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PH.D. SCHOOL OF ENVIRONMENTAL SCIENCES

Ph.D. Thesis

TAXONOMICAL AND ENVIRONMENTAL BIOLOGICAL STUDIES ABOUT A *STREPTOMYCES* STRAIN COLLECTION

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I. Actuality and significance

Nowadays, one of the most challenging goals is to eliminate anthropogenic chemical and biological contaminations of the environment. Toxic materials get into the environment by the industry, agriculture, traffic and communal waste. Contaminants cause problems not only by themselves but the interactions of various pollutants result in additional threats to organisms and their habitats. To address such environmental issues, various methods exist to relieve the damage including physicochemical, thermic, isolation and biological methods. The biological technique that invokes the biological transformation and degradation of pollutants in soils, waters and sediment is called bioremediation. At the selection of the remediation technology, not only the environmental and economic considerations come into play but also the risk mitigation regarding the environment, the ecology and human health during and as a result of the remediation. One type of bioremediation is bioaugmentation – that is the deployment of such bacterial strains relocated into the environment that are able to degrade these toxic materials and consequently cease the contamination and its toxic effects partially or even completely. The major advantage of this method against others is that if the degradation is complete, the degraded substance will be completely harmless to the environment.

Thanks to their wide-range metabolic repertoire, their significant environmental tolerance and intense reproduction rate, bacteria and microscopic fungi are the most common objects for bioremediation processes. In case of biodegradation of toxic materials, it is important to investigate whether the decrease in toxic concentration can be attributed to the biological degradation itself because toxins can occasionally be bound to cell-wall of microbes. It is also important to examine how toxicity changes after biodegradation as secondary metabolites may be generated which could be more toxic than the original compound.

An aspect of consideration however should be that, certain bacterial strains are capable of degrading materials which are either harmful or even toxic to the environment but not necessarily applicable to biodegradation processes due to their behaviors as facultative or obligate pathogens or occasionally they may produce substances with antimicrobial effects (e.g. antibiotics). If a bacterial strain relocated into the environment has antimicrobial effects, it may disturb the natural microbiota of the given environment and may also raise additional environmental health issues (e.g. development and expansion of resistance). Therefore, its application may result in severe risk rather than benefits of the biological degradation.

II. Background and aims

The biggest challenge in present-day plant production is the appearance of such mold fungal species in food and forage whose mycotoxin production raises health hazards to human beings. Mycotoxins are secondary metabolites and produced by certain mold fungal species. The fact that these mycotoxins accumulated in produces and products can directly or indirectly contaminate the whole system of food chain poses an additional risk and raises its hazardousness. Therefore, it is needed to raise awareness of mycotoxin decontamination efforts regarding food commodities, forage and other agricultural products. As a result of the climate change, such intensive toxin-producing mold fungal species, that were previously present in tropical and subtropical regions only, got into our homeland. Based on available published data, several bacterial strains prove to be capable of degrading mycotoxins thus bioaugmentation may be a possible solution to decrease the damage caused by mycotoxins. In my experiments on the basis of their international occurrences, I worked with biodegradation of two significant mycotoxins – the aflatoxin-B1 which is produced by *Aspergillus* species (AFB1), and the zearalenone mostly produced by *Fusarium* (ZEA). Carcinogen, mutagen, teratogen and immunosuppressive effects of the former were confirmed, while ZEA has hormonal effects similar to that of estrogen, so is responsible for biological disorders of reproduction in both animals and humans.

In my dissertation, I studied the AFB1 and ZEA mycotoxin degradation abilities of almost 500 members of a strain collection, which mostly belongs to the *Streptomyces* genus. Unlike the current studies, I also examined their detoxification abilities. From biotechnological perspective, the *Streptomyces* constitute probably the most important prokaryote group. They are remarkable sources of industrial enzymes (hydrolase, transferase, esterase) and pharmaceutical commodites. The *Streptomyces* produce antibiotics in an excessively wide spectrum but may also be the source of antifungal, antiviral and anticancer substances. *Streptomyces* strains belong to the *Streptomycetales* order are globally used by the pharmaceutical industry to produce antibiotics, but their biodegradation abilities have not been revealed extensively as of yet.

Based on the discussions above, my aims are the following:

1. Taxonomical identification of a strain collection consisting of almost 500 members (mostly *Streptomyces*) with molecular biological methods (using 16S rDNA sequencing and analysis).
2. Examination of the members of the strain collection (mostly *Streptomyces* strains) for their AFB1 and ZEA biodegradation and biodetoxification ability with immune-analytical and toxicity-profiling bio-tests.
3. Examination of antimicrobial effects of those identified and from a biodegradation perspective known strains against six test organisms (three bacterial and three microscopic fungal species).
4. Comparison of toxin degrading abilities and antimicrobial effects of the examined *Streptomyces* strains.

III. Materials and methods

Firstly, I revitalized, identified and then performed phenotypical examination on the 494-member strain collection of the Department of Environmental Safety and Ecotoxicology at Szent István University, which consist species mostly from the *Streptomyces* genus. In accordance with my aims, the taxonomically identified strains were screened for their AFB1 and ZEA mycotoxin degradation abilities, analyzed their toxin biodetoxification abilities and finally defined their antimicrobial effects as well.

III/1. Revitalization

Before the identification, as the source of revitalization, a 494-member strain collection was at my disposal, which was isolated during earlier works related to the department. The execution of the task was made a bit more difficult due to the fact that I had only one specimen from each item, which were marked only with a code number. In other words, I had no information regarding the optimal medium and temperature for incubation. After opening the vial tubes, the dissections were injected into GYM medium. The incubation was performed at various temperatures (24, 28, 45°C). In order to achieve my first goal, after the revitalization, 16S rDNA gene sequencing was performed for the identification and then the strains were maintained with lyophilization and cryo storage at -80°C.

III/2. Toxin degradation and detoxification

In accordance with my second goal, the mycotoxin biodegradation ability of 124 species belonging to the *Streptomyces* genus was studied in a bitoxin and monotoxin experiments. During the initial screening at the mycotoxin biodegradation experiment, the AFB1 and ZEA biodegradation abilities of *Streptomyces* genus were tested in a bitoxin system. In the bitoxin system, 1 mg/l final concentration from both toxins was set, without replications. After the sampling, the samples were centrifuged and supernatant and pellet fractions were stored separately. Effectiveness of the toxin biodetoxification was tested by SOS-Chromotest for AFB1 and by BLYES-test for ZEA from supernatant fraction. In case of SOS-Chromotest values of induction factor lower than 1.5 indicated the elimination of AFB1 toxicity. The bioluminescence intensification (BI%) of ZEA's estrogen effect was measured with BLYES-test where the decrease in bioluminescence intensification values meant the decrease or cease of estrogen effects (lowest values compared to the control value).

The 10 most effective strains in biodetoxification from the bitoxin experiment were involved to the monotoxin degradation tests where the solution is contained only one toxin in 1 mg/l final concentration. Here the other difference compared to the bitoxin experiment was that the toxicity-profiling bio-tests were supplemented with ELISA-test capable of analytical measurement as well. In this method the toxin concentration was colorimetrically detected based on antigen-antibody reaction and the extent of the degradation was able to be calculated.

During the biodegradation studies, it must be also taken into consideration whether, besides biochemical processes of focal bacteria, other effects (e.g. adsorption) decrease the concentration of toxic materials. Pellet fraction of the samples containing bacterial cells were also analyzed with ELISA-test to rule out these effects from biodegradation of AFB1 and ZEA.

III/3. Examination of the antimicrobial effect

In order to achieve my third goal, an antimicrobial effect assessment was performed on selected members of the strain collection with cross streak method and agar diffusion test using six selected test organisms. The prokaryote group was represented by *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (the latter one for its significant environmental and clinical role), while the eukaryotes by three fungal species frequently used in biological experiments. The *Saccharomyces cerevisiae* was selected for its great resistance against antifungal chemicals, the *Aspergillus fumigatus* for its human medical relevance and the *Fusarium ploriferatum* for its role in plant pathology. All six test organisms were part of the departmental strain collection.

For the cross streak method, potato dextrose agar (PDA) was used to which a streak of pure culture of a *Streptomyces* strain was plated into the centrum of the agar. Test organisms mentioned above were plated perpendicularly to the *Streptomyces* streaks but not physically touched them. The experiment contained both the bacterial and fungal species on separate agar plates. Antimicrobial activity of the focal *Streptomyces* strain was detected by measuring the inhibition zones where the test organism did not grow in millimeters.

For the agar diffusion test, *Streptomyces* strains were injected into flasks containing GYM broth, and then a filtrate was prepared from them to get a solution of derivatives with presumable antimicrobial effect. Then, suspension from the culture of each test organism was prepared and added to a Petri dish containing PDA and then homogenized. In each solidified plate, 3 holes were cut using a sterile plug drill. The inhibitory effect of the filtrate was measured in length by the diameter of the inhibition zone around the holes (in mm).

IV. Results

IV/1. Revitalization and molecular biological identification

87 of the 494 strains were not revitalized, so the subsequent studies consist of the remaining 407 strains. 380 from the 407 revitalized strains contained 131 different *Streptomyces* species based on the results of the 16S rDNA identification procedure. Besides, 11 strains were from the *Rhodococcus* genus and 8 from other genera (namely *Micrococcus, Nocardia, Nocardiopsis, Pseudonocardia, Thermoactinomyces* and *Ureibacillus*). Eight strains are still under identification. At the time of finishing my thesis, 287 pieces of 16S rDNA sequences from the *Streptomyces* strain collection have been published at the NCBI GenBank database.

NOVEL SCIENTIFIC RESULT: (Thesis 1) After the revitalization, members of the *Streptomyces* strain collection were identified using the 16S rDNA method, defined their temperature optimum and other physiological parameters and then a strain collection of 380 items (of which 131 belongs to various *Streptomyces* species) was generated, and the strain collection is maintained sustainably in the long run.

IV/2. Toxin degradation and detoxification

Bitoxin degradation experiment

Based on the results of the SOS-Chromotest and the BLYES-test, several strains seemed to be efficient in the degradation of AFB1 and ZEA. According to the preliminary bitoxin experiment, ten *Streptomyces* strains eliminated the biological effects of both the mycotoxins in their mixture which were selected for further monotoxin degradation experiments (Table 1).

Table 1. Induction factor values (IF) of SOS-Chromotest and intensification percentage values (BI%) of BLYES-test of the 10 most effective *Streptomyces* strains during the bitoxin experiment.

Strain code	Species name	SOS-Chromotest (IF)	BLYES-test (BI%)
K145	*St. rimosus*	1,16	-3,63
K234	*St. cacaoisubsp.asoensis*	0,81	3,09
K128	*St. spiroverticillatus*	1,18	4,00
K136	*St. violaceoruber*	1,06	13,83
K144	*St. luteogriseus*	1,20	14,09
K189	*St. rimosus*	1,09	15,16
K139	*St. sanglieri*	1,18	21,16
K236	*St. cinereoruber*	1,24	22,43
K129	*St. violarus*	1,06	22,57
K116	*St. baarnensis*	1,08	24,14
Control		**2,38**	**760,14**

Monotoxin degradation experiment

After the ten selected *Streptomyces* strains degraded the two mycotoxins separetly in monotoxin systems, the concentration of the remaining AFB1 toxin was measured by the ELISA test and the biological effect was tested by SOS-Chromotest. Based on the ELISA test, biodegradation was detected under 5% for two strains (*St. spiroverticillatus* K128 and *St. violaceoruber* K136), ranging from 50 to 70% for five strains (*St. baarnensis* K116, *St. violarus* K129, *St. cinereoruber* K236, *St. sanglieri* K139, *St. Remosus* K189), and over 70% for three strains (*St. rimosus* K145, *St. luteogriseus* K144, *St. cacaoi subsp. asoensis* K234). Mean value of induction factor of control samples was 2.25 (this is equivalent to the 1 ppm concentration without degradation), therefore, in a non-strain solution, high genotoxicity was measured due to the lack of AFB1 degradation (Table 2).

Table 2. SOS Chromotest induction factor (IF) value (mean ± sd, n=3) and AFB1 concentration and degradation percentage based on the ELISA test in the monotoxin system (supernatant fraction).

Strains	Species	SOS Chromo test (IF)	ELISA AFB1 (mg/L)	ELISA AFB1 (degradation %)
K234[Δ]	***St. cacaoisubsp.asoensis***	**1,37 ± 0,26**	**0,132 ± 0,177**	**88,34 ± 15,62**
K144	*St. luteogriseus*	1,76 ± 0,25	0,337	79,93
K145	*St. rimosus*	1,83 ± 0,07	0,337	79,93
K189	*St. rimosus*	2,14 ± 0,08	0,455	68,13
K139	*St. sanglieri*	2,20 ± 0,04	0,522	61,43
K236	*St. cinereoruber*	2,04 ± 0,14	0,551	58,52
K129	*St. violarus*	2,23 ± 0,31	0,613	52,33
K116	*St. baarnensis*	2,34 ± 0,06	0,627	50,90
K136	*St. violaceoruber*	1,86 ± 0,35	1,088	4,79
K128	*St. spiroverticillatus*	2,30 ± 0,32	1,120	1,60
Control[Δ]		**2,25 ± 0,18**	**1,136 ± 0,129**	**0,00**

[Δ] The ELISA test was performed with three repetitions (mean ± sd, n=3).

Effectiveness of ZEA detoxification of the 10 selected strains from bitoxin experiment was tested using the BLYES test method. The residual estrogenic effects of the degradation samples were performed with a percentage of bioluminescence intensification (BI%) compeared to the control (Table 3). The decrease in the estrogen effect detected by BLYES test is therefore real and is the result of the activity of *Streptomyces* strains. The analytical measurement of the concentration of ZEA residuals was carried out by ELISA test. Of the ten strains, ZEA degradation rate was below 50% for the *St. violarus* K129, between 50-80% for six strains (*St. cinereoruber* K236, *St. sanglieri* K139, *St. baarnensis* K116, *St. violaceoruber* K136, *St. luteogriseus* K144 and *St. spiroverticillatus*

K128), and over 80 % for three strains (*St. cacaoi subsp. asoensis* K234 and *St. rimosus* K145 and K189).

Table 3. The BLYES test bioluminescence intensification percent (mean ± sd, n=3) and the ZEA concentration and breakdown percentage based on the ELISA test in the monotoxin system (supernatant fraction).

Strains	Species	BLYES (BI%)	ELISA ZEA (mg/L)	ELISA ZEA (degradation %)
K145$^{\Delta}$	***St. rimosus***	**-29,72 ± 5,27***	**0,0035 ± 0,0017**	**99,62 ± 0,18**
K189$^{\Delta}$	***St. rimosus***	**-30,14 ± 7,36***	**0,0033 ± 0,0022**	**99,64 ± 0,23**
K234$^{\Delta}$	***St. cacaoi subsp. asoensis***	**307,61± 142,99***	**0,1125 ± 0,0619**	**87,85 ± 6,68**
K128	*St. spiroverticillatus*	687,74 ± 119,49	0,2245	76,01
K144	*St. luteogriseus*	665,16 ± 25,44	0,2555	72,70
K136	*St. violaceoruber*	553,46 ± 71,52	0,3965	57,64
K116	*St. baarnensis*	710,67 ± 31,50	0,4200	55,13
K139	*St. sanglieri*	478,35± 41,85	0,4330	53,74
K236	*St. cinereoruber*	708,22 ± 62,17	0,4555	50,84
K129	*St. violarus*	610,42 ± 23,26	0,6040	35,47
Control$^{\Delta}$		**610,27 ± 61,49**	**0,9265 ± 0,0602**	**0,00**

$^{\Delta}$The ELISA test was performed with three repetitions (mean ± sd, n=3).
*Significant differences from the control system (one-way ANOVA, $F_{10,22}$=49.46; p<<0.001).

According to the pellet fraction experiment, bound rate of AFB1 toxin compound to cell-wall of *Streptomyces* strains was less than 30%. In the pellet fraction, the lowest toxin concentration (0.05 ppm) was detected for the most effectively degrading strain (*St. cacaoi subsp. asoensis* K234) from monotoxin experiment of the AFB1 degradation. Therefore, the 88% decrease in AFB1 concentration was not due to adsorption but actual biodegradation. For ZEA pellet fraction studies, ZEA adsorption rate values were never higher than 12.7%. No adsorption was detected for those strains (*St. rimosus* K145 and K189) that were the most effective in degrading ZEA and eliminating estrogen effects proving that the effectiveness of these strains is factual.

NOVEL SCIENTIFIC RESULT: (Thesis 2) Based on the results of the ELISA and SOS-Chromotests, the *Streptomyces cacaoi subsp. asoensis* K234 effectively detoxifies the aflatoxin-B1 mycotoxin, it is able to eliminate its genotoxic effect *in vitro* and that is not attributable to toxin binding to cell-wall proven by pellet fraction study.

NOVEL SCIENTIFIC RESULT: (Thesis 3) Based on the results of the ELISA and SOS-Chromotests, the *Streptomyces rimosus* K145 and K189 strains effectively detoxify the zearalenone mycotoxin, they are able to eliminate its estrogenic effect *in vitro* and that is not attributable to toxin binding to cell-wall proven by pellet fraction study.

IV/3. Antimicrobial effect examination

Cross streak method

In the cross streak method, 57 strains out of 160 (2 strains included only in the agar diffusion test) did not show any antimicrobial effect. 39 strains showed only antibacterial effects. 6 of these strains inhibited the growth of the three test bacterial species (Figure 1), and the remaining 33 strains showed antibacterial activity against only one or two tested bacterial species. Among the latter, *St. rangooensis* K103 and K111 strains should be highlighted because no literature data exists for the antibacterial effects of this species, but in my experiments, its strains inhibited the growth of *Bacillus subtilis* DSM 347 and *Escherichia coli* K12. The *St. turgidiscabies* species has been described as a herbal pathogen, however, in my experiment, *St. turgidiscabies* K36 strain showed good inhibition against *Bacillus subtilis* DSM 347 and *Escherichia coli* K12.

27 strains showed antifungal activity only, eight strains inhibited the growth of three test species, and 19 strains showed only one or two species with antibiotic effect. No information about antifungal compounds is available in the literature on *St. baliensis*, but it's strain K73 inhibited all three fungal species in my experiment.

A total of 37 strains showed antimicrobial activity against any members of both groups (selected bacteria and fungi). *St. caniferus* K176 of these strains should be highlighted which inhibited the growth of all test organisms except for *Pseudomonas aeruginosa* P18, while the available literature mentions only a weak antifungal effect of this species. 4 of the 37 strains inhibited all three bacterial species and 10 strains were active against all three species of fungi. 5 strains had antimicrobial effects against all six test organisms. *St. rimosus* K145 and K189 strains exhibited outstanding antifungal and antibacterial properties as these strains inhibited the growth of all test organisms and caused the longest zones of inhibition (in mm). The effective antimicrobial ability of the species has already been revealed. It's inhibitory effects stand out of other *Streptomyces* strains for the inhibition of *Pseudomonas aeruginosa* P18 bacteria. The strains of *P. aeruginosa* bacterial species are globally characterized by multi-resistance to different antibiotics. As a result, it is not surprising that in my studies only a few (25 out of 162) *Streptomyces* strains showed inhibitory activity. Therefore, the highly effective inhibitory effect of *St. rimosus* K145 and K189 deserves particular attention in clinical practice in the fight against *P. aeruginosa*.

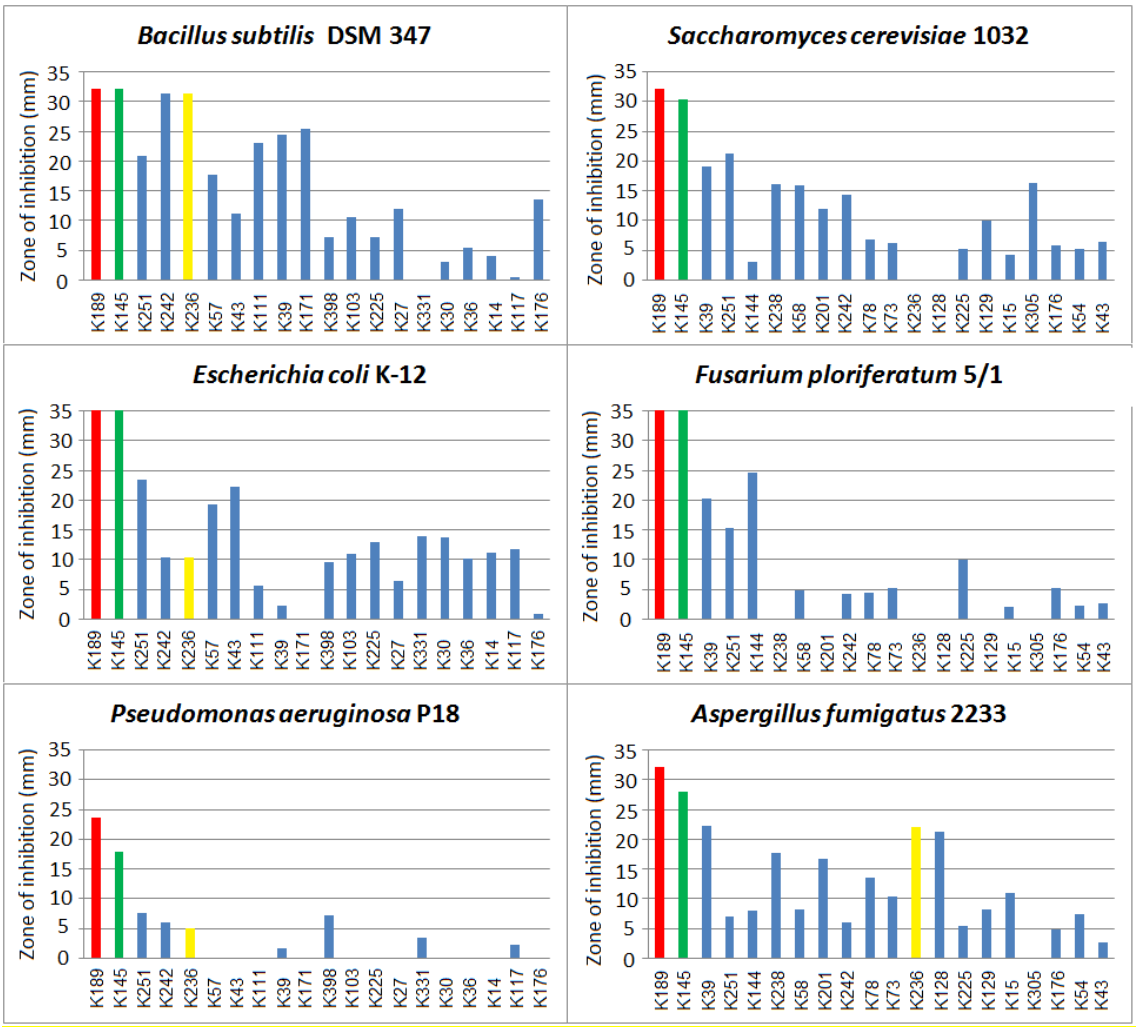

Figure 1. Zone of inhibition shown by *Streptomyces* strains in mm in the cross streak method.

The bar charts on the left shows the 20 *Streptomyces* strains which were the most effective against the three test bacterial species and the bar charts on the right those 20 *Streptomyces* strains which were the most effective against the three test fungal species. Highlighted are the three most effective antibiotic strains: *Streptomyces rimosus* K145 with green, *Streptomyces rimosus* K189 with red, and *Streptomyces cinereoruber* K236 with yellow.

Agar diffusion test

40 strains out of 74 did not show any antimicrobial effects at the agar diffusion test. Results are illustrated in the same way as for the cross streak method (Figure 2). 9 strains inhibited the growth of bacteria but these strains reduced the growth of only individual species of bacteria. Most of the *Streptomyces* strains (19) produced antimicrobial substances against *Bacillus subtilis* DSM 347 and the most effective strain was *St. flavogriseus* K53. Earlier researches already revealed the antibacterial effect of this *Streptomyces* species.

14 strains showed inhibitory against fungi solely but there were no strains that would inhibit the growth of all three fungi species. 11 strains affected both groups (bacteria and fungi). *St. rimosus*

K145 of these strains showed inhibitory against all fungal species in this test as well. Based on the results of the agar diffusion test, only the *St. rimosus* K189 strain inhibited the growth of all test organisms and this strain was the only one that had a little inhibitory effect on *P. aeruginosa* P18. Therefore, the results at this strain, with respect to the inhibited groups, were consistent with the results of the cross streak method.

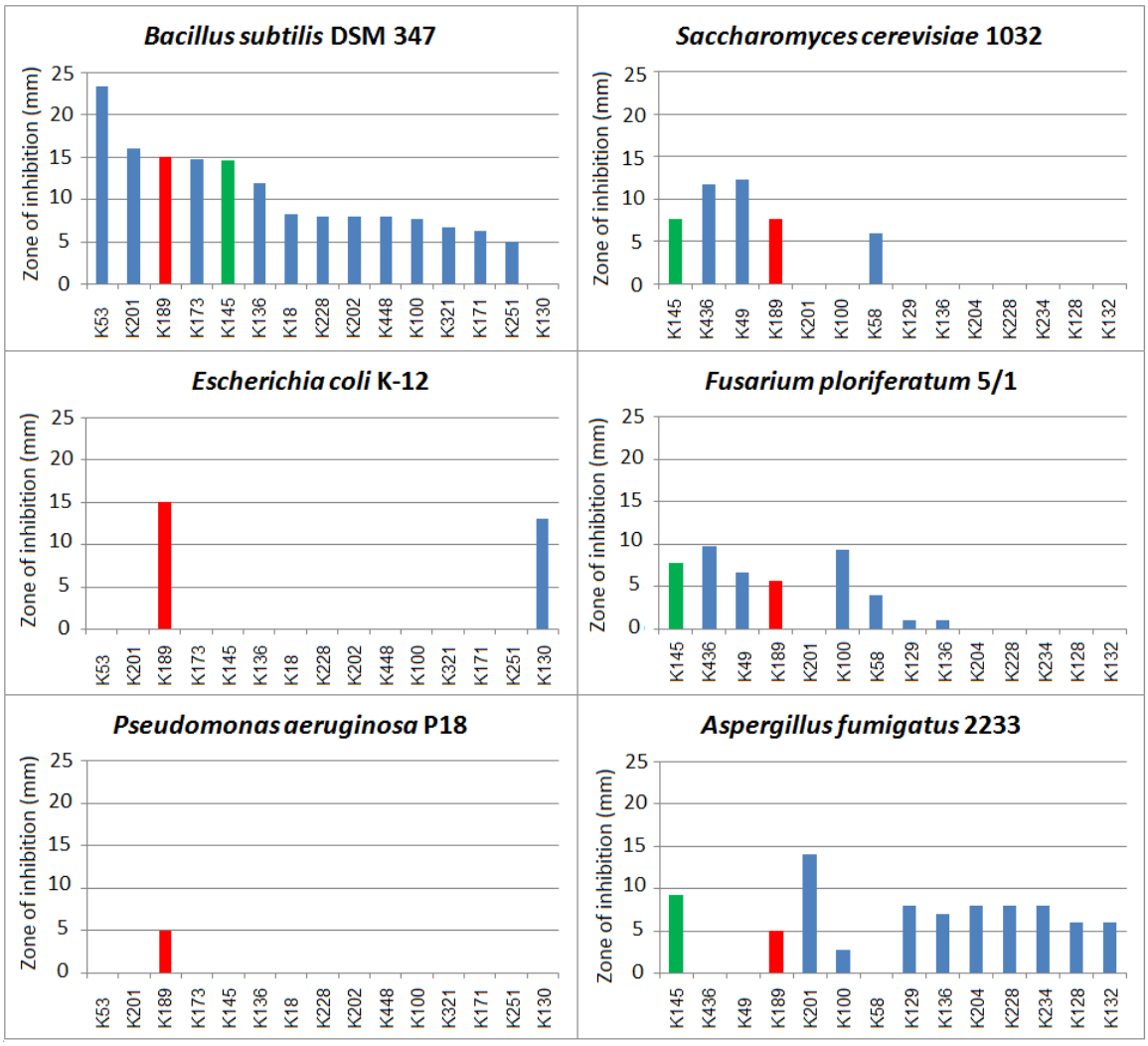

Figure 2. Zone of inhibition shown by *Streptomyces* strains in mm in the agar diffusion test.

The bar charts on the left shows the 15 *Streptomyces* strains which were the most effective against the three test bacterial species and the bar charts on the right those 15 *Streptomyces* strains which were the most effective against the three test fungal species. Highlighted are the two strains with wide inhibition spectrum: *Streptomyces rimosus* K189 with red, and *Streptomyces rimosus* K145 with green.

IV/4. Comparison of antimicrobial effect and biotoxification results

St. rimosus K145 strain successful at ZEA degradation and detoxification showed an effective and broad spectrum of antimicrobial effects by the cross streak method. *St. rimosus* K189 strain, which

also excelled at ZEA degradation, was the only one that had inhibitory effects on each test organism in both tests. However, *St. cacaoi subsp. asoensis* K234 effective in both the AFB1 and ZEA degradation and detoxification showed only insignificant antimicrobial effects. For this *Streptomyces* strain, at the cross streak method, minimal inhibitory effects were detected against *Bacillus subtilis* DSM 347, which is especially sensitive against antibiotics, and at a small degree against *Aspergillus fumigatus* 2233 at the agar diffusion test.

Table 4. Comparison of correlations between detoxification and antimicrobial results. Results of both the cross streak method and the agar diffusion test are shown separately including those of the bitoxin and monotoxic experiments. The sums of the bacterial and fungi inhibition zones were separately analyzed. Abbreviations: cross = cross streak method, hole = agar diffusion test, n = number of strains in the test.

Comparison	Detoxification test	Antimicrobial test organism	variable$_1$	variable$_2$	r	p	n
Detoxification and cross streak method	bitoxin	Bacteria	SOS-Chromo [IF]	cross [mm]	-0.11	0.3144	92
		Bacteria	BLYES [BI%]	cross [mm]	-0.16	0.1383	92
		Fungi	SOS-Chromo [IF]	cross [mm]	-0.287	0.0059**	92
		Fungi	BLYES [BI%]	cross [mm]	-0.361	0.0004**	92
		Aspergillus	SOS-Chromo [IF]	cross [mm]	-0.36	0.0004**	92
		Aspergillus	BLYES [BI%]	cross [mm]	-0.51	0.0000002**	92
	monotoxin	Bacteria	SOS-Chromo [IF]	cross [mm]	0.45	0.2298	9
		Bacteria	BLYES [BI%]	cross [mm]	-0.59	0.0916	9
		Fungi	SOS-Chromo [IF]	cross [mm]	0.07	0.8801	9
		Fungi	BLYES [BI%]	cross [mm]	-0.13	0.7476	9
Detoxification and agar diffusion hole test	bitoxin	Bacteria	SOS-Chromo [IF]	hole [mm]	-0.28	0.0297*	60
		Bacillus	SOS-Chromo [IF]	hole [mm]	-0.26	0.0461*	60
		Bacteria	BLYES [BI%]	hole [mm]	-0.05	0.7169	60
		Fungi	SOS-Chromo [IF]	hole [mm]	0.07	0.5862	60
		Fungi	BLYES [BI%]	hole [mm]	-0.23	0.0812	60
	monotoxin	Bacteria	SOS-Chromo [IF]	hole [mm]	0.02	0.9628	9
		Bacteria	BLYES [BI%]	hole [mm]	-0.65	0.0576	9
		Fungi	SOS-Chromo [IF]	hole [mm]	-0.04	0.9134	9
		Fungi	BLYES [BI%]	hole [mm]	-0.71	0.0322*	9

*The correlation stops at the removal of two effective strains: *Streptomyces rimosus* K145 and K189.
**The correlation remains ($p<<0,05$) without outstanding values (K145 and K189).

Therefore, strains effective at mycotoxin degradation do not necessarily exert an antimicrobial effect. In addition, many strains were proven to be effective against test bacterial and fungal species but did not eliminate the biological effects of mycotoxins. These observations were also supported

by analysis of correlation (Table 4). In most cases, no correlation was found between detoxification and antimicrobial properties. However, weak correlations were found caused by outliers of the two effective antimicrobial strains of *St. rimosus* but this did not prove the general correlation between the two properties. The only significant correlation resulting not from the outliers was between the detoxification values of the bitoxin experiment and the antimicrobial effects against the *Aspergillus fumigatus* 2233 test organism at the cross streak method (Table 4). The correlation found for fungal test organisms is also caused by *Aspergillus fumigatus* values. Accordingly, the lower genotoxic or hormonal effects caused by a given strain in the bitoxin experiment, the longer zone of inhibition was generated in the cross streak method experiment against the *Aspergillus* species. Therefore, according to the bitoxin system, inhibition against *Aspergillus* may be related to the detoxification property.

NOVEL SCIENTIFIC RESULT: (Thesis 4) *Streptomyces turgidiscabies* K36 strain generated antibodies against *Bacillus subtilis* DSM 347 and *Escherichia coli* K-12 bacterial species and that is not confirmed by literature data.

NOVEL SCIENTIFIC RESULT: (Thesis 5) *Streptomyces baliensis* K73 strain inhibited all three test fungi species (*Saccharomyces cerevisiae* 1032, *Fusarium ploriferatum* 5/1, *Aspergillus fumigatus* 2233) although no antifungal effects have been shown in regards to them so far.

NOVEL SCIENTIFIC RESULT: (Thesis 6) *Streptomyces gibsonii* K100 against *Bacillus subtilis* DSM 347, *Fusarium ploriferatum* 5/1, and *Aspergillus fumigatus* 2233 test organisms; *Streptomyces rangoonensis* K103 on *Bacillus subtilis* DSM 347, *Escherichia coli* K-12, *Saccharomyces cerevisiae* 1032, *Fusarium ploriferatum* 5/1, and *Aspergillus fumigatus* 2233; finally, *Streptomyces glomeroaurantiacus* K132 strain exerts an antimicrobial effect on *Bacillus subtilis* DSM 347 and *Fusarium ploriferatum* 5/1 test organisms, which have not been reported so far.

NOVEL SCIENTIFIC RESULT: (Thesis 7) *Streptomyces caniferus* K176 strain exerts an antimicrobial effect on *Bacillus subtilis* DSM 347 and *Escherichia coli* K-12 test bacteria, yet their antifungal activity was shown before.

NOVEL SCIENTIFIC RESULT: (Thesis 8) *Streptomyces coelicoflavus* K305 effectively inhibits *Saccharomyces cerevisiae* 1032 yeast strains, yet their antibacterial effect was shown before.

NOVEL SCIENTIFIC RESULT: (Thesis 9) Based on analyses of correlation, no correlation was found between the detoxification and antimicrobial effects of *Streptomyces* strains. One exception is the positive correlation of the detoxification properties of the strains and the inhibitory effect of *Aspergillus fumigatus* 2233 in the cross streak method.

V.Conclusions and suggestions

The *Streptomyces* genus of *Streptomycetales* order has been a bacterial group known for its production of antibiotics. From the environmental elements, many of these strains have now been identified and their antimicrobial properties investigated. This group is rather soil-dwelling and holds extremely diverse biochemical properties and its functions could be employed at biodegradation have remained largely unexplored yet. In my studies, a multiphase analysis of 380 *Streptomyces* strains was performed in which they were genotyped, and their AFB1 and ZEA mycotoxin degradation and biodetoxification ability and their antimicrobial activity were investigated.

The two mycotoxins frequently occur together in nature, so at first a bitoxin degradation experiment was performed. Then, ten such strains of *Streptomyces* were selected which effectively detoxifies AFB1 and ZEA in the presence of both toxins. In order to confirm their degradation and detoxification abilities, these strains were investigated also in a monotoxin degradation experiments. For three strains, it was proven that they are able to eliminate the biological effects of mycotoxins. The different results of the bitoxin and monotoxin experiments are supposed to be due to that the mycotoxins may function differently in the presence of each other. The interaction of different mycotoxins may enhance but may also reduce their toxic or hormonal effects depending on their concentration. In addition, various toxin molecules may activate similar metabolic pathways in the enzymatic system of the degrading bacterium, which could stimulate the degradation process in the presence of multiple toxins.

At the individual degradation of both AFB1 and ZEA mycotoxins, several strains degraded toxin molecules, but only a few eliminated the harmful (genotoxic or hormonal) effects of them based on the toxicity-profiling bio-tests. For biotechnological use, it is not enough to justify the degradation of the molecular structure of a toxin as it may produce metabolites (degradation products) which may have more toxic effects than the original compound. In my experiments, the *St. cacaoi spp. asoensis* K234 was the only strain degrading AFB1 mycotoxin most effectively and eliminating its biological effects. This strain effectively degraded the ZEA mycotoxin molecule also, but did not completely eliminate its hormonal effects. In the degradation of ZEA, the *St. rimosus* K145 and K189 strains proved to be the most effective as the biological (estrogenic) effect was eliminated besides the extensive degradation rate of the mycotoxin molecule.

In alignment with the statement above, it has been demonstrated that, in addition to high degradation efficiency, genotoxic and hormonal effects caused by toxic substances remaining after

degradation should be investigated as well. In addition to biodegradation processes, monitoring the biological effects is also necessary.

Several *Streptomyces* strains are used in the pharmaceutical industry for their high antibiotic activities. My further studies aimed at analyzing their antimicrobial effects and the results supported the fact that many *Streptomyces* strains effectively inhibit the growth and reproduction of microorganisms. In my dissertation, inhibitory effects of strains were studied by cross streak method and agar diffusion test. With these microbiological tests, several strains were investigated whose antimicrobial effects had already been demonstrated, but such were analyzed either of which this ability has not been mentioned in the literature before.

In cross-streak method and agar diffusion experiment, a total of 41 strains inhibited only bacteria, 28 strains only fungi and 43 strains both groups. Among them, the *St. rimosus* K145 and K189 strains – also highlighted at the ZEA degradation – stood out again inhibiting all tested test organisms in growth. In addition, it is worth to highlight the broad spectrum antimicrobial effect of *St. cinereoruber* K236, *St. albidofla*vus K242 and *St. lavendulae* K251 strains.

The antimicrobial effects of several *Streptomyces* species have not been described previously. For example, *St. cinereoruber* K36 and *St. baliensis* K73 strains, which have not been detected against bacterial or fungal species until now. In addition, it has not been published for the species *St. rangoonensis*, *St. gibsonii*, *St. glomeroaurantiacus*, *St. caniferus* and *St. coelicoflavus* that they inhibit bacterial or fungal species.

Although there was no correlation between the detoxification and antimicrobial properties of *Streptomyces* strains, the antimicrobial effect of the genus may query the utilization of strains against mycotoxins in bioremediation, as the inhibition effects of *Streptomyces* bacteria in the environment may raise questions of human and environmental safety. Based on the results of the two different experiments (biodetoxification and antimicrobial activity), the outstanding AFB1 biodetoxifying *St. cacaoi subsp. asoensis* K234 strain inhibited minimally *Bacillus subtilis* DSM 347 in the cross-streak method and had a weak inhibitory effect against *Aspergillus fumigatus* 2233 in the agar diffusion test. However, according to other literature data, the species has a more effective and broad spectrum antimicrobial effects. For example, it had an inhibitory effect on an antibiotic-resistant *Staphylococcus aureus* strain.

Additionally, *St. rimosus* K145 and K189 strains with effective ZEA degradation also emerged in the production of antimicrobial agents. They effectively inhibited all the bacterial and fungal test organisms according to the results of both antimicrobial experiments. The *in vivo* use of the

Streptomyces strains proven to be effective in biodetoxification may lead to human health risks, therefore, the next step may be the determination and isolation of enzymes taking part in degradation of mycotoxins.

The future applicability of biodegradation (bioremediation) properties is also dependent on the antimicrobial ability of a strain. Hence despite of its good degradation ability, it is hazardous to relocate a bacterial strain with a broad spectrum of antimicrobial effects to the environment as it may interfere with natural microbiota or induce antibiotic resistance. In order to reduce the risk of antimicrobial activity and resistance dissemination, it is highly recommended using the mycotoxin-degrading enzymes produced by bacterial strains instead of using strains *in vivo*. Enzymes can be produced in large quantities by fermentation, under laboratory conditions, then extracted and purified, and finally used specifically. Within the biotechnological use of the strains, development of such fermentation methods should be promoted which extract and multiply enzymes responsible for degradation to make advance in bioremediation of mycotoxins. Production of an effective enzyme of a strain by other genetically engineered bacteria with no antimicrobial effect may provide a safe solution to eliminate mycotoxins. However, genetically modified organisms may be permitted to use *in vivo* only in limited way; therefore, these organisms should be used only for production of their enzymes. Such methods are still used in production of antibiotics, so adapting them to extraction of enzymes may be applicable.

From the perspective of enzyme production, also important information on a bacterial strain is whether the mycotoxin degrading trait is characteristic to the bacterial species, i.e. it is coded in its original DNA or is an acquired trait from its habitat or a plasmid. For degradation of AFB1 and ZEA, it has also been revealed that degrading enzymes are encoded in the DNA of the studied (non-*Streptomyces*) strains. However, in the case of a *Pseudomonas* strain, ZEA fragmentation was detected with plasmid-encoded enzymes. However, the foregoing does not provide sufficient evidence that the toxin-degrading abilities of *Streptomyces* strains are traits encoded in their original DNA or they are consequence of gene transfer events associated with their original habitat.

Based on a report of UN World Health Organisation, among others, carbapenem-resistant *Pseudomonas aeruginosa* is one of the most known problems of antibiotic resistance in the world today. To prevent the spread of resistance, development of new antibiotics would be important. Although *Pseudomonas aeruginosa* P18 strain used in this thesis work was not carbapenem-resistant, but *St rimosus* K145 and K189 strains effectively inhibited it, therefore, further antimicrobial experiments are suggested to investigate carbapenem-resistant environmental and clinical isolates of *Pseudomonas aeruginosa* with these *Streptomyces* strains.

It is suggested to maintain the strains of the collection and to preserve their biochemical activity in order to carry out the remaining biodegradation and antimicrobial experiments. To do this, it is imperative to determine and optimize the maintenance under appropriate conditions. Therefore, it is recommended to carry out optimization experiments on the medium, to determine the appropriate rate of carbon and nitrogen content and a favorable pH range.

V. Publications

Scientific paper:

Harkai, P., *Szabó, I.*, *Cserháti, M.*, *Krifaton, C.*, *Risa, A., Radó, J., Balázs, A., Berta, K.*, *Kriszt, B.* (2016) Biodegradation of aflatoxin-B1 and zearalenone by *Streptomyces* sp. collection. International Biodeterioration & Biodegradation, 108, 48-56. **(IF: 2,962)**

Krifaton Cs., B. Kriszt, A. Risa, S. Szoboszlay, ***P. Harkai****, M. Cserháti, M. Eldridge, J. Wang, J. Kukolya*, (2013) Application of a yeast estrogen reporter system for screening zearalenone degrading microbes, Journal of Hazardous Materials, 244, 429-435. (**IF: 4,173**)

Kaszab, E., Kriszt, B., Atzél, B., Szabó, G., Szabó, I., ***Harkai, P.****, Szoboszlay, S.* (2010) The occurrence of multi drugresistant *Pseudomonas aeruginosa* on hydrocarbon contaminated sites. Microbial Ecology, 59, 37-45. (**IF: 2,558**)

Proceedings:

P. Harkai*, K. Berta, I. Szabó, Cs. Krifaton, A. Balázs, J. Radó, A. Risa, B. Kriszt* (2014) Screening for mycotoxin (aflatoxin-B1, zearalenon) biodegradation from actinomycetes strain collection. Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, 2014. október 15-17., Keszthely (Absztraktfüzet p.20.).

Krifaton Cs., Kukolya J., Risa A., ***Harkai P****., Cserháti M., Szoboszlay S., Kriszt B.* (2011) Screening of zearalenone degrading microbes by yeastoestrogen reporter system (BLYES). In: Nagy K.,Márialigeti K. (szerk.) ActaMicrobiologica et Immunologica Hungarica,**58** (Suppl.): 175.

Kukolya J., Krifaton Cs., Cserháti M., Háhn J., ***Harkai P****., Sebők F., Dobolyi Cs., Szoboszlay S., Kovács B., Bakos K., Urbányi B., Kriszt B.* (2011) Biomonitoring rendszerek alkalmazása mikotoxin biodetoxifikációjára alkalmas mikroorganizmusok szelekciójára. In: Darvas B. (szerk.) I. Ökotoxikológiai konferencia, Budapest, pp. 16-17.

P. Harkai, *P. Veres, M. Farkas, J. Kukolya, S. Szoboszlay, B. Kriszt* (2010) Screening for new taxa from *Actionomycete* strain collection built in the early seventies. Magyar Mikrobiológiai Társaság 2010. évi Nagygyűlése, 2010. október 13-15., Keszthely (Absztraktfüzet p.28.).

***Cummulative impact factor* (IF) *of publications*: 9,693**