, such as Bacilli, in farm animal production has increased considerably over the last 25 years. Probiotics are live bacteria, which when administered in an adequate amount confer beneficial physiological effects to the host, thus resulting in improvements in the health and growth performance of animals.

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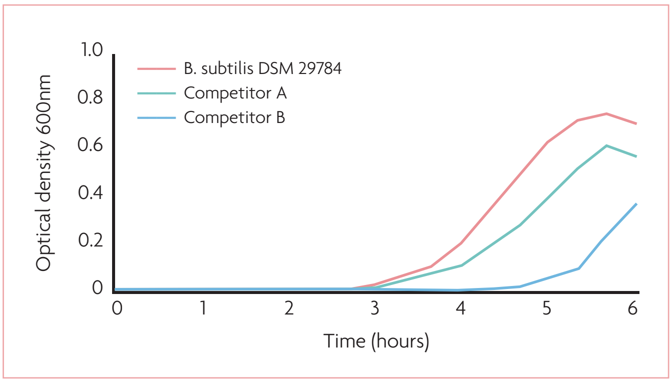


Fig. 1. Germination kinetics of different Bacillus strains.

Bacillus cells have two distinct morphologies; endospores (spores) and vegetative cells, depending on the environmental conditions. Bacilli are fed to animals as spores that can sustain a harsh feed processing and long storage.

Spores are metabolically quiescent and are formed in response to environmental stresses as a survival mechanism.

They are a non-reproductive form of bacteria and are the most durable known viable biological structure. Spores are robust and can survive UV radiation, extreme temperatures, pressure, toxic chemical exposure and acidity.

After a spore is ingested by an animal, it must germinate and grow to become metabolically active, and thereby provide its full benefits to the animal. A spore germinates in the presence of germinants, which bind to specific receptors on the inner membrane of the spore.

Bacillus spores have a variety of such receptors, which allow them to bind to several types of germinants, nutrients or non-nutrients, and this leads to favourable environmental conditions (temperature, pH).

When a sufficiently strong signal is generated, germination begins. The receptors can also be inhibited but, once fully triggered, germination is irreversible.

Unlike spores, vegetative cells divide, multiply, communicate and are motile. They are metabolically active and produce metabolites that provide the full effects of the probiotic to the host.

Germination of Bacillus subtilis DSM 29784 has been proven in vitro and more importantly, visualised in vivo.

The capacity of most Bacillus strains to germinate and grow in vivo is unknown. As a result of the complexity of the germination process, especially in a very complex environment, namely the digestive lumen content, the in vivo confirmation of germination remains a challenging task.

The germination and growth of Bacillus subtilis DSM 29784 has been investigated in detail, using a variety of complementary methods, such as an in vitro time course, fluorescent technologies and in vivo metabolite production assessment.

In vitro germination

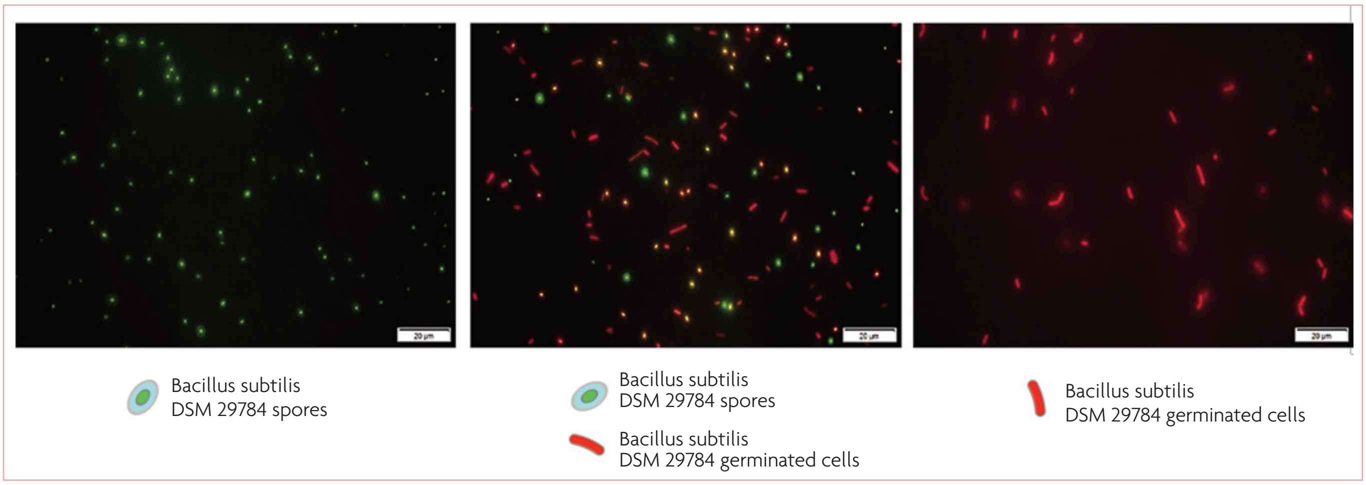
The Bacillus subtilis DSM 29784 strain was selected because of its superior germination capabilities in gut-like and feed conditions.

In a preliminary study, Bacillus subtilis DSM 29784 was grown in vitro, together with a range of feed components and additives, in order to assess their impact on its germination speed, which was assessed by means of optical density (OD).

The germination profile of Bacillus subtilis DSM 29784 was similar over time for wheat, corn and soy supplementation. Moreover, the inclusion of organic acids, coccidiostats and a range of additives showed no impact on the germination.

The germination kinetics of Bacillus subtilis DSM 29784 has also been compared in vitro with other Bacillus strains.

Fig. 2. B. subtilis DSM 29784 fluorescent spores and vegetative cells observed by microscopy at various times.



Testing another Bacillus subtilis strain and a Bacillus licheniformis, under similar conditions, clearly demonstrated that the germination kinetics varied to a great extent between the strains, with Bacillus subtilis DSM 29784 showing a clearer germinating superiority (Fig. 1).

In vivo germination

The germination of Bacillus subtilis DSM 29784 has been observed by developing a unique and innovative fluorescent dual-reporter system which tracks the germination process in the bird's gut.

The first step consists of integrating fluorescent markers in the Bacillus subtilis DSM 29784 genome, more specifically two genes, one encoding Green Fluorescent Protein (GFP) and the other encoding for Red Fluorescent Protein (dsRed) to express a green signal for a dormant spore and a red signal after germination and growth, respectively.

Once the variant was created, fluorescence was verified by means of microscopy analysis. A 96-well plate was inoculated with Bacillus subtilis DSM 29784 spores and fluorescence was observed at various times, as shown in Fig. 2.

The dormant spores of Bacillus subtilis DSM 29784 fluoresce green. Upon germination, the green fluorescence rapidly disappears, and the germinated cells fluoresce red.

The third step consists of visualising germination in the bird's intestine.

A 13-day trial was conducted to verify the germination of Bacillus subtilis DSM 29784 under in vivo conditions. Broiler chicks were fed either a control or a treatment diet to which dual-labelled Bacillus subtilis DSM 29784 reporter spores had been added 72 hours prior to slaughtering.

At the end of the study, the ileum contents were sampled, analysed and processed via an InCell Analyzer to check for fluorescence in the samples.

A red colour indicates that most spores have germinated and are actively growing, thereby supporting the health effects of broilers (Fig. 3).

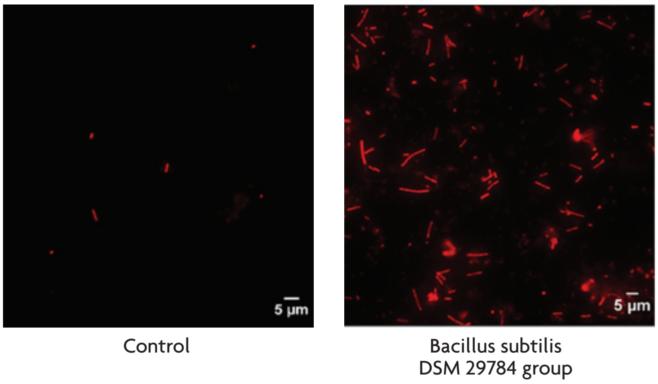


Fig. 3. Germination visualised via red fluorescence in ileal samples.

In vivo metabolites production

A complementary approach that can be used to investigate the in vivo activity of Bacillus subtilis DSM 29784, after germination and growth, is to monitor its production of metabolites.

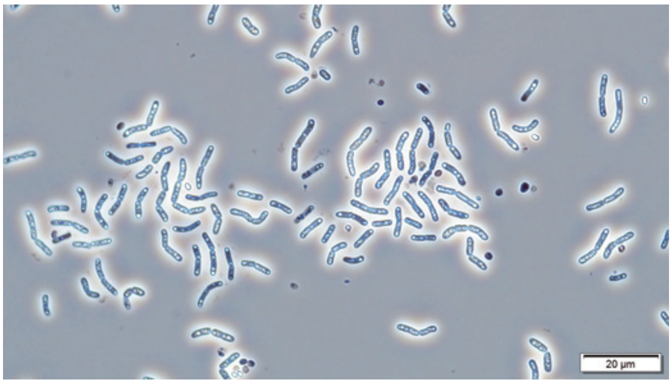
Bacillus subtilis DSM 29784's metabolites were first studied in vitro and three metabolites were identified as produced in high and consistent quantities (niacin, pantothenate, hypoxanthin).

The concentration of these metabolites was then measured in the digestive contents of birds that had received Bacillus subtilis DSM 29784. The study clearly showed that the concentration of the metabolites was closely related to the number of detected Bacilli (Fig. 4).

Furthermore, it was confirmed that the activity of Bacillus subtilis DSM 29784 is higher in the upper parts of the gut, thus reinforcing the data obtained in the germination study. These data clearly support the fact that Bacillus subtilis DSM 29784 is active in the gut of birds.

Sustaining animal resilience

The metabolic activity of Bacillus subtilis DSM 29784 in the gut supports the beneficial in vivo effects on the performance, health and microbiota of the host.



Once germinated and activated, Bacillus subtilis DSM 29784 sustains the resilience of an animal by acting through three intimately connected lines of defence: ensuring a resilient microbiome, strengthening the barrier function and preserving gut integrity and, finally, guaranteeing an optimal inflammatory control and immune response.

In an in vivo study, Bacillus subtilis DSM 29784 has been shown to positively influence bacterial populations, by creating a beneficial effect on the microbial activity: for instance, it has been shown to increase polysaccharides degrading populations as well as butyrate-producing bacteria.

Showing that Bacillus subtilis DSM 29784 is also active in vivo, allows us to be confident about the in vitro data. Bacillus subtilis DSM 29784 can strengthen the gut barrier by increasing the expression of tight junction proteins.

Moreover, it reinforces and controls the inflammatory response through the inhibition of IkB degradation, thus preventing NF-κB translocation. By doing this, the expression of pro-inflammatory compounds, such as IL-8 and the iNOS enzyme, is controlled.

Conclusion

Thanks to the unicity of its strain, its formulation and its timely germination, Bacillus subtilis DSM 29784 becomes metabolically active where it matters most: in the animal's gut. This is where it produces its metabolites and acts on the three lines of defence: the microbiota, the gut mucosa and the inflammatory response to sustain animal resilience. This scientifically proven probiotic provides significant and consistent improvements in gut health, the growth rate and feed utilisation, thereby saving resources and improving the sustainability of the animal farming industry. ■

References are available from the authors on request

Fig. 4. Relative percentage of Bacillus spp. and hypoxanthine concentration (nmol/g faeces) for the B. subtilis DSM 29784 supplemented birds vs control.