# Lactic acid bacteria as protective cultures for plant-based food

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The increasing popularity of plant-based foods has prompted a re-evaluation of food safety strategies. Plant-based foods are inherently exposed to a variety of microorganisms, with *Bacillus* spp. (spore-forming bacteria) being one of the most common contaminants. Various representatives, such as *Bacillus cereus,* produce heat-stable toxins that lead to food-born disease. Several lactic acid bacteria (LAB) produce a variety of antimicrobial compounds, including bacteriocins, which target specific microbial contaminants, thereby mitigating the risk of spoilage or disease while minimizing the need for chemical preservatives or harsh processing methods. LAB as protective cultures have been an emerging solution in the dairy and meat industry. In this study, we aimed to develop protective cultures of LAB against *Bacillus* spp. that can be applied in our model plant-based matrix made from lupin beans.

## Nisin and Pediocin activity against *Bacillus* spp.

The supernatants (SN) of *Pediococcus pentosaceus* (FAM-20650, pediocin producer) and *Lactococcus lactis* subsp. *lactis* (FAM-17921, nisin producer) were tested in an agar-well diffusion assay. Antimicrobial activity against *Bacillus subtilis* (FAM-1470) was observed for **Nisin** SN, but not for **Pediocin** SN, as shown on Figure 1.


**Figure 1**. Inhibition of *B. subtilis* (FAM-1470) in agar-well diffusion test. Inside the wells 50 µl of each SN were added, the plates were kept overnight at 4 °C and then incubated at room temperature for 24 h.

To determine the SN concentration needed to inhibit FAM-1470, we measured its growth ($OD_{600}$) over a period of 48 h in dilutions of each SN (up to 1024x diluted) as seen in Figure 2.

For **Pediocin** SN, inhibitory effect was seen until the four-times diluted sample; nevertheless, here the strain FAM-1470 began to grow after 24 h.

The inhibitory effect determined in the **Nisin**-containing SN was stronger, less nisin might be needed to inhibit FAM-1470. Inhibition was determined up to the eight-times diluted sample, where *B. subtilis* started growing after 24 h.

Additionally, we observed a partial deactivation of the bacteriocins in the samples treated with proteinase K, indicating a need for protocol refinement or the use of alternative enzymes to ensure optimal deactivation (Figure 2).

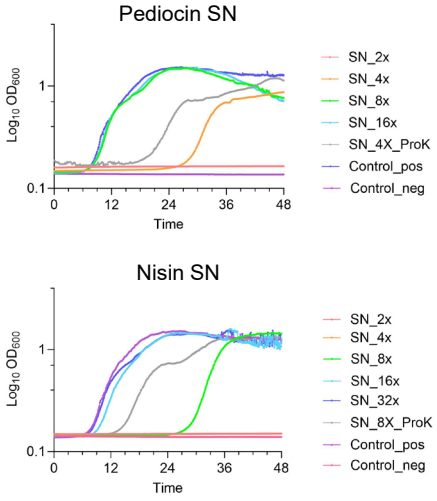



**Figure 2**. Growth curves of *Bacillus subtillis* FAM-1470 using dilutions of each SN. 2x indicates two-times diluted SN and so forth. ProK refers to the Proteinase K treated SN. The $OD_{600}$ measurements are shown in logarithmic scale, incubation was done at 25 °C for 48 h (n=2).

## Synergistic effect of bacteriocins, heat and fermentation against *Bacillus subtilis* in lupin matrix

Pasteurized lupin matrix was mixed with the SN of either FAM-20650 or FAM-17921 (15 or 10 ml per 50 gr matrix, respectively) and then heated at 90°C for 15 minutes (Figure 3). The treated lupin matrix was then spiked with *B. subtillis* (FAM-1470) and the combined effect with VeganMix starter culture was tested.

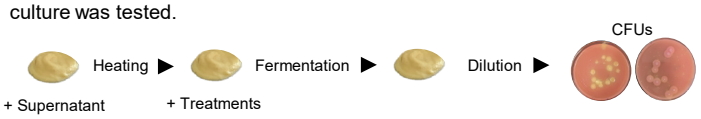
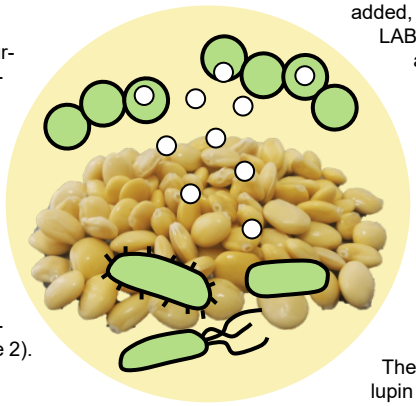



**Figure 3.** Matrix preparation and treatments, which include adding of *B. subtillis,* starter culture, mix of both and controls.

As shown in Figure 4, the pH decreased where the starter culture was added, indicating that neither bacteriocin inhibits the VeganMix LAB strains. Interestingly, in the Lupin matrix with **Nisin** SN and the heated control, the pH was lower when *Bacillus* was co-inoculated with the VeganMix.



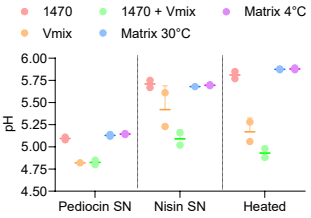



**Figure 4.** pH measurements of the lupin matrix after 18 h incubation at 30 °C (n=2).

The re-isolation of the spiked *Bacillus* (FAM-1470) from the lupin matrix showed a reduction of 4 orders of magnitude for **Pediocin** SN and 1 for **Nisin** SN compared to the heated sample as seen in Figure 5.

Remarkably, when the FAM-1470 and the starter culture VeganMix were co-inoculated, almost no *Bacillus* were recovered in both SN supplemented lupin matrices. In contrast, in the only heated sample $10^6$ *Bacillus* were recovered (Figure 5). Hence, the combined effect of bacteriocins, heat and fermentation has proven effective in preventing *Bacillus subtilis* contaminants.

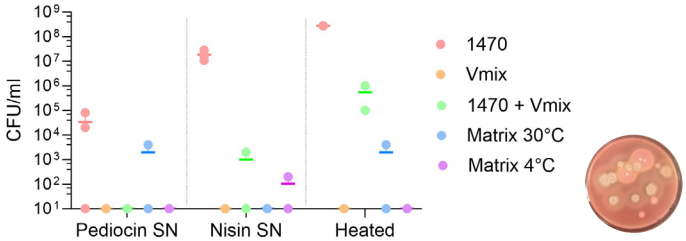



**Figure 5.** CFU/ml of the spiked *B. subtillis* (FAM-1470) and native contaminants (*Bacillus* spp.) detected in the Lupin matrix. Dilutions were plated in SC and MYP media (n=2).

## Conclusions & Outlook

**Nisin** SN exhibited higher antagonistic activity in *in vitro* assays compared to **Pediocin** SN. However, when Pediocin SN was added to the lupin matrix, greater inhibition was observed. This might suggest a matrix-dependent effect.

Future experiments will include the combination of various bacteriocins SN, the integration of protective cultures into fermentation, the assessment of subtilin bacteriocins, and testing the antagonistic effect of these **bacteriocins against *Bacillus cereus***.

Exploiting the antagonistic properties of LAB as protective cultures, in combination with **heat** and **fermentation**, is key to effectively outcompete and suppress undesirable microorganisms in plant-based foods and ingredients, increasing their safety and ensuring food security.

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