

Inhibition of toxin-producing *Aspergilli* by *Lactobacillus animalis* and *Propionibacterium freudenreichii*

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Introduction: Lactic acid bacteria (LAB) and propionibacteria are well known to be used in the production of different types of fermented feed and food, thanks to their active role in the inhibition of spoilage and pathogenic bacteria and fungi. Silage is fermented feed largely used in ruminant nutrition. Moldy silages containing different genera of fungi (e.g. *Aspergillus*, *Penicillium* and *Fusarium*) able to produce different types of mycotoxins that can impact the health and productivity of animals significantly. In particular, *A. fumigatus* has been suggested by Owaki *et al.* 2015, as one of the potential causes of hemorrhagic bowel syndrome (HBS) (together with *Clostridium perfringens*). Silage inoculants and/or probiotics can be used as bio preservation agents for controlling yeasts and molds during the silage making process, fermentation and at feed out. This work aimed at studying the *in vitro* inhibition of toxin-producing *Aspergilli* strains by two probiotic *Lactobacillus animalis* (LA51) and *Propionibacterium freudenreichii* (PF24) strains which may be used as bio preservation agents for animal feed.

Materials and Methods: *Lactobacillus animalis* (LA51) and *Propionibacterium freudenreichii* (PF24) were cultured in Man Rogosa Sharpe broth (MRS, Difco DF2881-30) under anaerobic conditions at 37 °C until the exponential growth phase (8-9 log cfu/ml). The fungi *Aspergillus flavus* (ATCC 46283) and *Aspergillus fumigatus* (ATCC 96918) were cultured in Potato Dextrose broth (PDB, Oxoid CM0962), incubated for 3 days at 25 °C. Fungal fresh cultures were inoculated in Potato Dextrose Agar plates (PDA, Oxoid CM0139) and incubated at 25 °C for 7 days aerobically. Spore suspensions of *A. flavus* and *A. fumigatus* were obtained by adding 10 ml of sterile water, containing 5% of tween 80, and shaking to dislodge the spores. The spore suspensions were counted by hemocytometer and adjusted to 4-6 log spores/ml. Fresh cultures of LA51 and PF24 were spotted on MRS agar plates (6 µl per spot, two spots for each plate) and incubated anaerobically at 30°C for 3 days. The standardized spore suspensions were added to melted PDA soft agar at 50 °C and the spore-containing suspension was poured on the surface of the MRS plates containing the bacterial spots. Plates were incubated aerobically at 25 °C up to 5 days. The test was performed in triplicate and the inhibition zones were evaluated after 1, 2, 3, 4 and 5 days of incubation.

Results and Discussion: The inhibition spectrum of the tested probiotic strains against the selected fungi are reported in Table 1. LA51 exhibited large inhibition zones against both tested *Aspergillus* strains, while the PF24 strain showed no inhibitory influence against the tested fungi. LA51 inhibited the growth of *A. flavus* starting from the first day of incubation (Figure 1), confirming the suitability of the strain for improving food safety (Ware *et al.* 2012). The inhibitory effect of LA51 towards *A. fumigatus* was observed after 48 hours of incubation (Figure 2) which could be

due to secondary metabolites. Several *Lactobacillus* strains have been reported capable of inhibiting fungal growth and reducing the aflatoxin production, through different organic acids (Dalie *et al.* 2010; Ahlber *et al.* 2015; Abbaszadeh *et al.* 2015). On the contrary, the probiotic PF24 strain showed no inhibition effect against neither *A. flavus* or *A. fumigatus* growth (Figure 1 and 2), as previously reported by Abdul Rahman (2017).

Table 1 Antifungal inhibition spectrum of the probiotic strains against *A. flavus* and *A. fumigatus*

Probiotic strains	<i>A. flavus</i>	<i>A. fumigatus</i>
<i>Lactobacillus animalis</i> (LA51)	+++	++
<i>Propionibacterium freudenreichii</i> (PF24)	-	-

-(no inhibition); ++ (inhibition from 3.0 to 8.0 % of the petri dish); +++ (inhibition > 8.0 % of the petri dish)



Figure 1 Effect of *L. animalis* LA51 (left) and *P. freudenreichii* PF24 (right) on *A. flavus* after 1 day incubation.



Figure 2 Effect of *L. animalis* LA51 (left) and *P. freudenreichii* PF24 (right) on *A. fumigatus* after 2 days incubation.



Conclusions: The present study confirms the suitability of the LA51 probiotic strain as a bio preservation agent for controlling yeasts and molds growth. Further studies are ongoing to characterize the active compounds responsible for the inhibition of *Aspergillus* strains.

References

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