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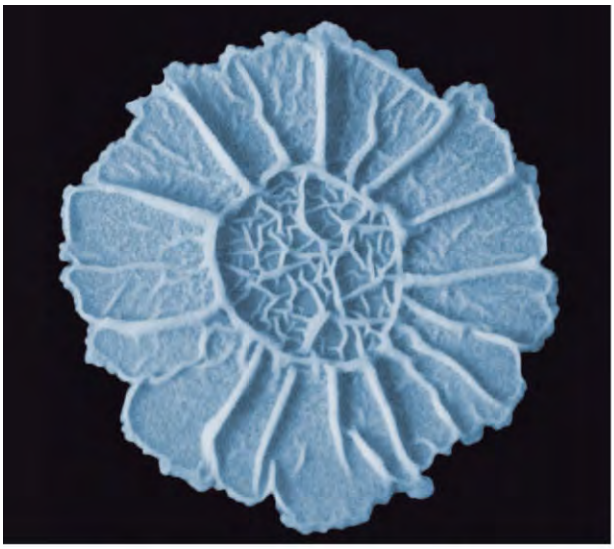

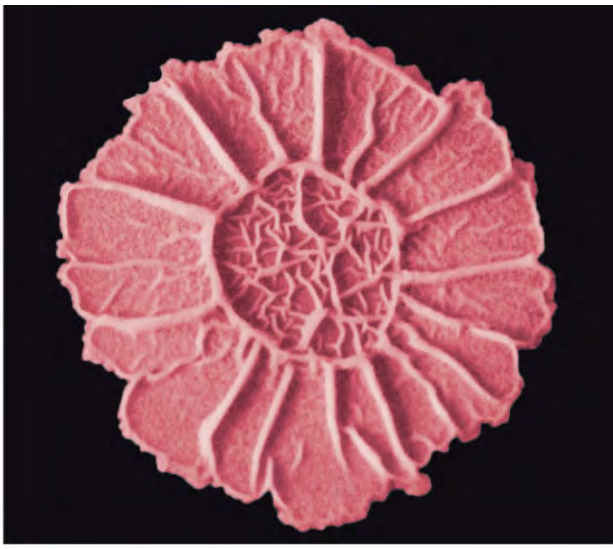

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MIP21

Horizontal gene transfer and genetic engineering of linear plasmid pLMA1 in *Micrococcus luteus* Fleming strain

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Micrococci are Gram-positive, G+C-rich, nonmotile, non-spore-forming actinomycetous bacteria. *Micrococcus* strains have proven to play important roles in the biodegradation of xenobiotics, bioremediation processes, production of biotechnologically important enzymes or bioactive compounds, as test strains in biological assays for lysozyme and antibiotics. They can also cause infections in immunocompromised humans.
We recently reported the isolation and characterization of the first linear plasmids in different strains of *Micrococcus* from extreme environments in Argentina [1,2]. Among them, linear plasmid pLMA1 (110 kb) proved to be linked to an erythromycin resistance phenotype. In this report we show that this plasmid can be transferred to the type strain of *M. luteus* (NCTC 2665) in a process most closely resembling conjugation. However, clear differences from the classical conjugative plasmid transfer were observed, indicating the existence of a specialized system. We were further able to perform genetic manipulations on the transferred plasmid, which will allow the functional investigation of the elements involved in the transfer process.
In addition, the transfer ability of the linear plasmid pLMA1, together with the rather efficient method for its manipulation, can establish it as an useful tool for biotechnological purposes in the genus *Micrococcus*
The authors would like to acknowledge the support from the Bayerisches Hochschulzentrum für Lateinamerika (BAYLAT).

1. Dib JR, Wagenknecht M, Hill R, Farías M E, Meinhardt F. 2010. Plasmid; 63: 40-45.
2. Dib JR, Liebl W, Wagenknecht M, Farías ME, Meinhardt F. 2013. Appl Microbiol Biotechnol. 97(1): 63-75.

MIP22

Diazotrophic *Beijerinckiaceae* as symbionts of the conifer *Lepidothamus fonkii* (Phil.) in Patagonian peatlands, Chile - a new nitrogen fixing symbiosis in Gymnosperms

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Biological nitrogen fixation is the dominant process for the provision of plant-available nitrogen in many nutrient-limited peatlands. Evidence is building up that methanotroph associated nitrogen fixation is important in such systems. *Lepidothamnus fonkii* (Phil.) is a small conifer of the family Podocarpaceae that thrives in nutrient-limited Patagonian peatlands and has structures similar to root nodules of plants hosting symbiotic diazotrophs. However, evidence for a symbiotic nitrogen fixation associated with *L. fonkii* and its potential importance for the N-cycle in peatlands is lacking to date. Thus, electron microscopy, acetylene reduction assays, $[^{15}N]_2$-tracer studies, *nifH* (encoding nitrogenases) gene and transcript as well as 16S rRNA sequence analyses were applied. *L. fonkii* roots were densely covered by nodules hosting bacteroid like structures. Nitrogen fixation potentials of roots were significantly greater than those of rhizosphere peat. Given a root biomass of 220 g m^{-2}, annual nitrogen fixation rates of up to 2 g m^{-2} were estimated, exceeding background N-deposition by far. Illumina sequencing of RNA derived 16S rRNA gene amplicons indicated an enrichment of active *Acetobacteraceae, Beijerinckiaceae*, *Bradyrhizobiaceae*, and *Planctomycetaceae* associated with roots compared to rhizosphere peat. *Acidobacteria*-related sequences dominated amplicon libraries of rhizosphere peat. Root rather than rhizosphere peat associated transcripts of nifH almost exclusively affiliated with nifH of Beijerinckiaceae. Diversity estimates of root associated nifH genes and transcripts were significantly smaller than those of the rhizosphere peat. The collective data suggest that a novel mutualistic symbiosis of diazotrophic *Beijerinckiaceae* and *L. fonkii* is essential for nitrogen input in Patagonian peatlands.

MIP23

The influence of MyD88 on the microRNA profile of murine macrophages during *Legionella pneumophila* infection

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Introduction: The gram-negative, facultative intracellular bacterium *Legionella pneumophila* (*L.p.*) naturally resides in water-living amoeba. During *Legionella* pneumonia, contracted by inhalation of bacteria-containing water droplets, alveolar macrophages serve as an atypical host for *L.p.* in the lung. Macrophages primarily sense *L.p.* by Toll-like receptors (TLR), which centrally rely on the adaptor protein MyD88 for downstream signaling. TLR activation stimulates macrophages and affects their gene expression profile.
Objectives: The aim of this study was to investigate the impact of the MyD88 knockout on the microRNA (miRNA) profile of murine macrophages during *L.p.* infection.
Materials and Methods: Bone marrow derived monocytes of wild type and $MyD88^{-/-}$ mice were differentiated into macrophages *in vitro*. Their activation status was determined by CD206 and iNOS FACS analysis and by microscopic morphology assessment. A comparison of infected wild type and knockout cells was performed by gene expression analysis and ELISA. Furthermore, the miRNA profile was investigated by Taqman Low Density Array (TLDA), and single miRNAs were validated by qPCR. Finally, synthetics of identified miRNAs were used to evaluate their regulatory capacities on chosen mRNA targets.
Results: Uninfected $MyD88^{-/-}$ cells showed no differences vs. wild type in terms of activation and morphology. After infection, differential regulation of pro-inflammatory cytokines, e.g. KC, IL-1α and TNFα, was observed in $MyD88^{-/-}$ cells. TLDA analysis and subsequent qPCR validation revealed miR-125a-3p to be regulated in a MyD88 dependent way during *L.p.* infection. Additional treatment with Cytochalasin D, Actinomycin D and the IKK XIII inhibitor illustrated an impact of *L.p.* uptake, gene transcription and signal transduction on this miRNA in response to *Legionella*. Analysis of predicted mRNA targets for miR-125a-3p (NTAN1 and GM9705) by synthetic pre-miRNA administration revealed no functional interaction so far.
Conclusion: While the miRNA profile of bone marrow macrophages is at least in part reproducibly changed upon infection with *Legionella pneumophila*, a functional relevance of miR-125a-3p remains to be established. The MyD88-dependency of this miRNA suggests it´s involvement in pro-inflammatory macrophage activation.

MIP24

Genotypic and functional profiling revealed plant-probiotic functions as a key factor that shape endophytic bacterial community in rice (*Oryza sativa* L.)

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Introduction: Endophytic bacterial strains exert several beneficial effects on host plants such as stimulation of plant growth, N_2-fixation, phosphate-solubilization, siderophore-production, synthesis of phytohormone and induction of plant resistance to pathogens. Rice is one of the most important staple foods for the world's population. However, the ecological role played by bacterial rice endophytes and the factors that contribute for their recruitment is not completely understood.
Objectives: The richness, diversity and dynamics in terms of rice endophytic bacteria and their plant-probiotic functions in two soil-types were tested to understand the aspects that shape endophytic community.
Methods: *Oryza sativa* cvs. TCN1, TCS10, TK8 and TN71 were cultivated in greenhouse using non-sterile acidic and near-neutral paddy soils. Seed-borne endophytes were characterized through PCR-DGGE. Root, stem and leaf tissues were screened for culturable endophytes and their plant-probiotic features. The richness, Shannon-Weiner diversity and evenness in terms of endophytic strains and their plant-probiotic features were estimated.
Results: A total of 52 distinct bacterial endophytes affiliated to 20 discrete genera differentially exhibiting plant-probiotic features were isolated, whose distribution fluctuated with soil-type, tissue-type and cultivars. Class *Bacilli* was prevalent in TCS10, TK8 and TN71, whereas *Gamma-proteobacteria* was dominant in TCN1. High strain diversity did not