



13<sup>th</sup> International Conference on

## **Microbial Interactions & Microbial Ecology**

July 19-20, 2018 | Rome, Italy

# Posters

Microbial Interactions 2018

13<sup>th</sup> International Conference on

# Microbial Interactions & Microbial Ecology

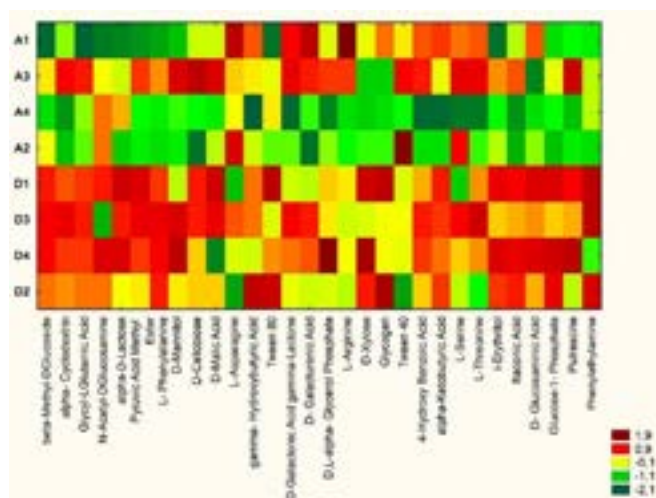
July 19-20, 2018 | Rome, Italy

## Determination of structural biodiversity and functional and metabolic profiles in soil under long-term maize monoculture

Anna Gałązka<sup>1</sup>, Karolina Gawryjolek<sup>1</sup> and Jarosław Grządziel<sup>1</sup>

Institute of Soil Science and Plant Cultivation – State Research Institute, Poland

Microbial diversity in soil may be limited under natural conditions by inappropriate environmental factors such as: limited food resources, environmental and physical factors, tillage system and interspecies interactions prevent the occurrence or maintenance of the species in the environment. The aim of this work was to determinate structural biodiversity and functional microorganisms in in soil under long-term maize monoculture. The reaction of maize cultivated in perennial monoculture for the direct sowing was investigated and compared to full tillage monoculture and crop rotation full tillage cultivation in the following phases: six leaves, twelve leaves, flowering phase, before harvest and after harvest. Three objects were included into this research: maize cropped continuously monoculture - zero tillage, maize monoculture cropped continuously – full tillage, crop rotation (spring barley, winter wheat, maize) – full tillage. The evaluation of the structural biodiversity of the soil was based on the next-generation sequencing (NGS). The functional and metabolic profiles in soil were determined by Biolog EcoPlate System. The research methods used in this subject have contributed to a better understanding of genetic diversity and composition of the population of microorganisms in the soil environment under the influence of the changes that have taken in soil under long-term maize monoculture.



### Recent Publications:

1. Gałązka A., Gawryjolek K., Gajda A., Furtak K., Książniak A., Jończyk K. 2018. Assessment of the glomalins content in the soil under winter wheat in different crop production systems. *Plant Soil and Environment*, 64(1), 32-37; doi: 10.17221/726/2017-PSE
2. Gałązka A., Gawryjolek K., Grządziel J., Frąc M., Książak J. 2017. Microbial community diversity and their interaction of soil under maize growth in different cultivation techniques. *Plant Soil and Environment*, Vol. 63, No. 6: 264–27; doi: 10.17221/171/2017-PSE
3. Gałązka A., Grządziel J. 2018. Fungal genetics and functional diversity of microbial communities in the soil under long-term monoculture of maize using different cultivation techniques. *Frontiers in Microbiology*, Research Topic: Soil Fungal Biodiversity for Plant and Soil Health, *Front. Microbiol.* 9:76; doi: 10.3389/fmicb.2018.00076

4. Gałązka A., Gawryjolek K., Grządziel J., Książak J. 2017. Effect of different agricultural management practices on soil biological parameters including glomalin fraction. *Plant Soil and Environment*, Vol. 63, No. 7: 300–306; doi: 10.17221/207/2017-PSE.

### Biography

Anna Gałązka is a head of the Department of Agricultural Microbiology at IUNG (since 2013). She is specialized in the evaluation of microbial diversity of soils associated with agricultural practice and environmental protection (research in molecular biology; evaluation of genetic differentiation and identification of microorganisms and characterization of metabolic profile of bacteria and fungi). She is participating in international and several national projects in IUNG, coordinating research project on biological activity and determination of microbial diversity of soil. She was a task manager in Aiming Project „Developing New system of tillage for sustainable agriculture” WND-POIG. 01.03.01-00-042/09 (2010-2013) – Determination of biological activity in soils and executor in projects: N305 080 32/2776 Evaluation of usefulness of *Azospirillum* spp. and *Pseudomonas stutzeri* inoculum in increasing of phytoremediation; project CZ.3.22/1.2.00/12.03445, Risk and benefits of application of exogenous organic matter on soil. Operational programme of cross-border co-operation Czech Republic – Poland 2007-2013. She was training of „Genomic of Nitrogen-Fixation Organisms” in 2008, Ghent, Belgium and participated in numerous courses and trainings in the field of molecular biology and microbiology. She works with NJF Nordic Association of Agricultural Scientists (keynote speakers on Seminar (483) 22-25.09.2015 Vezaiciai, Lithuania “Application of soil biological and biochemical parameters as indicator of soil health and fertility as influenced by different agrotechnical practices”). Member of Polish Microbiological Society since 2004 and author over 50 publications.

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## The genetic and functional diversity of bacterial community in soils contaminated with crude oil

Anna Gałązka<sup>1</sup>, Karolina Gawryjolek<sup>1</sup> and Jarosław Grządziel<sup>1</sup>

Institute of Soil Science and Plant Cultivation – State Research Institute, Poland

Soil contamination with crude oil, especially in the area of oil wells, is a serious environmental problem. Restoring the long-term pollution soil to its original state is very difficult. Under such conditions, the unique group of bacterial communities develops in the soil. These communities are adapted to the contamination conditions. Analysis of the structure and function of these microorganisms can be a source of valuable information. The aim of the study was to evaluate functional and structural diversity of bacterial communities in soils with long-term impacts from crude oil. Samples were taken from four oil wells: one oldest and still working (W01, working from 1888) and three younger (W02, W03, W04, working from the beginning of 20th century) still working but with periodic breaks – these oil wells work only a few months every year. Soil samples were collected at two distances: within a radius of 0.5 m of the oil wells (W01R, W02R, W03R, W04R) and within a radius of 3 m from the oil wells (W01, W02, W03, W04). The next-generation sequencing technique (V3-V4 16S rRNA) was accompanied with the community level physiological profiling (CLPP) method in order to better understand knowledge of both genetic and functional structure of soils collected under several oil wells. The significant differences of bacterial community structure between soils were obtained. The soils taken directly from oil wells were characterized by different composition of bacteria. The highest activity of carbon utilization patterns were observed in soils taken directly from oil wells.

### Recent Publications:

1. Gałązka A., Król M., Perzyński A. 2012. The Efficiency of Rhizosphere Bioremediation with *Azospirillum* sp. and *Pseudomonas stutzeri* in Soils Freshly Contaminated with PAHs and Diesel Fuel. Polish Journal of Environmental Studies Vol. 21, No. 2, 345-353.
2. Gałązka A., Gałązka R. 2015. Phytoremediation of polycyclic aromatic hydrocarbons in soils artificially polluted using plant-associated-endophytic bacteria and *Dactylis glomerata* as the bioremediation plant. Polish Journal of Microbiology, 64(3): 239-250.
3. Gałązka A., Grządziel J. The Molecular-Based Methods Used for Studying Bacterial Diversity in Soils Contaminated with PAHs (The Review). Soil Contamination - Current Consequences and Further Solutions. Edited by Marcelo L. Larramendy and Sonia Soloneski, ISBN 978-953-51-2816-8, Print ISBN 978-953-51-2815-1, 354 pages, Publisher: InTech, Chapters published December 21, 2016. <http://dx.doi.org/10.5772/64772>.

### Biography

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## Competence development and natural transformation in *Micrococcus luteus*

Antoni Lichev, Angel Angelov and Wolfgang Liebl  
Technical University of Munich, Munich, Germany

Natural genetic transformation has been documented in more than 80 bacterial species, including members of nearly all major taxonomic groups. Being one of the main routes for horizontal gene transfer, this process incorporates the internalization and the chromosomal integration of exogenous DNA during a genetically preprogrammed differentiated state called competence. In spite of the longstanding investigation of this phenomenon, so far only little is known about the regulatory mechanisms involved in genetic transformation and notably so in the case of representatives of the *Actinobacteria* phylum (high GC Gram-positive bacteria). In this report we focus on the competence development in the *Actinobacteria* member *Micrococcus luteus*. We provide evidence that nutritional limitation, provoked by the absence of amino acids in the growth medium, induces natural transformation in this species. Paradoxically, we also show that amino acid auxotrophy strongly inhibits competence development. We demonstrate the negative impact of a deletion of a RelA/SpoT-like homologue (Mlut\_12840) and a putative novel (p)ppGpp synthetase (Mlut\_22200) on natural transformation and we therefore speculate the involvement of the stringent response in the complex regulation of the competent state of *Micrococcus luteus*. Furthermore we investigate the specific regulatory function of branched-chain amino acids (BCAAs) and signify them as modulators of competence that alter expression of transformation-related genes. To our knowledge our data provides the first general insights into the regulation of natural transformation in a member of the *Actinobacteria* phylum and may also prove to be pertinent to a number of important pathogens belonging to the same taxonomic group.

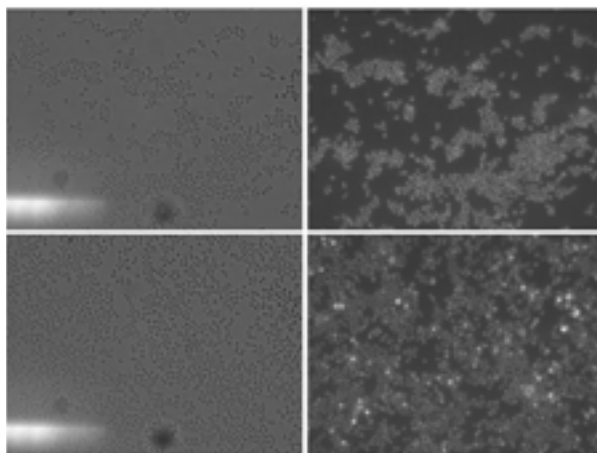


Figure 1: Competence development under nutritional limitation is a bimodal population trait occurring in only 5-10 % of the cells

### Recent Publications:

1. Angelov A., Bergen P., Nadler F., Hornburg P., Lichev A., Übelacker M., et al. (2015). Novel Flp pilus biogenesis-dependent natural transformation. *Front. Microbiol.* 6:84.

### Biography

Antoni Lichev has finished his Bachelor's and his Master's Thesis in Molecular Biotechnology at the Technical University of Munich. Since 2015 he has been doing his PhD in Microbiology at the Department of Microbiology at the Technical University of Munich. His previous work has been published in the journal "Frontiers in Microbiology".

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## Seed-borne bacterium interacts with air-borne fungus in rice fields

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The air-borne ascomycete fungus *Fusarium graminearum* causes head blight in many cereals and produces mycotoxins such as trichothecenes and zearalenone. The seed-borne bacterium *Burkholderia glumae* causes bacterial panicle blight in rice and produces toxoflavin that has antimicrobial activity and phytotoxicity. Disease symptoms caused by two pathogens are very similar and could often cause false diagnosis. In this study, we showed that two pathogens frequently co-isolated in rice heads and *F. graminearum* is resistant to toxoflavin produced by *B. glumae* while other fungal genera are sensitive to the toxin. We have tried to clarify the resistant mechanism of *F. graminearum* against toxoflavin and the ecological reason of co-existence of the two pathogens in rice. We found that *F. graminearum* resistance to toxoflavin is related to production of triacylglycerides containing linolenic acid. Co-cultivation of two pathogens resulted in increased conidia and trichothecene by *F. graminearum*. Bacteria physically attached to fungal conidia, which protected bacterium cells from UV light and allowed disease dispersal. Chemotaxis analysis showed that bacterial cells moved toward the fungal exudation. Disease severity on rice heads was significantly increased by co-inoculation rather than single inoculation. This study provides evidence of the two pathogens cooperatively interacting, with *F. graminearum* gaining the opportunity to induce disease progression efficiently and *B. glumae* achieving aerial dispersal.



### Recent Publications:

1. Jung B. et al. (2018) Cooperative interactions between seed-borne bacterial and air-borne fungal pathogens on rice. *Nature Communications* 9:31.
2. Jung B. et al. (2014) A putative transcription factor pcs1 positively regulates both conidiation and sexual reproduction in the cereal pathogen *Fusarium graminearum*. *The Plant Pathology Journal* 30:236-244.
3. Jung B. et al. (2013) Development of a selective medium for the fungal pathogen *Fusarium graminearum* using toxoflavin produced by the bacterial pathogen *Burkholderia glumae*. *The Plant Pathology Journal* 29:446-450.
4. Son H. et al. (2012) Mannitol induces the conversion of conidia to chlamydospore-like structures that confer enhanced tolerance to heat, drought, and UV in *Gibberella zeae*. *Microbiological Research* 167:608-615.
5. Son H. et al. (2011) A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. *PLoS Pathogens* 7:e1002310.

### Biography

Jungkwan Lee has completed his Ph.D. from Kansas State University, USA and postdoctoral studies from Seoul National University, Korea. He is an associate professor in Dong-A University, Korea. He has been working in the interaction between plant pathogenic fungi and bacteria in rice plants. He published more than 40 papers in reputed journals and has been serving as an associate editor for *The Plant Pathology Journal*.

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## Rubber Gloves Biodegradation by a Natural Soil Consortium

**Kamontam Umsakul<sup>1</sup>, Chairat Nawong and Prachparin Janvadee**<sup>1</sup>Prince of Songkla University, Faculty of Science, Department of Microbiology, Hat-Yai, Songkhla, Thailand

An increasing production of natural rubber (NR) products has led to major challenges in waste management since the degradation of NR is normally extremely slow. In this study, the degradation of rubber latex gloves in a mineral salt medium (MSM) using a natural soil consortium was studied. Soil consortia can degrade rubber gloves much more quickly than those of the single culture using the same conditions. The weight loss of the rubber gloves after incubation with a soil consortium of up to 14 days was as high as 59%. In contrast with using a single strain, only 9% of rubber glove weight loss was obtained. Determination of carbon dioxide evolution and viable cell number during cultivation with rubber gloves as a sole carbon source, revealed mineralization of the rubber materials during the increase of biomass. Scanning electron microscopy demonstrated an adhesive growth behavior of the consortium on the rubber surfaces. The results from Fourier transform infrared spectroscopy revealed the decrease in number of cis-1,4 double bonds, the formation of carbonyl groups indicating an oxidative attack at the double bonds.

### Biography

Kamontam Umsakul associated with Department of Microbiology, Prince of Songkla University, Thailand. She has published several papers in reputed journals. Her research interest is Microbiology.

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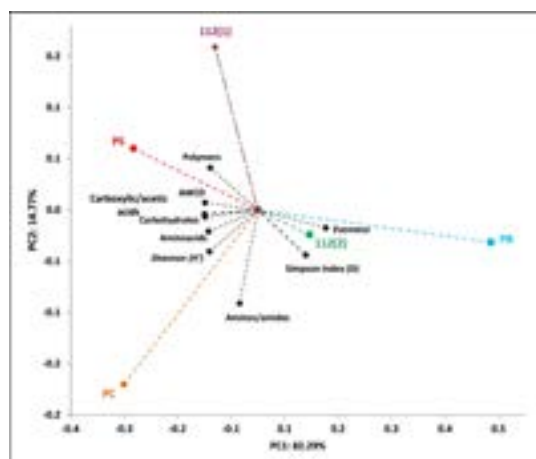
# Microbial Interactions & Microbial Ecology

July 19-20, 2018 | Rome, Italy

## Analysis of functional and structural microbial diversity of *Paulownia* spp. leaves

Małgorzata Woźniak<sup>1</sup>, Anna Gałązka<sup>1</sup>, Jarosław Grządziel<sup>1</sup> and Magdalena Frąc<sup>2</sup><sup>1</sup>Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation - State Research Institute, Puławy, Poland<sup>2</sup>Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland

The *Paulownia* spp. is a fast growing variety of deciduous tree that belongs to the Paulowniaceae family. These trees are used to produce biomass and reduce carbon dioxide concentrations in the atmosphere. *Paulownia* spp. leaves have a high content of protein, fats, sugar and nitrogen, phosphorus, potassium (NPP). The purpose of this study was to determine the functional and genetic diversity of the microbial of the *Paulownia* spp. Next Generation Sequencing (NGS) and Biolog EcoPlates were used to evaluate microbial diversity. The research materials were leaves of trees *Paulownia* spp. Plant samples were taken from Podkamins (a plantation established on arable soil) and Otrębusy (a plantation established on degraded soil). The samples were the clones of the same hybrid *Paulownia elongata* and *Paulownia fortunei*. Additional hybrid of *Paulownia tomentosa* and *Paulownia fortunei* was included. The functional analysis was carried out using statistical methods: cluster analysis by Ward's method, taking into account Euclidean square of distance. Moreover, the Shannon-Wiener ( $H'$ ) biodiversity index, R Richness, E Evenness, and AWCD (average well color development) were calculated. The obtained sequences were subject to bioinformatics analyses, such as clustering and separation of operational taxonomic units (OTU). The samples from the plantation established on degraded soli revealed that the most dominant phylum was *Bacteroidetes*, whereas from the arable soil, Proteobacteria. Comparisons of carbon source utilization and the diversity indices showed differences in the microbial community of composition. Among the five carbon sources studied, carbohydrates were the most frequently used group of compounds.



### Recent Publications:

1. Ayan S., Sadlam I., and Sivaciödlü A. (2003) *Paulownia* Sieb. & Zucc: A new exotic genus for multi-purpose uses in Kastamonu-Turkey. *Decision Support for Multiple Purpose Forestry* 4: 23-25.
2. Whipps J., Hand P., Pink D., and Bending G. D. (2008) Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology* 105(6): 1744-1755.
3. Yadav N. K., Vaidya B. N., Henderson K., Lee J., Stewart, W. M., Dhekney S. A, and Joshee, N. (2013) A review of *Paulownia* biotechnology: A short rotation, fast growing multipurpose bioenergy tree. *American Journal of Plant Sciences* 4: 2070-2082.



4. Garland J. L. and Mills A. L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* 57: 2351-2359.

### **Biography**

Małgorzata Woźniak obtained a master degree in biology with specialization microbiology in 2015. She studied at Maria Curie-Skłodowska University (UMCS) in Lublin, Poland. She work in Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation in Poland. She is principal Investigator (head of the project) in two projects: the research project in the statutory activity of IUNG-PIB, Title of project: "Molecular and biochemical identification of the endophytic bacteria and it application in plant growth promoting" and Preludium project, the National Science Center, Title of project: "The influence of fast-growing *Paulownia* Clon In Vitro 112 (*P.elonagata* x *P.fortunei*) on microbiological and physico-chemical properties of the soil in Poland".

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## Bacterial endophytes in crops – molecular identification and biodiversity

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Bacterial endophytes reside in a large number of plant species as part of their microbiome. Endophyte community structure exhibits a high diversity of species within a plant. This diversity is determined by abiotic and biotic factors such as soil conditions, biogeography, plant species, microbe–microbe interactions and plant–microbe interactions. The purpose of this study was to assess the degree of genomic diversity, relationship and molecular identification of bacteria isolated from the endosphere of maize, broad bean, wheat, rye, horsetail and burdock (root and stem). The tests were performed on 45 strains. The genetic identification of bacterial population was performed based on comparative sequence analysis of the 16S rDNA. On the basis of 16S ribosomal RNA sequences, all strains of bacteria were assigned to 10 genera: *Rhizobium* (19 isolates), *Delftia* (11 isolates), *Agrobacterium* (5 isolates), *Stenotrophomonas* (3 isolates), *Brevundimonas* (2 isolates), *Novosphingobium* (1 isolate), *Variovorax* (1 isolate), *Collimonas* (1 isolate), *Achromobacter* (1 isolate) and *Comamonas* (1 isolate). The assessment of the diversity of potential plant growth promoting bacteria, including 11 species *Delftia* sp, was conducted based on the BOX-PCR, ERIC-PCR and PCR-DGGE techniques. The genetic profile shows similarity among tested bacteria strains. The microorganism group was related to the type of the host plant.

### Recent Publications:

1. Costa L. E. D. O., Queiroz M. V. D., Borges A. C., Moraes C. A. D. and Araújo E. F. D. (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). Brazilian Journal of Microbiology 43: 1562-1575.
2. De Bruijn F. J. (1992) Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergeneric consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. Applied and Environmental Microbiology 58: 2180-2187.
3. Katara J., Deshmukh R., Singh N. K. and Kaur S. (2012) Molecular typing of native *Bacillus thuringiensis* isolates from diverse habitats in India using REP-PCR and ERIC-PCR analysis. The Journal of General and Applied Microbiology 58: 83-94.
4. Sun L., Qiu F., Zhang X., Dai X., Dong X. and Song W. (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. Microbial Ecology 55: 415-424.

### Biography

Małgorzata Woźniak obtained a master degree in biology with specialization microbiology in 2015. She studied at Maria Curie-Skłodowska University (UMCS) in Lublin, Poland. She work in Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation in Poland. She is principal Investigator (head of the project) in two projects: the research project in the statutory activity of IUNG-PIB, Title of project: "Molecular and biochemical identification of the endophytic bacteria and it application in plant growth promoting" and Preludium project, the National Science Center, Title of project: "The influence of fast-growing *Paulownia* Clon In Vitro 112 (*P.elonagata* x *P.fortunei*) on microbiological and physico-chemical properties of the soil in Poland".

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## Interactions of nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* with rhizosphere micro-organisms associated with Tomato

**Martina Franchini**

University of Nottingham &amp; Azotic Technologies Ltd., UK

*Gluconacetobacter diazotrophicus* (Gd) is a non-nodulating endophytic nitrogen-fixing bacterium isolated from the intercellular spaces of sugarcane. The aim of this study was to investigate the effect of this bacterium on Tomato plants in presence and absence of *Trichoderma asperellum* (T34 commercial strain). *Trichoderma* spp. is one of the most widely employed biological control agents used against plant pathogens and is an opportunistic avirulent plant symbiont. This fungus is known to antagonize other soil fungi either parasitizing on them or inhibiting their growth due to the production and release of cell wall degrading enzymes. Furthermore, *T. asperellum* has been shown to synthesize a wide range of other plant protective molecules that can enhance the plant's response to pathogens. However, the details of its modes of action still remain to be clarified. To investigate the effect of the combined action of T34 and Gd on crop plants, Tomato Money Maker seeds were treated with Gd and sown into Levington M3 soil in presence and absence of *T. asperellum*. T34 biocontrol agent from FarGro® was mixed to the soil before sowing. Four months after sowing, differences in plants' strength and biomass production were observed: plants grown in presence of both Gd and T34 showed healthier phenotypes and higher leaf and biomass production in comparison to untreated control plants or to plants that were inoculated with Gd only. Flowering and fruiting were also showed to be positively affected in plants co-inoculated with both the Gd and *T. asperellum* with higher fruit yield. This preliminary experiment provides promising data for further investigating the mechanisms that lie behind the interaction between Gd, T34 and crops, and may potentially lead to the formulation of a new enhanced biofertilizer.

### Recent Publications:

1. Evdokia Syranidou et al., "Biodegradation of Weathered Polystyrene Films in Seawater Microcosms," Scientific Reports 7, no. 1 (2017): 1–12.

### Biography

Martina Franchini main interests in Biology have driven her to concentrate her studies in the microbiological field. During the years, her expertise has been focused on the investigation of the environmental main issues and how Biology, and more specifically, Microbiology, can approach them. Bioremediation has been the subject of her latest studies: from the use of bacteria for reduction of toxic oxianions with concurrent bioproduction of Nanoparticles to a participation in a study aimed to isolate a bacterial consortium of plastic biodegraders. Martina has worked in international contexts, learning the importance of constructive team work and open knowledge sharing.

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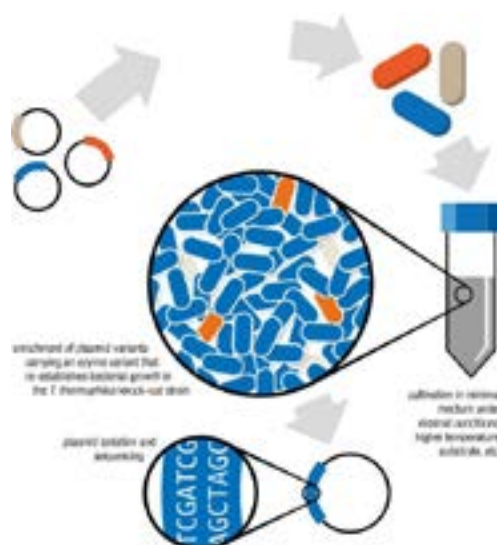
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## Growth-based selection of glycoside hydrolases in the extreme thermophile *Thermus thermophilus*

Matthias Moerch and Angel Angelov

Wolfgang Liebl Technische Universität München, Munich, Germany

The constant depletion of fossil energy sources and the raising demand for more eco-friendly alternatives challenges science to develop new technologies. The utilization of plant biomass is a promising substitute for conventional systems by being a rather inexpensive energy carrier and of high sustainability at the same time. Its availability through cultivation of energy crops and by usage of agricultural/forestry waste account for great industrial value. In particular, the valorisation of waste by converting it to fermentable substrates has the advantage not to threaten food security. Since plants themselves consist primarily of lignocellulosic fibres (a mixture of cellulose, lignin and hemicellulose), the first step in the process of fuel production is the hydrolysis of this bigger structures into smaller, more soluble sugars. Its depolymerization into glucose monomers is often facilitated by using cellulases – an enzyme group consisting of endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and  $\beta$ -glucosidases (EC 3.2.1.21) which work synergistically together. With increasing market share of new bio fuels, the future demand for (novel) cellulases – which today already make up 8 % of worldwide industrial enzyme demands – will therefore rise as well constantly. In this work we developed a system to select for thermostable glycoside hydrolase enzymes (GH) using the extremely thermophilic bacterium *Thermus thermophilus*. Unlike mesophilic bacteria (e.g. *E. coli*), *T. thermophilus* provides an overall more suitable enzymatic background and thereby greater potential to express thermostable recombinant proteins properly. This increases the probability of detecting novel thermostable cellulases when transformed in this organism; compared to the commonly used host *E. coli*. As an advantage, growth-based selection approaches already result in favourable enzyme variants, compared to traditional screening methods which require testing of every single clone. In order to obtain a GH-negative strain, we constructed a *T. thermophilus* knock-out strain which lacks four glycosidases. As confirmed by para-nitrophenol (pNP) enzyme assays and incubation of cell extract with X-Gal and X-Glu, these deletions reduced the hosts ability to cleave  $\beta$ -glycosidic and  $\beta$ -galactosidic bonds to a minimum. Without these GHs, the knock out strain is not able to grow in minimal medium. Complementation with the hosts own  $\beta$ -glucosidase via the shuttle vector pMK18 re-established growth of the knock out strain. For purpose of following system verification, cglT – a glycosyl hydrolase belonging to GH family 1 from the thermophilic bacterium *Thermoanaerobacter brockii* – was transformed in the *T. thermophilus* knock-out strain. This novel approach of complementation-based selection in an extreme thermophilic organism is a promising tool to look through big meta genomes or mutagenesis libraries, selecting for enzyme variants of higher thermostability and/or other substrate specificity in a highly efficient manner.



**Recent Publications:**

1. Angelov A.\*, Pham VTT.\*, Übelacker M., Brady S., Leis B., Pill N., Brolle J., Mechelke M., Moerch M., Henrissat B., Liebl W. A metagenome-derived thermostable  $\beta$ -glucanase with an unusual module architecture which defines the new glycoside hydrolase family GH148. *Sci Rep.* 2017 Dec 11;7(1):17306 \*These authors contributed equally to this work.
2. Leis B.\*, Held C.\*, Bergkemper F., Dennemarck K., Steinbauer R., Reiter A., Mechelke M., Moerch M., Graubner S., Liebl W., Schwarz WH., Zverlov VV. Comparative characterization of all cellulosomal cellulases from *Clostridium thermocellum* reveals high diversity in endoglucanase product formation essential for complex activity. *Biotechnol Biofuels.* 2017 Oct 23;10:240 \*These authors contributed equally to this work.

**Biography**

Matthias Moerch has completed his Masters in Molecular Biotechnology at the Technical University of Munich in 2014, comparing different protein digestion methods for shotgun proteomics in his Master's Thesis. Since 2015 he pursues his doctorate at the Department of Microbiology at the Technical University of Munich establishing the extreme thermophilic bacterium *Thermus thermophilus* as an alternative host for metagenome analysis.

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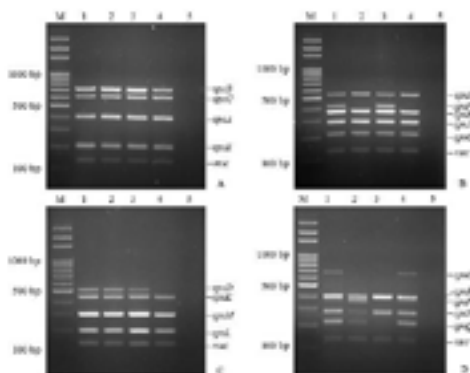
# Microbial Interactions & Microbial Ecology

July 19-20, 2018 | Rome, Italy

## Detection of genes encoding cell wall-associated proteins in *Staphylococcus pseudintermedius* isolates from dogs, humans, and the environment

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The detection of 18 genes encoding cell wall-associated (CWA) proteins in *Staphylococcus pseudintermedius* named *spsA-spsR* were performed by using the new development of a set of multiplex PCRs (mPCRs). The distribution of these genes were detected in isolates from dogs (n=70), humans (n=25), and the environment of a veterinary hospital (n=40). The new 4 sets of mPCR comprising of 4-5 genes per set, including *nuc* gene as an internal control were developed. The mPCR sets could detect at least 1 pg/μl of DNA template. We found 23 *sps* gene profiles among the 135 isolates, with diverse gene combinations. *spsD*, *spsE*, *spsI*, *spsO*, *spsP*, and *spsQ* were variable detection but not statistically significant difference in each sources of isolates. Only *spsP* and *spsQ* encoded protein A or Spa were more frequently detected in the canine isolates from infected sites than from carriage sites suggested to play a role in pathogenicity. Interestingly, the positive amplicons of *spsR* gene in three human isolates showed gene deletions that were similar to the sequence in *S. aureus* ST398. The variation and difference of surface protein genes between human and animal *S. aureus*, including deletions, insertions, and truncation or pseudogenes have been reported and suggested to differentially affect host-specific adaptation.



### Recent Publications:

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2. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, Kennedy AD, Barbian KD, McCarthy AJ, Street C, Hirschberg DL, Lipkin WI, Lindsay JA, DeLeo FR and Lowy FD. 2012. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *mBio.* 3(2).
3. Phumthanakorn N, Chanchaithong P and Prapasarakul N. 2017. Development of a set of multiplex PCRs for detection of genes encoding cell wall-associated proteins in *Staphylococcus pseudintermedius* isolates from dogs, humans and the environment. *J Microbiol Methods.* 142: 90-95.

### Biography

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## Protective Effect of Exopolysaccharides Produced by *Lactobacillus plantarum* LRCC5310 on Rotavirus-Induced Diarrhea and Safety Assessment in Mice

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Rotaviruses are the most important cause of diarrhea in infants and children worldwide. However, to date, no specific antiviral drugs for the treatment of rotavirus infection have been developed. We isolated *Lactobacillus* strains from the Korean traditional fermented food, Kimchi. Among them, *Lactobacillus plantarum* LRCC5310 strain, specifically, the exopolysaccharides (EPS) produced from the strain, were shown to have an antiviral effect against human rotavirus Wa strain in vivo. The oral administration of EPS led to a decrease in the duration of diarrhea, viral shedding and the destruction of enteric epithelium integrity in the infected mice. Also, to assess safety, oral administration to mice for 14 days had no adverse effects on liver, heart, stomach, lung, kidney and intestine through histology and blood analysis. The findings indicate that the strain *L. plantarum* LRCC5310 does not raise safety concerns in mice, up to 5,000 ppm concentration. Thus, this EPS is likely to be safe for human consumption and it can be used for the effective control of rotavirus infection. Further studies in humans should be conducted.

### Recent Publications:

1. KiyoungK (2018) Exopolysaccharide from *Lactobacillus plantarum* LRCC5310 offers protection against rotavirus-induced diarrhea and regulates inflammatory response. Dairy Science(to be published).
2. N.S.Oh, J.H.Koh, M.R.Park(2016) Hypolipidemic and anti-inflammatory effects of fermented Maillard reaction products by *Lactobacillus fermentum* H9 in an animal model. Dairy Science 99(12):9415-9423.
3. N.S.Oh(2015) Dietary Maillard reaction products and their fermented products reduce cardiovascular risk in an animal model. Dairy Science 98(8):5102-5112.

### Biography

Miri Park has completed her master's degree in life science from Korea University, Republic of Korea. She is the researcher of Ingredients and Solution Team at Lotte R&D center, Republic of Korea. She is responsible to search for useful food materials, especially probiotics, to evaluate efficacy and apply it to products.

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# Accepted Abstracts

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# Microbial Interactions & Microbial Ecology

July 19-20, 2018 | Rome, Italy

## Phenotypic and genetic characterization of *Lactobacillus plantarum* strains isolated from Chinese Sourdough

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Sourdough has been used for centuries to leaven breads made with different flours. The quality of the product highly depends on the microbial composition and their characteristics. Therefore, selection of right strains for fermentation is a dire need of a successful fermentation. *Lactobacillus plantarum* is a plant-adapted bacterium that provides desirable technological and functional characteristics to sourdough breads including good aroma, texture and taste, apart from enhancing nutritional characteristics. A total of 165 strains of *Lactobacillus plantarum* isolated from Chinese sourdoughs were genotyped based on RAPD and RFLP and a total of 7 and 8 genotypes, respectively, were found. The ability of strains to metabolize different carbohydrates was also determined and a significant variation, in agreement with different genotypes, was noticed. A variation among strains for acidification, peptidase activity and quotient of fermentation was noticed, which explain the reason why different breads having the dominance of this species have variable quality characteristics. In the future, it is important to select strains based on desirable fermentation characteristics.

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July 19-20, 2018 | Rome, Italy

## Characterization and technological properties of newly isolated *Streptococcus Macedonicus* strains from different regions of Italy

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The aim of this study was to discover more about *S. Macedonicus* and its abilities by comparing it with a very well-known commercial bacterium like *S. Thermophilus*. As a member of the LAB, *Streptococcus Thermophilus* is identified as a thermophilic group and use as a commercial starter in industry. On the other hand, although *Streptococcus Macedonicus* was first described more than 16 years ago but still the exact role of this bacteria in the industry is not distinct. In this study, eight new isolated strains of *S. Thermophilus* and *S. Macedonicus* were applied to be compared with each other from the technological point of view. Analysis such as Growth in different PH, temperature and sugars, Antimicrobial susceptibility test and Inhibitory activity test were performed and the results were evaluated by using General linear model (GLM) in SAS 9.4. In all the comparison like growing in different PH, temperature and sugars between these two species, *S. Macedonicus* indicated the better growth rate which shows more functional bacteria. .

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July 19-20, 2018 | Rome, Italy

## Enhancing the constitutive resistance in *Capsicum annuum* L. fruits, as an environmentally friendly approach against a fungal disease

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One of the great challenges for food security in the 21st century is searching for possibilities to improve yield stability through the development of disease-resistant crops, against the most problematic diseases. The present study was undertaken to enhance natural disease resistance of *Capsicum annuum* L. against anthracnose disease, through some selected pre- and postharvest treatments. The fungal pathogen, *C. acutatum*, was isolated from naturally infected chili with anthracnose, collected from the study area. Potassium silicate (Kasil®) was applied as a postharvest treatment at concentrations, 0 (control), 100, 200, and 400 mg/l and found that the concentration at 200 mg/l was effective. Elicitor treatment, at 200 mg/l, was done once in a week for 4 weeks, it resulted in significantly reduced anthracnose disease when the harvested fruits were challenge-inoculated with *C. acutatum*. Postharvest application of potassium silicate (Kasil®), at the same concentration reduced anthracnose lesion area by 25 - 100%, compared to the untreated controls. Postharvest spray treatment of chili at mature, green stage with potassium silicate at 200mg/l, reduced the severity of anthracnose development by 34-100%. Spore germination assay revealed that potassium silicate has no antifungal effect on conidia of *C. acutatum*. Potassium silicate (Kasil®) treated fruits, inoculated with *C. acutatum* after harvest, showed greater accumulation of phytoalexins. Related (PR) Proteins such as  $\beta$ -1, 3-glucanase are considered to play an important role in plant disease resistance. .

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## Molecular regulation of adhesion and biofilm formation in *Bacillus licheniformis* high and low biofilm producers using RNA-Seq

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**B**acillus licheniformis is a dominant spore-forming microbial contaminant of the dairy industry throughout the world. A variety of studies suggest that the ability of this species to form biofilms is the key reason for its widespread prevalence. However, despite its apparent prevalence and spoilage potential, there is no information regarding the molecular determinants involved in biofilm formation by *B. licheniformis*. We used the transcriptome sequencing (RNA-seq) technology to reveal over- and underrepresented transcripts in the transition from the planktonic (logarithmic culture) to the biofilm state (24 h old) in a high and low biofilm forming *B. licheniformis* dairy strains. Genes, representing 28.3 % and 36.3 % of the whole *B. licheniformis* gene content were upregulated in the biofilm state as compared to the planktonic state in high and low biofilm producers, respectively. The gene expression patterns of several groups of genes differed significantly ( $P < 0.05$ ) under the two growth conditions. The genes related to chemotaxis proteins (CheA, CheB, CheC, CheD, CheV, CheW, CheY, MotA, MotB) and flagellar assembly were exclusively upregulated in the planktonic phenotype as compared to biofilms in both strains. In terms of Kyoto Encyclopedia of Genes and Genomes (KEGG) based pathways, metabolic pathways were significantly ( $P < 0.05$ ; Q-values  $< 0.05$ ) downregulated in the planktonic state as compared to the biofilm state in both strains which shows that biofilm formation is an energetically expensive process. Lipid and sugar metabolism seemed to play an important role in the matrix production. Overall, several genes involved in adhesion, matrix production and matrix coating were either absent or less expressed in the biofilm state of low biofilm producer as compared to the high biofilm producer. Interestingly, the genes related to sporulation and extracellular polymeric substances were concomitantly expressed in the biofilm state of both strains which suggests that sporulation is coupled with the biofilm formation in this species similar to *B. subtilis*. Collectively, our results provide a comprehensive insight into biofilm formation in *B. licheniformis* that will be helpful for future research into mechanisms and targets..

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## Enhancement of PGP activities and host colonization by *Gluconacetobacter diazotrophicus* using co-inoculation with *Terribacillus saccharophilus*

**Inmaculada del Castillo**

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*Gluconacetobacter diazotrophicus* (Gd) is a plant growth promoting (PGP) nitrogen fixing microorganism found in association with a variety of crops, such as sugarcane, coffee, rice, tomato and wheat (Luna, et al. 2010, Eman and Rasha 2015, Stephen, et al. 2015). In addition to nitrogen fixation, Gd has been reported to produce plant-growth promoting hormones including IAA. Gd colonisation has also been associated with phosphate and zinc solubilisation and siderophore production (Eskin, Vessey and Tian 2014). In previous research at Azotic Technologies Ltd [AT], we have detected important genotypic and phenotypic differences between strains, that are characterized by features associated with host interaction. A characteristic associated with the proprietary strain identified by AT, is the ability to colonise plant tissues intracellularly. This is one of the key features that differentiates the AT variant from the type strain, PAL5 (Cocking, Stone and Davey 2006). The above characteristics allow the AT strain to develop a more intimate symbiotic relationship with the host, facilitating the supply of fixed nitrogen directly to host cells. Although originally discovered in sugar cane (Cavalcante and Döbereiner, 1988) and largely associated with sucrose-rich plant species, AT has developed a range of formulations with Gd that can be applied to the seeds and cut surfaces of a large number of crops including rice, maize and wheat. The use of PGP bacteria in non-native hosts presents Gd with additional challenges to the establishment of successful colonisation that can be overcome using formulation additives including co-inoculation with other beneficial microorganisms. In this study we have demonstrated how the co-inoculation of Gd with the gram-positive microbe, *Terribacillus saccharophilus* (Ts) enhances the endophytic colonisation of *Brassica napus* seedlings by Gd concomitantly enhancing additional plant-growth-promoting responses including nitrogen fixation. Using the acetylene reduction assay (ARA), nitrogenase activity levels up to 1.6-fold higher were recorded for mixed, compared to Gd or Ts-only cultures. The current research demonstrates a synergetic effect associated with co-inoculation with Gd and Ts that is able to enhance the nitrogen-fixing performance of Gd in the plant promoting increased seedling biomass two weeks after application with the mixed inoculant.

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# Microbial Interactions & Microbial Ecology

July 19-20, 2018 | Rome, Italy

## Numerical modeling of bacterium interaction at tangential direction

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Understanding of bacterium interaction behavior opens a new possibility to analyze various bacterial cultures. Also, to understand phenomena related to a motion of bacterium. As an example, one of the phenomena can be related to bacterium motion in the human circulatory system, sticking to a surface or infection transmission in the air, where bacterium is transmitted in a water droplet. The process of bacterium motion is solved numerically, applying the motion describing various models. Here was prepared numerical experiment, in which it is found the change of acting forces during bacterium interaction at a distance as well during bacterium deformation. Moving towards the surface bacterium motion is described with force components, which are acting at normal and tangential directions. Usually bacterium has oblique interaction and in this case the theoretical tangential interaction needs to be considered. The problem is that unlike normal direction, description of adhesive dissipative bacterium interaction at tangential direction is not known and limited knowledge available in known literature. This problem was analyzed, a theoretical model is considered. By applying this model, there are given numerical experiments results as time dependent acting forces as well as a change of an interacting bacterium displacement, velocity.

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July 19-20, 2018 | Rome, Italy

## Simultaneous microbial and electrochemical reductions of vanadium (V) with bioelectricity generation in microbial fuel cells

**Song Wang** and **Baogang Zhang**

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Simultaneous microbial and electrochemical reductions of vanadium (V) with bioelectricity generation were realized in microbial fuel cells (MFCs). With initial V(V) concentrations of 75 mg/L and 150 mg/L in anolyte and catholyte, respectively, stable power output of  $419 \pm 11$  mW/m<sup>2</sup> was achieved. After 12 h operation, V(V) concentration in the catholyte decreased to the value similar to that of the initial one in the anolyte, meanwhile it was nearly reduced completely in the anolyte. V(IV) was the main reduction product, which subsequently precipitated, acquiring total vanadium removal efficiencies of  $76.8 \pm 2.9\%$ . Microbial community analysis revealed the emergence of the new species of Deltaproteobacteria and Bacteroidetes as well as the enhanced Spirochaetes mainly functioned in the anode. This study opens new pathways to successful remediation of vanadium contamination.

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## The role of microbial modification of bile acids for host-microbe cross talk in a cohort of Crohns disease and Ulcerative Colitis

Alvaro Lopez Gallardo, Carolyn Murray, Emilo Laserna Mendieta, Carthage Moran, Cormac Gahan, Marcus Claesson, Fergus Shanahan and Susan Joyce  
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The GI tract is recognised as a super organ where coevolved mutualistic relationships benefits both the microbial residents and human health. For instance, while the liver is responsible for bile acid synthesis and conjugation, the gut microbiota is responsible for the diversity of bile moieties. Bile moieties are more than just emulsifiers of lipid and liberators of vitamins from dietary components. They act as signalling molecules that can exert their effects both locally and systemically, the most potent signalling molecules are those generated through microbial conversion. Here, we have examined metabolites from biological material from an Irish cohort of IBD to include Crohns disease and Ulcerative Colitis and matched controls (n=182). We have stratified, based on volunteer demographics and analysed metabolites, including bile moieties, hormones and cytokines in these patients. We link bile modifications with bile acid signalling and the incidence of bile acid diarrhoea (BAD) in these patients. We show that BAD is elevated in incidence of Crohns disease irrespective of BMI and that this incidence is due to increased levels of microbial produced secondary bile acids and to aberrant hormonal signalling but not absorption. Taken together, these data indicate that bile acid signalling is altered among Crohns disease sufferers with an elevation in bile acid diarrhoea irrespective of BMI in re-sectioned individuals.

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## Enhancement of bacterial denitrification for nitrate removal in groundwater with electrical stimulation from microbial fuel cells

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Electricity generated from the microbial fuel cell (MFC) is applied to the bioelectrical reactor (BER) directly as electrical stimulation means for enhancement of bacterial denitrification to remove nitrate effectively from groundwater. With maximum power density of 502.5 mW m<sup>-2</sup> and voltage outputs ranging from 500 mV to 700 mV, the nitrate removal is accelerated, with less intermediates accumulation, compared with control sets without electrical stimulation. Denitrification bacteria proliferations and activities are promoted as its number and Adenosine-5'-triphosphate (ATP) concentration increased one order of magnitude ( $3.5 \times 10^7$  in per milliliter biofilm solution) and about 1.5 folds, respectively. Effects of electricity from MFCs on enhancement of bacterial behaviors are demonstrated for the first time. These results indicate that MFCs can be applied in the in-situ bioremediation of nitrate polluted groundwater for efficiency improvement.

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