Theses of doctoral dissertation

Judit Kosztik

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Identification and characterisation of lactic acid bacteria isolated from exotic animals for biotechnological utility

Judit Kosztik

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Name of the doctoral school: Doctoral School of Food Sciences

Discipline: Food Science

Leader: **Prof. Dr Livia Simonné Sarkadi**

University Professor, DSc

MATE Doctoral School of Food Sciences

Supervisor: **Dr Ildikó Vidács Batáné**

Senior research fellow, PhD

Research Group for Food Biotechnology

MATE Institute of Food Science and Technology

Approval signature of the leader of the doctoral school and the supervisor:

The candidate fulfilled all the conditions prescribed in the Doctoral Regulations of Hungarian University of Agriculture and Life Sciences, the remarks and suggestions made during the workshop discussion were taken into account during the revision of the dissertation, therefore the dissertation can be submitted for public debate.

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Leader of the doctoral school Supervisor

1. Introduction and objectives

Lactic acid bacteria produce lactic acid during carbohydrate fermentation. Many bacterial species are capable of this, but these bacteria are not necessarily related. Due to their lactic acid formation, they have long been used in the food industry to preserve various dairy products, vegetables and meat products. Lactic acid lowers the pH of the medium, which inhibits the growth of spoilage microbes.

The use of lactic acid bacteria for mycotoxin depletion has also been researched for a long time. Mycotoxins are secondary metabolites produced by mould strains that can have adverse health effects on higher vertebrates. They can mainly cause kidney and liver damage, but their cytotoxicity and carcinogenicity are also known. Due to these properties, the prevention of their appearance in feed and food is extremely important from both health and economic points of view. It is, therefore, important to develop the most effective methods for preventing the appearance of mycotoxins in food and feed and of inhibiting their harmful effects on health.

Some strains of lactic acid bacteria are known to be able to inhibit the growth and multiplication of other microorganisms through their low molecular weight metabolites. Lactic acid bacteria, which inhibit the growth of moulds, can help in the removal of mycotoxins, since if the mould is unable to grow, it cannot produce mycotoxins. It happens that the presence and growth of moulds cannot be prevented and the mycotoxin is already in the feed or food product. Although mycotoxin-degrading microorganisms exist, their use in feed and food on a large scale is not known. In this case, too, some strains of lactic acid bacteria may be useful, because based on literature data, although they cannot degrade toxins, several strains are able to bind mycotoxin molecules on their cell surface. Surface-bound toxin molecules can pass intact through the intestinal tract of the consuming animal / human, thereby helping to clear the toxin from the body.

Recent research suggests that vertebrates pass on their specially adapted lactic acid bacteria to their offspring during labour and laying.

This allows the emergence of species-specific microbial strains within vertebrates. Due to this mechanism, even exotic animals kept in zoos for generations retain specialised strains of lactic acid bacteria, which may include previously undiscovered species or strains with special abilities.

The aims of my work:

1. ***Building of a strain collection of a large number of lactic acid bacteria***. For this purpose, I planned the isolation of lactic acid bacteria from the faeces of 15 mainly herbivorous exotic zoo animals by classical microbiological methods, and then the identification of the isolates by molecular microbiological methods (genomic DNA extraction, repetitive PCR (rep-PCR), 16S rDNA PCR).

2. ***Investigation of the possible inhibitory effect of lactic acid bacteria strains on aflatoxin B1-producing*** **Aspergillus flavus** ***strains.*** For this, I planned to use an agar diffusion method, to culture the mould and the lactic acid bacterium strain simultaneously.

3. ***Investigation of aflatoxin B1 (AFB1) and sterigmatocystin (ST) binding capacity of the members of the strain collection***. The literature deals with the AFB1-binding ability of some lactic acid bacterial strains, but there are no data on ST, so I also planned to test and compare the adsorption capacity for AFB1 and ST of my strain collection.

2. Materials and methods

Creating a stock collection: During my work I worked with the faecal samples of the following 15 exotic animals of the Budapest Zoo and Botanical Garden: Aldabra giant tortoise (*Geochelone gigantea*), Bengal stick insect (*Medauroidea extradentata*), Naked mole-rat (*Heterocephalus glaber*), Ring-tailed lemur (*Lemur catta*), Indian crested porcupine (*Hystrix indica*), Koala (*Phascolarctos cinereus*), Mhorr gazelle (*Gazella dama mhorr*), Southern cassowary (*Casuarius casuarius*), White rhinoceros (*Ceratotherium simum*), Northern bald ibis (*Geronticus eremita*), Vombat (*Vombatus ursinus tasmaniensis*) and Red panda (*Ailurus fulgens*). The selected animals are mainly herbivores, although some of them also consume insects. Nutrition played a role in the selection, because to deal with the mycotoxins ingested with plant-derived foods, these animals may have developed a special microflora.

After sampling, I obtained a pure culture from each strain using classical microbiological methods. Genomic DNA was extracted from the biomass and then rep-PCR was performed, from which a pattern was obtained by agarose gel electrophoresis. I grouped the strains based on the pattern and sequenced the 16S rDNA PCR amplicon of 2-3 strains from each group at Baseclear Inc. I compared the sequence data with the 16S rRNA gene sequences in the EzBioCloud database, which made it possible to identify each group at the species level.

Inhibition of *Aspergillus flavus* growth by lactic acid bacteria: Suspensions of 10^6 spores / ml concentration were prepared from four different aflatoxin B1-producing *A. flavus* mould strains (Zt31, Zt41, Zt55 and Zt80) in peptone water containing Tween 80. The mould strains were isolated in 2009 from corn silage. Agar plates were poured in two steps. First 10 ml agar was poured into the Petri dish, and after it solidified, a sterile plastic cap with a diameter of 12 mm was placed in the middle, and then another 10 ml of medium inoculated with the mould was poured around it. After solidification, the cap was removed and 100 µl of a 10^8 cell / ml suspension of a fresh culture of the lactic acid bacterium strain studied was pipetted into the resulting well. This

method made it possible to prevent the bacterial suspension from leaking between the agar and the Petri dish, which could lead to an inaccurate result. Petri dishes were incubated in a 37 ºC thermostat for 72 h. Then the radius of the inhibition zone around the hole was measured.

Mycotoxin binding assays: The identified samples of our lactic acid bacterium collection and the type strains of 25 *Lactobacillus* species ordered from BCCM (Belgian Coordinated Collections of Microorganisms) were also tested for their aflatoxin B1 and sterigmatocystin binding capacities.

To determine the optimal experimental parameters, preliminary experiments were performed with strains of different *Lactobacillus* species. The growth of each LAB strain in the presence of the toxin and the effect of the incubation time on the binding of the toxin were investigated. Also the lowest LAB cell concentration at which the toxin binding is detectable was determined.

Based on the results of the preliminary experiments, the tested lactic acid bacterium strains were grown in 15 ml of MRS broth and supplemented with 0.2 ppm AFB1 or ST toxin. MRS broth was used as negative control, while MRS broth containing 0.2 ppm toxin was used as positive control. The tubes were vortexed and incubated for 10 min at room temperature. They were then centrifuged at 4,000 rpm for 40 min to separate the supernatant and biomass. The supernatant was transferred to a sterile Falcon tube and placed in an ultra-freezer at -80 °C until further analysis. The AFB1 or ST content of the biomass was determined by HPLC.

Quantification of aflatoxin B1 and sterigmatocystin contents by HPLC: In case of the controls, 1 ml of the broth was added to a 2 ml Eppendorf tube to extract the toxin, followed by the addition of 1 ml of dichloromethane. It was shaken for 20 minutes, while the toxin dissolved from the broth into the dichloromethane phase. From this, 0.2 ml was pipetted into a 2 ml Eppendorf tube, and then the samples were evaporated at 50 °C for the HPLC measurement. For the LAB samples, the centrifuged biomass was shaken with a mixture of 0.2 ml of

methanol and 1.8 ml of dichloromethane for 20 minutes and then centrifuged. One millilitre of the methanol-dichloromethane phase was evaporated at 50 °C for the HPLC measurement.

HPLC measurement: The evaporated samples were resolved in 1 ml of eluent solution and filtered through a 0.45 µm pore size hydrophilic polytetrafluoroethylene filter before the measurement. Following isochromatic liquid chromatographic separation, the amount of mycotoxin in the samples was determined by UV detection at 365 or 240 nm for AFB1 and 325 nm for ST. Separation was performed on a Brisa (Technochroma, Spain) C18 column (5 µm, 15 cm x 0.46 cm) at 30 °C. Biomass values were compared to those obtained for the positive control (0.2 ppm AFB1 and ST-containing MRS broth).

3. Results

Composition of microbes isolated from faecal samples of exotic animals on MRS medium

The dominant bacteria from a faecal sample of exotic herbivorous animals were cultured on MRS (lactic acid bacterium selective) agar medium. Eight to one hundred isolates per animal were collected. The lowest number of isolates obtained was from the stick insect (8) and the highest from the ring-tailed lemur (89). When examining the patterns, it was surprisingly found that strains isolated from an animal species, even if they belonged to one species, sometimes showed different patterns. This means that patterns can also vary at the level of strains within a bacterial species. This fact alone could call into question the usefulness of the method, however, though there may be pattern differences, overall the patterns of most strains show the same picture within a given species, so the use of this method still leads to significant cost savings. Very different microbial compositions were observed for the animals examined. For some animals, such as the gorilla, the gray giant kangaroo, or the emu, only strains of different species belonging to one genus dominated the microbiome. In contrast, for the wombat, the white rhinoceros, and the northern bald ibis, a fairly diverse microbiota could be isolated. In the case of the koala, an even more diverse microbiota was found, but unfortunately, there were also strains belonging to pathogenic species, e.g. *Shigella flexneri* or the opportunistic pathogenic *Escherichia coli*. Due to the MRS medium (which is selective for lactic acid bacteria), with a few exceptions, strains belonging to lactic acid bacteria were isolated. On average, although 100 colonies per animal (with the exception of the Bengal stick insect, where only 5 colonies grew after spreading) were isolated, only about 60% of the isolates became permanently sustainable. Strains that were more sensitive to freezing could not be revived later, despite the addition of glycerol before freezing.

Aldebra giant tortoise (*Geochelone gigantea*): From the isolated 100 colony, only 32 samples grew in MRS broth. Forty-one percent of the isolates belonged to 3 different species of the genus *Lactobacillus*,

among which *L. paraplantarum* dominated. The only species of the genus *Weisella*, *W. soli*, was represented in the samples in a proportion of 22%. To the genus *Enterococcus* belonged 9% of the strains in equal distribution among 3 different species, while the genus *Pediococcus* had the 9% of the strains, though in this case each strain belonged to 1 species, *P. pentosaceus*. The remaining 19% of the isolates belonged to the species *Staphylococcus gallinarium*, despite MRS broth being selective for lactic acid bacteria.

Bengal stick insect (*Medauroidea extradentata*): 12 colonies grew from the stool sample on the surface of the MRS agar. From these, 5 isolates were able to grow in MRS broth, 80% of which were strains belonging to the species *Enterococcus hormaechei*, while 20% of the samples, i.e. 1 strain, belonged to the species *Cronobacterium zurichensis.*

Naked mole-rat (*Heterocephalus glaber*): Of the 100 isolated colonies, 85 grew well inoculated into MRS broth. Ninety-nine percent of these strains were members of the genus *Enterococcus*. Among the species, *E. faecalis* predominated, with 91% of all strains belonging to this species. Only 1 strain belonged to the *Staph. epidermis* species, which is not a lactic acid bacterium, not even part of the gut microbiota, may have entered the faeces from the skin of the animal.

Emu (*Dromaius novaehollandiae*): Of the 100 isolated colonies, 45 were able to grow in MRS broth. All of these belonged to the genus *Enterococcus*. The strains were evenly distributed among 5 species. Strains of *E. mundtii* (26%) predominated among the samples.

Gorilla (*Gorilla gorilla*): Of the 100 isolated colonies, 51 grew in MRS broth. Each of these belonged to the genus *Lactobacillus*, distributed among 6 different species, with *L. kitaatonis* as dominant species, with 45% of the samples belonging to that.

Ring-tailed lemur (*Lemur catta*): Eighty-eight of the isolated colonies were able to grow in MRS broth. Eighty-one percent (71) of the strains belonged to *Lactobacillus paracasei*, so this species dominated this microbial community. Like the gorilla, the genus *Lactobacillus* was

dominant here. Eleven percent of the strains belonged to *L. lactis* of the genus *Lactococcus*. *Leuconostoc lactis* was represented by 6% of the strains, *Escherichia coli* and *E. pallensis* by 1-1%.

Indian crested porcupine (*Hystrix indica*): From the isolated samples, 37 colonies were able to grow in MRS broth. Seventy-three percent of the strains belonged to the genus *Lactobacillus*. *L. paraplantarum* dominated the overall sample composition. The genus *Pediococcus* provided 24% of the strains (21% *P. pentosaceus*, 3% *P. stilesii*).

Koala (*Phascolarctos cinereus*): Fifty-five of the isolated colonies grew in MRS broth. Unfortunately, the largest proportion of the samples (45%) were strains belonging to the pathogenic species *Shigella flexneri*. Fifteen percent of the strains belonged to the species *Enterococcus avium*. Twelve percent of the strains belonged to two species of the genus *Escherichia*. *E. coli* is an opportunistic pathogenic bacterium. The genera *Lactococcus, Citrobacterium, Leuconostoc, Acinetobacterium* and *Weissella* were also represented by 2-2%.

Mhorr gazelle (*Gazella dama mhorr*): Thirty-seven of the isolated colonies could be cultivated in MRS broth. Fifty-one percent of the strains belonged to two species of the genus *Pediococcus. P. acidilactici* was the more dominant species overall, 43% of the strains belonged there. Another 46% of the strains belonged to the genus *Enterococcus,* and *E. lactis* dominated within the genus. Only 3% of the strains belonged to *L. mucosae* of the genus *Lactabacillus*.

Southern cassowary (*Casuarius casuarius*): From the 100 isolated colonies, 43 were able to grow in MRS broth. The vast majority of the strains (72%) belonged to the genus *Lactobacillus*, including *L. salivarius* (58%). Strains of species of the genus *Enterococcus* were also found in the sample, 12% *E. faecalis*, 7% *E. faecium*, 5% *E. hirae*, and 2% *E. lactis*. Only 2% of the strains belonged to the genus *Pediococcus*.

White rhinoceros (*Ceratotherium simum*): Of the isolated colonies, 40 grew in MRS broth. Most of the strains belonged to *L. mucosae* (20%), *L. graminis* (13%) and *L. equi* (15%) of the genus *Lactobacillus* (48%). Within the genus *Pediococcus*, *P. pentosaceus* (18%) and *P. acidilactici*

(3%) were present. *E. hirae* and *E. mundtii* species of the genus *Enterococcus* were present in 15-15% and strains of *E. faecalis* in 4%.

Western gray kangaroo (*Macropus fuliginosus*): Of the 100 colonies isolated from a faecal sample, 69 grew in MRS broth. Ninety-three percent of the strains were distributed among 5 species belonging to the genus *Enterococcus*, most strains belonged to *E. faecalis*, 36% of all isolates. The genus *Lactobacillus* presented 7% of the isolated strains (*L. pentosus*).

Northern bald ibis (*Geronticus eremita*): From the 100 colonies inoculated into the MRS broth, only 32 were able to grow. Thirty-one percent of the strains belonged to species of the genus *Lactobacillus*. To *L. crustorum* belonged 19%, while to *L. paraplantarum* belonged 3% of the strains. Twenty-five percent of the strains belonged to the genus *Enterococcus*, while 22% to the genus *Pediococcus*.

Vombat (*Vombatus ursinus tasmaniensis*): Of the 100 colonies inoculated into MRS broth, 21 grew well. Forty-eight percent of the strains belonged to the genus *Pediococcus*, in equal proportions to *P. lolii* (24%) and *P. acidilactici* (24%). To the genus *Lactobacillus* belonged 23% of the strains, mostly to *L. reuteri* and *L. salivarius*. Twenty-four percent of the strains belonged to the genus *Enterococcus*, while 5% belonged to the *genus Leuconostoc*.

Red panda (*Ailurus fulgens*): Of the 100 colonies isolated from MRS agar plates, 47 also grew in MRS broth. Most of the isolated species belonged to the genus *Enterococcus*. Within the genus, the *E. casseliflavus* species dominated, 32% of the strains belonged there. The genus *Lactococcus* provided 21% of the strains, and within the genus *L. garvieae* species dominated. One species of the genus *Leuconostoc*, *Leuc. lactis*, could be isolated, and 13% of the strains belonged there.

Inhibition of the growth of *Aspergillus flavus* strains by lactic acid bacteria

The inhibitory effect of lactic acid bacterial strains on the growth of AFB1-producing *A. flavus* mould strains (Zt30, Zt40, Zt55, Zt80) was studied. In addition to live lactic acid bacteria, also the effect of lactic acid, acetic acid, ethanol, and cell-free supernatant produced by lactic acid bacteria were examined. No inhibitory effect on the growth of mould strains could be observed even for the supernatant of SK29 with good inhibitory ability. Similarly, lactic acid, acetic acid and ethanol did not show an inhibitory effect on the studied moulds. From these results, it can be concluded that the inhibition is not caused by organic compounds commonly produced by lactic acid bacteria, but possibly by bioactive peptides that are produced only in the presence of moulds. This was also confirmed by recent research on mould inhibition by lactic acid bacteria (Luz *et al.* 2017, Muhialdin *et al.* 2020). In the study, I examined 82 strains belonging to 19 different lactic acid bacterial species. Against the four *A. flavus* strains capable of producing aflatoxins, the strains belonging to the species *Lactobacillus salivarius, L. crustorum, L. paracasei, L. plantarum*, and *Pediococcus pentosaceus* had the best inhibitory ability. The largest inhibition zone was 5 mm wide and was obtained for a *L. salivarius* strain isolated from the Southern cassowary.

Summarising my results, it can be said that both the mould inhibitory effect of lactic acid bacteria and the susceptibility of moulds to the presence of lactic acid bacteria are strongly strain-dependent, but there are lactic acid bacterium species that are highly likely to inhibit mould growth.

Mycotoxin binding assays

To investigate the toxin-binding capacity of the large number of lactic acid bacterium strains from my collection, it was necessary to perform some preliminary experiments to find the optimal parameters.

***Effect of cell count on toxin binding*:** Toxin binding was only detectable at concentrations of 10^7 LAB cells/ml for both aflatoxin B1 and sterigmatocystin.

Effect of incubation time on toxin binding in lactic acid bacteria: Five different *Lactobacillus* strains were incubated with AFB1 toxin for 10 min and 48 h. For three strains, the length of the incubation time did not significantly affect the efficiency of toxin binding, in one case the longer and in one case the shorter incubation time was more efficient. Based on literature data and practicality, I used the shorter duration in the further experiments.

***Effect of toxins on the growth of lactic acid bacteria strains*:** As mycotoxins have a serious detrimental effect on the health of higher organisms, the question arises as to whether they also have any negative effects on bacteria. Based on my results, neither AFB1 nor ST caused a significant decrease in cell numbers of LAB compared to the control at the tested concentration.

AFB1 binding capacity of the genus *Lactobacillus*

Eighty strains from my own strain collection were tested for aflatoxin B1 binding. In order to test the AFB1 binding ability of species of the genus *Lactobacillus* as comprehensively as possible, I constructed a phylogenetic tree based on the 16S rDNA sequences of all *Lactobacillus* species found in the NCBI (National Center for Biotechnology Information) database. My own strain collection did not have stains of every larger clades, so I ordered type strains of 25 species from BCCM (Belgian Coordinated Collections of Microorganisms), which I included in the toxin binding studies. I also examined the ST binding abilities of the 20 strains with the best AFB1 binding capacity from my own strain collection and the 25 strains ordered from the BCCM strain collection. Of the 105 strains tested, *L. pentosus* TV3 (11.5%) and *L. plantarum* strains AT26, AT3, AT1, AT27 (8-9%) had the best AFB1 binding ability. From all strains tested, only 14 strains were able to bind the toxin above 5%. Phylogenetic tree was prepared based on the 16S rDNA sequences of the type strains of the examined *Lactobacillus* species found in the NCBI database. From this, it could be observed that the

Lactobacillus strains with the best toxin-binding ability are closely related. For another 33 strains, I obtained binding capacity between 3-4%. For the additional 58 strains tested, the binding was less than 3%.

Sterigmatocystin binding capacity of the genus *Lactobacillus*

In addition to the 14 best AFB1 binding strains form my own strain collection, I also tested the sterigmatocystin-binding ability of the 25 strains ordered from BCCM. There is no report in the literature that the ability of lactic acid bacteria to bind sterigmatocystin has been previously studied. The results show that *L. plantarum* strains TV1, AT1, AT3, AT5, *L. paracasei* MA8, and *L. pentosus* TV3 have the best ST binding abilities. These strains showed more than 20% binding at 0.2 ppm ST concentration. As with AFB1, strains with good binding abilities belong to closely related species, moreover, there is an overlap between the best AFB1 and ST binding species.

In the course of my work, I paid special attention to the study of the toxin-binding potential of the genus *Lactobacillus*. However, species of other genera also belong to lactic acid bacteria. For a more comprehensive picture, it is also worth examining the strains of these genera. My collection of lactic acid bacteria also includes several strains belonging to other genera than *Lactobacillus*, so I also studied their ability to bind AFB1.

AFB1 binding capacity of *Enterococcus* strains

I examined the AFB1 binding ability of twenty *Enterococcus* strains. Among the tested strains, *E. hirae* AT12 (4.62%) and *E. lactis* SK34 (3.40%) had the highest binding values. For the other strains, the binding was below 1.61%.

AFB1 binding capacity of *Pediococcus* strains

I examined the AFB1 binding ability of 24 strains of the genus *Pediococcus*. Of these, 8 strains belonged to *P. acidilactici*, 3 strains to *P. lolli*, 12 strains to *P. pentosaceus* and 1 strain to *P. stilesii*. Of the

strains tested, *P. acidilactici* strain OR83 proved to be the best AFB1 binder. The other strains showed binding values around 4% or less.

ST binding capacity of *Pediococcus* strains

Also the ST-binding ability of five strains belonging to the genus *Pediococcus* was studied. Binding values ranged from 9 to 18%. These results confirm what was observed for strains of the genus *Lactobacillus* that the ST-binding capacity of the lactic acid bacterium strains was double that of for AFB1. To date, no data have been published in the literature on the ST binding capacity of strains belonging to the genus *Pediococcus*.

AFB1 binding capacity of strains of the genera *Lactococcus* and *Weissella*

There are only a few strains belonging to these genera in my strain collection. Therefore, one strain of the species *Lactococcus formonensis*, one of *L. garviae*, and 3 strains of *W. soli* could be studied. *Lactococcus* strains bound between 2.2–2.5%, while AFB1 binding of *Weissella* strains ranged from 0.7–1.2%.

My new scientific results

1. By processing faeces samples of exotic animals of the Budapest Zoo and Botanical Garden, I have built a collection of lactic acid bacterium strains of more than 600 strains.

2. I successfully isolated a new microbial strain from faeces of the Indian crested porcupine, which turned out to be not only a new species but a new genus as well. The strain has been described to science as *Micrococcoides hystricis* (Tóth *et al*. 2017).

3. By examining 82 strains belonging to 19 different lactic acid bacterial species, I obtained a comprehensive picture of the inhibitory capacity of lactic acid bacterium strains on *Aspergillus flavus* AFB1 toxin-producing strains. Strains belonging to the species *Lactobacillus salivarius, L. crustorum, L. paracasei, L. plantarum*, and *Pediococcus pentosaceus* had the best inhibitory ability.

4. Based on the 16S rDNA sequence of the type strains of the genus *Lactobacillus*, I prepared a phylogenetic strain tree and tested the AFB1 binding ability of all major clades. The strains of *L. pentosus, L. plantarum* and *L. graminis* species had the highest (above 10%) AFB1 binding values.

5. To the best of my knowledge, this is the first study on the sterigmatocystin binding ability of lactic acid bacteria belonging to genera *Lactobacillus* and *Pedicoccus*. Strains of *L. plantarum* (23%) and *P. acidilactici* (18%) had the highest binding values, and the sterigmatocystin binding capacity of lactic acid bacterium strains was approximately double that of aflatoxin B1.

4. Conclusions and recommendations

A new species, new genus strain was isolated from the Indian crested porcupine, with which I proved that it is worth to study microbiomes of exotic animals.

Rep-PCR has made the identification of nearly 1000 strains at species level more cost-effective.

Some strains of lactic acid bacteria are actually able to inhibit the growth of moulds. It can be concluded that the presence of mould triggers the production of inhibitory metabolites from lactic acid bacteria.

The ability of lactic acid bacteria to bind mycotoxins was tested for aflatoxin B1 and sterigmatocystin. I obtained lower binding values for AFB1 compared to the literature data. In the literature, the studies were generally performed in PBS buffer, while I used MRS broth, which is more representative of the natural habitat of lactic acid bacteria. So, my results are not easily comparable with those in the literature. Another possible reason for the difference between the literature data and the results obtained here that the binding capacity of strains belonging to the same species can also be very different from each other, as described by Chapot-Chartier *et al*. His results published in 2010 also support this. It has been described that a cell wall polysaccharide covalently attached to the peptidoglycan layer is considered to play the most important part in toxin binding. The amount of this polysaccharide may vary from strain to strain.

Twice as much sterigmatocystin was bound by lactic acid bacterial cells than AFB1. This may be due to the fact that the molecular structure of sterigmatocystin differs from that of aflatoxin B1, and it is also possible that as ST forms aggregates in an aqueous medium, several ST molecules can bind to a single binding site (Jakšić *et al*. 2019).

My plans include also investigating the sterigmatocystin binding ability of strains belonging to the genera *Enterococcus*, *Lactococcus*, and *Weisella* in the strain collection.

Animal feeding trials are currently being carried out with our strains with the best toxin-binding abilities. The aflatoxin-enriched feed of cattle was supplemented with toxin-binding lactic acid bacteria, and the aflatoxin M1 content of the milk is measured in comparison with the control (lactic acid bacteria-free) feed. The goal is to verify that the lactic acid bacteria bind the toxin and it eventually passes through the animal's intestinal tract intact without the toxin getting into the milk.

The binding capacity of strains of the lactic acid bacterium strain collection reagrding other mycotoxins, e.g. patulin, zearalenone, ochratoxin should also be tested.

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PUBLICATIONS RELATED TO THE RESEARCH TOPIC

IF Journal articles

Bata-Vidács I., **Kosztik J**., Mörtl M., Székács A., Kukolya J. (2020): Aflatoxin B1 and sterigmatocystin binding potential of non-*Lactobacillus* LAB strains. *Toxins*, 12, 799. (Q1 - IF.: 3,531)

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