FEMS Central Office, Delftechpark 37a, 2628 XJ Delft, The Netherlands
T: +31 (0) 15 302 0050 E: fems@fems-microbiology.org W: www.fems-microbiology.org

FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES
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Report Form (FEMS Research Grant)

FEMS Early Career Scientist (full name):	**STANISLAVA KRÁLOVÁ**
Your business address:	Czech Collection of Microorganisms Kamenice 5, Bld.A25, Brno-Bohunice 62500 Czech Republic
tel. and email (fax):	+420 549 49 4897 stanci@mail.muni.cz
Microbiological Society:	CSSM
Name and address of FEMS Delegate:	Assoc.prof. MVDr. Renáta Karpíšková, Ph.D. Veterinary Research Insitute. Department of Bacteriology Hudcova 70 621 00 Brno
Name of host / supervisor:	Assoc. Prof. Mark R. Liles, PhD.
Host laboratory:	Auburn University Department of Biological Sciences
Period in host laboratory:	from: 01.01.2018 to: 31.3.2018
Date and signature of FEMS Early Career Scientist	Date: 31.05.2018 Signature:
Date and signature of Supervisor*:	Date: 31.05.2018 Signature:
* If you find it necessary, please add any other relevant information or comments on a separate sheet	
TITLE OF REPORT: Phylogenetic and biochemical classification of bacteria producing bioactive compounds.	

GUIDELINES:

1. The report (2-3 pages) should have the format of a scientific article, including abstract, introduction, results, discussion and reference sections.
2. The FEMS Research grantee should prepare his/her report with the host supervisor and submit her/his report together with the report form, in duplicate to the Delegate. The report is due within three months after completing the fellowship.
3. Please upload this report to the FEMS Grants Online System.
4. In case – part of – the results will be submitted for publication, you ought to acknowledge FEMS for the support (for your fellowship) in your paper and send a reprint to FEMS Business Office.

Final report

FEMS Research Grant

FEMS Early Career Scientist: RNDr. Stanislava Králová

Project Title: Phylogenetic and biochemical classification of bioactive compounds producing bacteria.

Abstract:

Aim of this project was to conduct a study on beneficial soil microorganisms that colonize and promote plant growth. This plant growth-promoting rhizobacteria (PGPR) are highly diverse and include different bacterial genera. Aim of this study was to find novel bacterial species isolated from soils that would be interesting from taxonomic as well as practical point of view, specifically for application for agricultural purposes. Initial analyses showed little or no activity of obtained bacterial cultures in meaning of enhancing plant growth, productivity or plant resilience to pathogens. However, these bacteria were subjected to screening for antimicrobial products with positive results. Five phylogenetic groups of novel actinobacteria were find that were shown to have antimicrobial activity against *Staphylococcus aureus* (MRSA strains), *Acinetobacter baumanii* and *Pseudomonas aeruginosa*. After initial phylogenetic analysis based on 16S rRNA sequences, it was shown that this 5 groups represent novel species that are now subjected to final analyses necessary for their official description.

Introduction:

In recent years many beneficial soil microorganisms that colonize plant roots and promote plant growth have been described (1, 2, 3, 4). These plant growth-promoting rhizobacteria (PGPR) are highly diverse bacterial group including different bacterial genera, such as *Agrobacterium*, *Arthrobacter, Azotobacter, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Azorhizobium, Bradyrhizobium, Rhizobium* and two predominant genera *Pseudomonas* and *Bacillus* (5).

As a matter of fact, it is also well known that bacteria occurring in soils (6, 7) or in marine environment (8, 9) are often successful producers of antimicrobial compounds acting against various genera of bacteria or fungi. More importantly, antibacterial compounds active against multidrug-resistant bacteria can be found among novel bacterial species isolated from these environments (10).

Results

Isolation and cultivation of soil bacteria led to creation of in-house bacterial collection at the Univrsity of Auburn, composed of hundreds of various isolates belonging to different bacterial genera. Initial screening of these isolates revealed only limited or zero activity regarding plant promoting properties. After several attempts to change conditions of testing and screening, aim of this study was altered due to limited amount of time of this internship.

Another burning issue in microbiology is antimicrobial resistance and increasing number of multidrug-resistant bacteria. Therefore, bacterial strains present in Auburn collection as well as other actinobacteria (obtained from marine environment) provided by SINTEF (Norway) were subjected to screening for antimicrobial compounds using the soft-agar overlay technique (11). This screening revealed five groups of actinobacteria showing antibacterial properties against multidrug resistant strains of *Staphylococcus aureus* (MRSA), *Acinetobacter baumanii* and *Pseudomonas aeruginosa*.

Initial phylogenetic analyses based on 16S rRNA gene sequences revealed separated and unique clusters of these bacteria in current actinobacterial phylogeny, revealing four clusters phylogenetically placed to the genus *Streptomyces* and one cluster being placed to the genus *Glycomyces*. Based on observed growth properties (some strains growing extremely slow), three out of these five groups were subjected to biochemical and physiological analyses at first. Group I contained only one strain of greenish coloured *Streptomyces* sp. P01-C11. The closest relatives based on 16S rRNA sequencing were DSM 42061^T *S. pratens*, DSM 42060^T (98.6%), S*. herbaceus* DSM 42062^T (98.3%) and *S. incanus* (98.2%). Group II consisted of two isolates (P16-A05, P38-E01) with slower growth (4-5 days) being related to DSM 42095^T *S. daliensis* (98.6%), B-2659 ^{T} *S. rimosus* ssp*. rimosus* (98.4%) and B-2317 ^{T} *S. sclerotialus* (98.3%). The last group III contained also two isolates (P01-B04, P01_F02) with closest phylogenetic neighbors DSM 41794^T *S. beijiangensis* (99.0%), B-24910^T *S. brevispora* (99.0%) and B-24909^T *S. laculatispora* (98.0%).

Based on polyphasic taxonomic approach using standard procedures for description of novel actinobacteria (12, 13) all these strains along with their closest relatives were subjected to extensive biotyping. These tests included macroscopic and microscopic morphology assessed after cultivation on different media; tolerance to different temperatures, salinity and pH; enzymatic activities (API ZYM), production of oxidase, catalase, urease; tests for hydrolysis of DNA, Tween, gelatin, starch, cellulose, xanthin, hypoxanthin, tyrosine and casein; production of melanine pigments. Whole spectrum of carbohydrate sources was tested as described previously (13). All tests were read daily

for up to 6 weeks. Testing for antibiotic resistance was done by standard disc diffusion method using Mueller-Hinton agar and read according to CLSI standards.

Discussion

Original aim of this study was to find PGPR strains that could be applied for agricultural purposes. After initial analyses of isolated and cultured soil bacteria, none of these isolates was suitable for further experiments. However, in-house collection of soil bacteria and collection of marine bacteria provided by SINTEF (Norway) enabled screening and finding of five groups of actinobacteria producing antimicrobial compounds that inhibit activities of multidrug-resistant pathogens, namely *S. aureus*, *A. baumanii* and *P. aeruginosa*.

Initial phylogenetic analysis showed separate phylogenetic position of these actinobacteria, leading to processes of their descriptions as novel bacterial species. Based on obtained results, three novel *Streptomyces* species will be described and published along with extensive information on their antimicrobial activities, biochemical and physiological properties, as well as deeply analysed whole genome sequences that will be provided in cooperation with SINTEF (Norway). Next analyses will be focused on isolation and characterization of antimicrobial products produced by these bacteria and expression of these products in heterologous hosts for better production and accessibility necessary for further studies.

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