Novel bioactive compounds produced by *Actinomycetales* through the mixed cultivation techniques

Applicant: Jan Bobek, Ph.D.

BACKGROUND

I. New laboratory of bacteriology at the Institute of Immunology and Microbiology will connect immunological and microbial disciplines.

The basic and applied research in the Institute of Immunology and Microbiology (IIM) covers studies on incompatible different aspects of virology, prionic research, parasitology, and immunology. Up today, the bacteriology research was in the shadow of the other fields in the institute. The applicant's vision is to build a strong bacteriological research team which will study the culture conditions and molecular mechanisms launching the production of novel bioactive compounds and their effect on the co-cultured cells (other bacteria, parasites, and human cells). The proposed project requires an interdisciplinary approach; for that reason it brings a potentiality for a more intense collaboration between bacteriological, parasitological, and immunological departments.

II. The interactions of streptomycetes with other organisms lead to a production of secondary metabolites.

In the last three decades, medicine around the world has dealt with exponentially increasing rates of antibiotic resistant infections. The latest example illustrating the alarming speed of a resistance gene dissemination is a "pan-drug" resistant CRE strain of *E. coli*, insensitive to the last choice antibiotic colistin, reported in 2015 in pigs and a few humans in China (Liu *et al.*, 2016). This April, it appeared in a patient with no contacts to China in the USA (McGann *et al*., 2016). As the number of newly approved antimicrobials is continuously declining, recent research concentrates on novel screening methodologies applicable both to traditional and novel natural producers.

The genus of streptomycetes keeps a strong position among the most valuable bioactive compounds producers. About one half of clinically-used antibiotics are produced by these bacteria. Other secondary metabolites serve as antihelmintics, insecticides, and herbicides. Moreover, some of them have recently been found to influence human cell behavior, as immunomodulators, anticancer agents, etc. These compounds are able to specifically target key regulators or enzymes of mammalian cells (van Keulen and Dyson, 2014).

The traditional reservoir of streptomycetes is soil; however, they can also be found in water, including oceans (Chater and Hopwood, 1993). In their natural environments, streptomycetes enface numerous interactions with other organisms, endeavoring for the similar nutrient sources or simply sharing the same ecological niches. The suppression of the microbial competitors is the reason why these organisms evolved such wide capacities to produce bioactive compounds - antibiotics, toxins, biosurfactants, volatiles and others (Tyc *et al.*, 2014). Thus, the secondary metabolism of streptomycetes provides a selective control of their surrounding environment.

Interestingly, there is an increasing evidence that *Streptomyces* and other *Actinobacteria* frequently colonize the skin, guts, respiratory, and genital tracts of animals, including humans (Osipov and Verkhovtseva, 2011; Collado *et al*., 2016), where they are expected to modulate the microbial communities. Our concern is whether such human colonizers are also able to produce secondary metabolites modulating host cell behavior. We theorize that they modulate not only the host microbiome, but they can directly influence human tissues or the immune system. Produced compounds may thus e.g. suppress the immune response against the streptomycete "intruders" or modulate cell behavior to provide friendlier environment. Such adaptations have been created during thousands years of co-evolution. Therefore, the produced compounds would be highly appreciated in medicine as we may expect their high specificity and efficiency, low cytotoxicity, and an easier entrance into human cells.

III. Awakening the biosynthetic potential of streptomycetes

Streptomycetes, as well as other microorganisms, have conventionally been investigated in pure cultures, in which a majority of their biosynthetic potential remains silent. Exponentially growing genomic data of streptomycetes confirm that only negligible number of biosynthetic gene clusters are active under standard laboratory conditions (e.g. 4 of at least 29 identified in the model streptomycete, *Streptomyces coelicolor* A3(2) (Bentley *et al.*, 2002; Pethick *et al.*, 2013; Ikeda *et al.*, 2003)). Awakening their biosynthetic potential thus opens novel possibilities for new drug discoveries.

Special attention is paid to mimicking natural microbial cell communication by inter-species interactions (the mixed-cultures cultivations or co-cultures) (Chiang *et al.*, 2011; Ochi and Hosaka, 2013); the technique has already proven the hypothetical expectations. Induction of antibiotic production by co-culture was reported between various streptomycete species (Yamanaka, 2005; (Traxler *et al.*, 2013), or between streptomycetes and fungi (*Aspergillus*; Wu *et al.*, 2015). Some experiments involved other soil bacteria representing natural interaction partners, such as *Bacillus subtilis* or bacteria containing mycolic acids in their cell walls (Onaka *et al.*, 2011; Traxler *et al.*, 2013), or two actinomycete endosymbionts of a single invertebrate (Dashti *et al.*, 2014). In the most extensive assay, Tyc and co-workers cultivated 146 different soil bacteria in pair-wise combinations and tested antimicrobial activity of produced metabolites on two clinical isolates of *Escherichia coli* and *Staphylococcus aureus.* When a streptomycete strain used in the assay was co-cultivated with *Burkholderia*, *Flavobacterium*, *Pseudomonas* and others, a production of unknown metabolites with bioactivity against the two clinical isolates was induced (Tyc *et al.*, 2014).

OBJECTIVES

In the course of this project we will explore metabolic activities of clinical isolates of streptomycetes (and other actinomycetes), when cultivated with selected human pathogens (bacterial, fungal, and protozoal) or with human cell/tissues **(listed in Table 1)**. The features and pharmaceutical attractiveness of newly isolated compounds will be investigated.

For this aim:

1. Pairwise cultivations (the microbial co-cultures) will be applied in combinations for each streptomycete strain and each microbial pathogen.

1a. Induction of secondary metabolism will be determined and antibiotic activity of the produced metabolites will be directly tested on the pathogenic bacteria (as well as on the other microbes).

2. Streptomycetes will be cultivated with human cells (specifically, the immune cells including dendritic cells, monocytes or pneumocytes).

2a. Potentials of the human cells to induce production of novel compounds by streptomycetes will be evaluated. The bioactivity of produced compounds against the human cells and the pathogenic bacteria will be determined.

2b. The effect of streptomycetes on the viability and functional capacity of human cells will be tested.

3. Novel bioactive compounds will be purified; their structure, genetic background, and bioactivities will be determined.

Table 1. Strains of *Actinomycetes*, inducing microbes, and inducing human cells used in the project

S. sp.anti-MRSA & antifungal	*S.sp ., Streptomyces* -like obscure & rare species			*S. sp.* clinical, human-assoc.		Pathogenic microbes	Human cell/tissue
BCCO1627	BCCO1585	BCCO1618	BCCO1658	TR1050	TR0950	*Escherichia coli* (CRE)	the immune cells (THP-1, dendritic cells)
BCCO1628	BCCO1587	BCCO1620	BCCO1690	TR1117	TR1144	*Clostridium*	pneumocytes
BCCO1075	BCCO1589	BCCO1624	BCCO1695	TR0978	TR1059	*Staphylococcus aureus* (MRSA)	the skin epithelium
BCCO1597	BCCO1597	BCCO1625	BCCO1701	TR1008	TR1120	*Salmonella*	
BCCO1627	BCCO1602	BCCO1633	BCCO1728	TR1292	TR1318	*Campylobacter*	
BCCO1632	BCCO1615	BCCO1636	BCCO1738	TR1049	TR1338	*Candida*	
BCCO1653	BCCO1616	BCCO1637	BCCO1741	TR1341	TR1428	*Aspergillus*	
BCCO1619	S. tsukubaensis NRLL18488 - positive ctrl					Acanthamoeba spp. Giardia intestinalis Leishmania spp.	
BCCO1680							
BCCO1638							
PRODUCERS						**INDUCERS**	

PRELIMINARY DATA, EXPERIMENTAL DESIGN AND METHODOLOGY

The experimental design will follow the presented flowchart:

Figure 1. Flowchart of the proposed project.

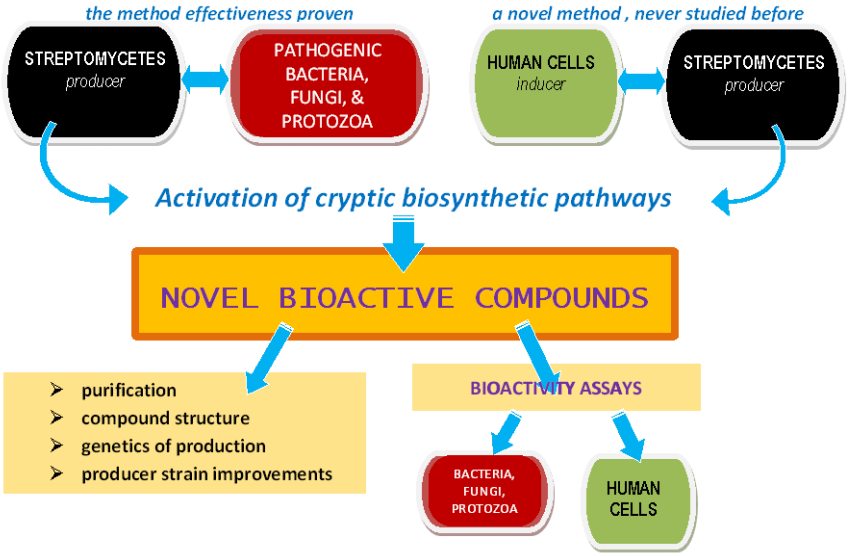
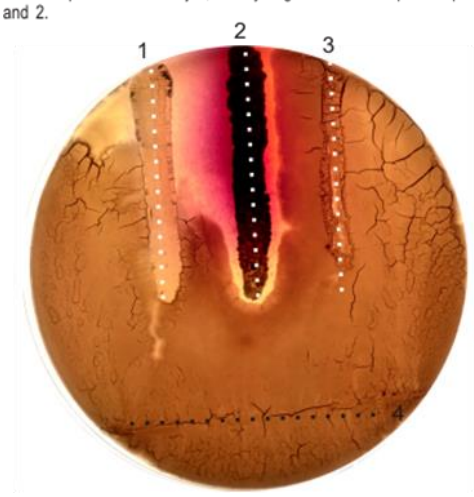


Figure 2. Interaction leading to a production of an antibiotic (preliminary result).

1. The microbial co-cultures

Example of interactions leading to production of secondary metabolites: Three actinomycetes were inoculated:1 *Streptomyces lividans* TK24, 2 *S. sioyaensis* BCCO981, 3 *Kribella catacumbae* DSM19601 in the lines indicated by white dots. Two days later, the *Bacillus* sp. was inoculated in the cross manner (black dots). After another 4 days we can see *Bacillus* growth inhibition by 2, the same strain producing an orange compound facing the *Bacillus* and a purple red compound facing the strain 1. The strongest inhibition of the fast-growing *Bacillus* can be seen in the region between 1 and 2. This suggests either induction of an antibiotic production of 2 by 1, or a synergic effect of compounds produced by 1 and 2.



For the experiments, new streptomycete isolates from unique biotopes will be used ("producer strains"). These will mainly originate from the collection of actinomycetes of the Biology Center AS CR in Ceske Budejovice (CCSACB, www.actinomycetes.cz), our collaborating partner. Their open collection of over 1500 new streptomycete and streptomycete-like natural isolates will provide pre-selected strains as a service. Some of these have already been proven active against MRSA, *Candida*, *Fusarium*, and *Aspergillus* (Fig. 2).

As a pilot assay, the selected streptomycete strains (listed in Table 1) will be co-cultured on agar plates in pairs with selected microorganisms ("inducer strains") - mostly pathogenic bacteria, fungi, and *Acanthamoeba spp.* (Table 1). Other *Protozoa* (*Giardia intestinalis, Leishmania spp.*) will be co-cultivated in suspensions in specific liquid media. Bacterial and fungal strains will include multiple resistant clinical isolates (e.g. *Escherichia coli, Clostridium, Staphylococcus aureus, Salmonella, Campylobacter; Candida,* and *Aspergillus,* resp.). Screenings will be performed on agar plates with proper timing of inoculation of both partners (slow-growers vs. fast-growers). Morphological and metabolic changes will be observed during 1-5 days of the co-culture, including *in situ* antimicrobial activity bioassays. The *in situ* bioassays are based on the observation of growth-inhibitory zones after overlaying the plate with a model sensitive microbe (*Escherichia, Bacillus* and yeast). Plates inoculated only with one interaction partner will be used as controls. In cases of positive bioactivity, liquid co-cultures will be set and their metabolomes analyzed by comparative metabolomics (pure inducer strain vs. pure streptomycete strain vs. the co-culture).

Both, post-cultivation media and biomasses will be analyzed, polar and non-polar metabolic fractions will be extracted by either aceton/ethylacetate or methanol extraction, respectively. For the initial comparison, TLC followed by bioactivity assay *in situ* will be performed to visualize the induced active compounds. Finally, the extracts will be analyzed using HPLC-MS accompanied with comparative metabolomics software. The fractions will be checked for the antibiotic activity using the inducer and other multi-resistant strains (mostly obtained from the Clinical microbiology and ATB center, Institute of Medical Biochemistry and Laboratory Diagnostics in Prague under the administration of MUDr. Vaclava Adamkova).

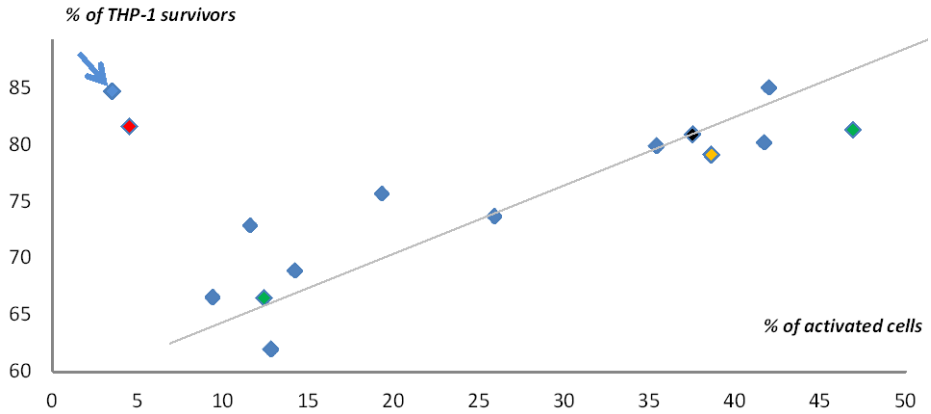
2. The Streptomyces – human cells interactions

Here we propose a completely novel approach where the methodology has to be developed from the very basics. The starting collection comprises fourteen human body-originated streptomycete strains **(listed in Table 1)**, which represent a unique collection from the NRL for Pathogenic Actinomycetes in Trutnov Hospital (Czech Republic). The species have been metabolically and phylogenetically characterized and, based on the 16S rRNA taxonomy, do not generally belong to any known pathogenic streptomycetes, though some fall to the same taxonomic group as the sub-Saharan Africa human pathogen *S. somaliensis* (according to comparison with sequences in EzTaxon database, http://www.ezbiocloud.net/eztaxon, Kim et al., 2012). This pilot set could be enlarged during the course of the project by targeted collection of streptomycetes occasionally appearing in clinical samples in the clinical microbiology laboratory of the applicant or in the National Reference Laboratory for Pathogenic Actinomycetes.

In our **preliminary co-culture experiment** (see the Graph below), the THP-1 human macrophages were co-cultured with pre-germinated spores of 14 human-originated streptomycete strains. Survival and activation of macrophages was assessed (Fig. 3). Effects of majority of the strains on the THP-1 seemed to follow a simple rule: the higher activation of macrophages, the higher was their viability after 30-90 minutes of co-culture (the

activation was assessed as the activation of the cells, the oxidative burst, FagoFlowEx kit, Exbio). Interestingly, in one case we observed that the streptomycete presence left macrophages without any obvious reaction, the cell survival was high and almost no activation was detected. We suppose that the unexpected behavior of the macrophages has been modulated by the streptomycete strain, which produced a specific metabolite(s) causing the strain being "invisible" for the immune response. This ability would be quite advantageous for a putative commensal microorganism. In another recent experiment performed with some of the strains, we have observed suppression of the PMA-mediated THP-1 activation by the presence of streptomycete strains, supporting the hypothesis of immunosuppressive effects of the bacteria.

Figure 3. The THP-1 viability and activation (preliminary result).



Activation and viability of THP-1 human macrophages after co-culture with 14 clinical-originated streptomycetes. *% of activated THP-1 cells and % of viable THP-1 cells are plotted for each particular streptomycete strain, represented by squares, after 90 minutes of co-culture. Negative control is shown in red (no streptomycetes added). The origins of strains indicated by colors: sputum in blue, uterus in green, conjunctival sac in yellow and hemoculture in black. Culture media did not contain any antibiotics to allow potential growth of streptomycetes. The major colinear trend of both parameters is fitted by the straight line. The arrow indicates the exceptionally behaving strain ("invisible", no activation, no-killing).*

We will perform similar pilot experiments with a wider collection of clinical actinomycete isolates (several tens strains of diverse origins, Table 1). As a control producer strain, *S. tsukubaensis* NRLL18488, producing an immunosuppressive drug tacrolimus (FK506), will be used in the experiments. The selection of human cells reflects the organs or parts of human body most frequently inhabited by streptomycetes (Table 1). Based on results, we will assay multiple parameters describing interaction of human cells with actinomycetes. Generally, human cells will be co-cultured with streptomycetes in the ratio 1:10 (1 human cell: 10 streptomycetes) for up to 24 hrs (optimal time interval will be determined for every cell type).

Dendritic cells represent the most potent antigen presenting cells capable to induce or polarize the immune response. The capacity of streptomycetes to promote dendritic cell maturation will be tested by co-culture of cord blood and buffy coat derived moDC with streptomcycetes and maturational status of dendritic cells will characterized by flow cytometry according to the presence of CD40, CD80, CD83, CD86, MHCII and qPCR (Taqman probes for CD40, CD80, CD86, MHCII, IDO – indolamine 2,3-dioxygenase, IL-10). IL-6 and TNF-alpha will be followed in cell culture supernatants. *In vitro* generated human moDC primed by streptomycetes will be co-cultured with naïve CD4 for 7 days followed by characterization of Th1 (CD4+T-bet+, IFN-gamma), Th2 (CD4+GATA3+IL-4, IL-13), Th17 (CD4+, RORgt+, IL-17A, IL17F, IL-22), Tregs (CD4+FoxP3+IL-10, TGF-beta) by flow cytometry. The capacity of streptomycetes primed dendritic cells to polarize immune responses will be followed by qPCR (gene expression of Th1 cytokine: IL-2, IFN-gamma, IL-12; Th2: IL-4, IL-13; Th17: IL-17A, IL-22 and regulatory cytokines: IL-10, TGF-beta). Cell culture supernatants will be inspected for Th1 (IL-2, IFN-gamma, IL-12), Th2 (IL-4, IL-13), Th17 (IL-17A, IL-22) and regulatory cytokines (IL-10, TGF-beta).

Viability and functional capacity of macrophages (THP-1) will be followed after the co-culture with streptomycetes by qPCR (gene expression of inducible NO synthase, IL-6, TNF-alpha, IL-10), concentration of IL-6, IL-10 and TNF-alpha in cell culture supernatants will be followed by ELISA, costimulatory molecules (CD80, CD86) and MHCII will be detected by flow cytometry.The effect on the viability and proliferation rate of pneumocytes and skin epithelium will be determined according to the presence of proliferation marker Ki-67.

Also, we will compare metabolic profiles of the co-cultured streptomycetes with negative controls incubated in the same way without the human cells. Highly sensitive techniques, as HPLC-MS, will be used for detection of the produced metabolites. Strains, showing either strong phenotypic effects on the human cells or producing novel metabolites upon interaction with them, will be subjected to fermentation (directly, or after a few rounds of co-culture with human cells) and produced metabolites will be extracted by standard organic solvents extraction as described above. The metabolic extracts, or their subfractions, will be applied to the target human cells (under normal or inflammation-stimulating conditions). Their effects on the immune response and cell survival will be examined. Viability of cells in tissue cultures will be followed by flow cytometry according to the propidium iodide incorporation in different time points (1hr, 3 hrs, 6 hrs, 12hrs, 24hrs, 48hrs and 72hrs). The effect of streptomycetes on the functional capacity of cells will be determined by estimation of the cytokines released into the cell culture media (ELISA), changes of gene expression (qPCR) and proliferation rate (CFSE dilution determined by flow cytometry). The strains producing metabolites with the strongest effects will be cultivated in a large scale (up to 4 liters of liquid cultures) and the active compounds will be isolated and purified using combination of liquid extraction, FLASH chromatography and HPLC chromatography. The structure of the compound will be checked first by high resolution LC-MS (known compound?) and then by NMR (novel compounds).

In general, the co-culture of streptomycetes with mammalian cells represents a completely novel approach. From our preliminary experiments (for more detailed information please visit our websites http://uim.lf1.cuni.cz/en/laboratory-of-bacteriology-research) we know that using common cell culture media without antibiotics, we can see survival of human cells for at least several hours and survival and growth of streptomycetes for at least 2 days. The pilot experiments should also clarify the minimal length of co-culture needed to activate metabolic changes in streptomycetes. The co-cultures collected in several time points (1, 2, 4, 10, 24 hrs) will be used as a seed cultures for small-scale fermentations and the metabolic changes will be assayed by comparative metabolomics using High Resolution LC-MS combined with a proper software.

3. Characteristics and biological activities of the produced compounds.

The active compounds with pharmacologically relevant features acquired in previous steps will be extracted and analyzed as specified above after large scales fermentations (up to 4 liters culture volumes) in the amounts and purity allowing NMR assessment of the structure and detailed activity tests. Pilot comparative metabolomics will be performed at low resolution LC-MS equipment. More detailed analyses of selected samples will be performed by high resolution HPLC followed by mass spectrometry in collaboration with Dr. Zdenek Kamenik, in the Institute of Microbiolgy, CAS. Fractionation of the extracts and active compounds purifications will be performed mainly by the flash chromatography, or preparative HPLC. Structure of novel compounds with pharmaceutically attractive features will be assessed by NMR and they will be subjected to patenting.

The antibacterial, antifungal, and antiprotozoal activities will be inspected first as comparison of growth inhibitory zones after application of the extracts, fractions or purified compounds to a target microorganism. Next, for the purified compounds, the minimal inhibitory concentrations will be assessed using standard protocols.

The effects of the compounds on human cells (immunomodulatory, pro-apoptotic, anti-proliferatory, etc.) will be assayed mainly by techniques and kits based on standard ELISA, flow cytometry or RT PCR methodology. The effect of streptomycetes on proliferation capacity of PBMC (peripheral blood mononuclear cells) will be tested by CFSE dilution using flow cytometry.

For the effective producer strain improvements, the biosynthetic gene cluster identification is crucial. This allows targeted upregulations of cluster expression, heterologous expression in specialized streptomycete hosts,

etc.. In cluster identification we can employ the growing knowledge on the biosynthetic pathways of secondary metabolites, which share numerous similar structures (polyketide or oligopeptide chains, various structural moietiesThe relevant genes can be targeted by PCR and used as a probe to isolate and clone entire biosynthetic gene clusters Specific heterologous producer strains with upregulated secondary metabolism are available in our laboratory (*S. lividans* K4-114, or *S. coelicolor* M1146 or M1154).

TIME SCHEDULE (as described in Table 2.)

Table 2. Time schedule

Year	Major tasks
2017	Strain selection based on preliminary data
	Microbiological and taxonomic characterizations of selected strains
	Optimization of microbial co-culture experiments (media, times, operations, etc.)
	Pilot co-cultures streptomycete-human cells
	Basic parameters human cells/strepomycete survival, basic response to co-culture
	Fermenation of pure cultures, pilot extractions of metabolites and bioassays
2018	Small scale fermentations of pure cultures and co-cultures
	Extraction of metabolites
	Fractionation of extracts, fraction analysis, purification of compounds
	Analysis of metabolites, comparative metabolomics
	Human cells response to actinomycetes - detailed studies (influence on immune response, survival, etc.)
2019	Optimization of human-streptomycete co-cultures to support production of novel compounds
	Fermentation, extractions of medium scale (co)cultures
	Genetic characterization of producers (incl. WGS sequencing or cosmid expression libraries, if needed)
	Purification of metabolites
	Structure assessments
	Detailed biological activity assays
	Data evaluation, preparation of manuscripts and patent applications

ANTICIPATED RESULTS AND IMPORTANCE OF THE PROJECT

Acquisition of novel compounds with pharmaceutically attractive features by means of activation of cryptic biosynthetic information, as the main aim, is a widely accepted technique. In the search for novel compounds we would like to specifically focus on the induction of new compounds active specifically against human pathogens, especially multiple resistant strains of bacteria and fungi, and against selected Protozoa.

The **main novelty of this project lies in the co-cultivation of streptomycetes with human cells.** The latest data indicate a persistent presence of streptomycetes in or on human bodies (data acquired by highly sensitive metagenomic or MALDI-TOF techniques; Osipov and Verkhovtseva, 2011; Collado *et al*., 2016). Even more, connection of their incidence to some chronic diseases, such as COPD (chronic obstructive pulmonary disease) or psoriasis, has been recently suggested (Zakharkina et al., 2013; Gao et al., 2008). Though, the regulatory impact of streptomycetes on the human microbiome is only expected and nothing is known about their direct interactions with human cells. On the other hand, streptomycetes are known to produce numerous compounds with specific and strong effects not only on other microorganisms, but also on behavior of human cells. Acquiring data documenting such interactions would have important impact on the view of their role within the human microbiome. Here proposed co-cultivation of streptomycetes with human cells brings a completely novel approach to specifically activate production of compounds targeting and modulating human cells, and therefore putatively attractive in medicine.

Indeed, any data indicating the direct interaction between human cells and actinomycetes are attractive enough for a highly valuated publishing. The expected results should lead to a submission of 3 manuscripts to

peer-reviewed international scientific journals. Novel compounds with desirable bioactivities or newly developed methodology of co-culturing will be subjected to patenting.

ELIGIBILITY & MAJOR RESPONSIBILITIES OF THE TEAM MEMBERS

The project will become the base workload of the novel Laboratory of bacteriology in our institute. The lab team is consisted from microbiology experts and Ph.D. students plus one collaborating immunology expert and one parasitology expert. The laboratory is equipped with all the instrumentation needed for molecular biology, fermentation, extraction, separation of metabolites and analytical techniques including LC-MS.

J. Bobek the head of Laboratory of bacteriology and the project leader; project coordination, data evaluation, optimization of microbial co-cultures, the pathogenic bacteria collection, writing papers; **J.B.** works in the field of the biology *Streptomyces* for about 20 years. His main focus is on the regulation of gene expression during developmental transitions, especially germination (Bobek et al., 2014). He has detected several novel regulatory RNAs that influence antibiotic production of the organism. He also performed several preliminary experiments showing the effect of co-cultures on the secondary metabolite production, for more detail see http://uim.lf1.cuni.cz/en/laboratory-of-bacteriology-research .

K. Petříčková microbial co-culture assays, heterologous expression, liquid co-cultures; she specializes on the molecular biology of actinomycetes for almost 30 years. The last 12 years they concentrate on the biosynthesis of secondary metabolites, especially on the anti-inflammatory and cancerostatic manumycins, from biochemical, genetic and evolutionary points of view.

M. Petříček medium to large scale fermentations, extractions of metabolites, fractionation, in situ bioassays

Postcoctoral fellow – heterologou expressions, gene expression analyses, fermentations

Ph.D. student – Dita Šetinová media preparation, co-cultivation design, fermentations, gene expression analyses, heterologous expressions

Ph.D. student – Matouš Čihák analytical chemistry, LC-MS, comparative metabolomics, evaluation of genomic and metagenomic data

J. Hrdý an immunologist; co-cultures of streptomycetes and human cells, evaluations of human cell responses, immunomodulatory activity assays, flow cytometry experiments; his current research is aspects of mucosal immunity and modulation of the interaction between microbiota and the immune system.

E. Nohýnková – a parasitologist; co-cultures of streptomycetes with Protozoa, bioactivity assays of produced metabolites against the protozoal parasites

COLLABORATIONS

The main applicant intensively collaborates with the laboratory of MUDr. Adamkova (Microbiology and ATB Centre) that routinely collects multiple-resistant clinical isolates of bacterial and fungal pathogens and performs ATB-sensitivity tests. These isolates will be accessible for the project. The international applicant's collaboration is mainly based on the partnerships in the streptomycete field with Dr. Marie Elliot, McMaster University, Canada. **J. Hrdý** collaborates with Lactic Acid Bacteria and Mucosal Immunity, Center for Infection and Immunity Lille, Institute Pasteur, France on the evaluation of the microbiota changes on the host immune system and with Instituto de Lactologie Industrial (UNL-CONICET), Facultad de Ingenieria Industrial Quimica, Sante Fe, Argentina on the effect of different bacterial strains on immunity (esp. a *Salmonella* infection model).

K. Petříčková collaborates for many years with Alica Chroňáková, mainly to screen natural isolates for novel bioactivities. During one of their previous projects, a unique collection of 1500 novel actinomycete isolates including rare strains isolated directly from human specimen had been arisen (Chroňáková et al., 2010). This collection represents valuable source of novel bioactivities, which is comparable to well-known German and Japanese streptomycete collections. The collaboration resulted in patenting of novel compounds and modern genetic screening techniques (e.g. recently accepted US patent application; Petříček et al, 2016). Further collaboration is with Dr. K. Ochi from the Hiroshima Institute of Technology, Japan on the induction of secondary metabolism in streptomycetes. Newly, they collaborate with groups of Prof. Grond (semisynthetic derivatives of manumycins) and of Dr. Kaysser (activation of genomic data scanning-based cryptic biosynthetic gene clusters) of the University of Tuebingen, Germany. Long term collaboration, supported by several grant projects, runs with a group of Prof. Ilja Stříž in the Institute of Clinical and Experimental Medicine in Prague on the anti-inflammatory effects of manumycins. NMR structure assessments are performed in the collaborations with the Institute of Microbiology AS CR in Prague.

REFERENCES

Bentley S.D., Chater K.F., Cerdeno-Tarraga A.M., Challis G.L., Thomson N.R., James K.D., et al. (2002). *Nature* 417, 141-147.

Bobek J, Strakova E, Zikova A, Vohradsky J. *BMC Genomics. 2014 Dec 23;15:1173.*

Chater, K.F., and Hopwood, D.A. (1993) "*Streptomyces*.," in *Bacillus subtilis and Other Gram-Positive Bacteria. Biochemistry, Physiology, and Molecular Genetics.*, eds. Sonenshein A.L., Hoch J.A., Losick R. (American Society for Microbiology), 83-100.

Chiang Y.M., Chang S.L., Oakley B.R. & Wang C.C. (2011). *Curr.Opin.Chem.Biol.* 15, 137-143.

Chroňáková, A., Krištůfek, V., Tichý, M., & Elhottová, D. (2010). Microbiological research, 165(7), 594-608.

Collado M.C., Rautava S., Aakko J., Isolauri E & Salminen S. (2016). *Scientific Rep.* 6:23129.

Dashti Y., Grkovic T., Abdelmohsen U.R., Hentschel U. & Quinn R.J. (2014). Mar Drugs. 12(5): 3046–3059.

Gao Z., Tseng C.-H., Strober B.E., Pei Z. & Blaser M.J. (2008). PLoS One 3(7), e2719.

Ikeda H., Ishikawa J., Hanamoto A., Shinose M., Kikuchi H., Shiba T., et al. (2003). *Nat. Biotechnol.* 21, 526-531.

Kaltenpoth M., Göttler W., Herzner G. & Strohm E. (2005). Curr Biol. 15(5):475-9.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., et al. (2012). Int J Syst Evol Microbiol 62, 716–721.

Liu Y.Y., Wang Y., Walsh T.R., Yi L.X., Zhang R., Spencer J., et al. (2016). The Lancet. Infectious diseases 16: 161-168.

Loria R., Kers J. & Joshi M. (2006). *Ann.Rev.Phytopathol.* 44, 469-487.

McGann P., Snesrud E., Maybank R., Corey B., Ong A.C., Clifford R *.et al. (2016).* Antimicrobial Agent Chemother. doi: 10.1128/AAC.01103-16

Mikulík K, Bobek J, Zídková J, Felsberg J. *Appl Microbiol Biotechnol. 2014 Aug;98(16):7185-97*

Ochi K. & Hosaka T. (2013). *Appl.Microbiol.Biotechnol.* 97, 87-98.

Onaka H., Mori Y., Igarashi Y. & Furumai T. (2011). *Appl.Environ.Microbiol.* 77, 400-406.

Osipov G.A. & Verkhovtseva N.V. (2011). Ben. Microbes 2, 63-78.

Pethick F.E., Macfadyen A.C., Tang Z., Sangal V., Liu T.T., Chu J., et al. (2013). Genome Announc. 1, e0006313

Petříček M, Petříčková K., Pospíšil S., Kuzma M, Chronakova A., Krištůfek V., Stříž I. Accepted US Patent Application No. US 14/570,862 (Patent No. to be announced) (2016)

Schatz A., Bugie E. & Waksman S.A. (1944). *Proc.Soc.Exp.Biol.Med.* 55, 66-69.

Seipke R.F., Kaltenpoth M. & Hutchings M.I. (2012). *FEMS Microbiol.Rev.* 36, 862-876.

Swiercz JP, Hindra, Bobek J, Bobek J, Haiser HJ, Di Berardo C, Tjaden B, Elliot MA. *Nucleic Acids Res. 2008 Dec;36(22):7240-51.*

Traxler M.F., Watrous J.D., Alexandrov T., Dorrestein P.C. & Kolter R. (2013). *MBio.* 4 (4), e00459-13.

Tyc O., van den Berg M., Gerards S., van Veen J.A., Raaijmakers J.M., de Boer W. & Garbeva P. (2014). *Frontiers in Microbiology* 5, 567.

van de Sande W.W.J. (2013). *Plos Negl.Trop. Dis.* 7,e2550.
van Keulen G. & Dyson P.J. (2014). *Adv.Appl.Microbiol.,* 89, 217-266.
Vetsigian K., Jajoo R. & Kishony R. (2011). *PLoS Biol.* 9, e1001184.
Wu C., Zacchetti B., Ram A.F.J., van Wezel, G.P., Claessen D., Choi Y.H. (2015). Sci Rep. 2015; 5: 10868.
Zakharkina T., Heinzel E., Koczulla R.A., Greulich T., Rentz K., Pauling J.K. et al. (2013). *PLoS One* 8(7):e68302.