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Next Generation Biodiversity Analysis: the Bacterial Metadatabase Bac*Dive*

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Microbial data and metadata are scattered throughout the scientific literature, databases and unpublished lab notes. This makes them difficult to access. Bac*Dive* mobilizes data from internal descriptions of culture collections and initial descriptions of novel taxa in the primary literature and currently offers data for 62.683 bacterial and archaeal strains. Here we describe exemplary mobilization projects like the Reichenbach collection of myxobacteria, where information on 12,535 typewritten index cards were digitized and a total of 37,156 data points were extracted by text mining. Another mobilization project targeted Analytical Profile Index (API®) tests on paper forms that were collected in culture collections over 30 years. Overall 8665 API® tests were digitized, which provide physiological data for 5059 microbial strains. Published at Bac*Dive*, this collection becomes the largest publicly available API® test data collection in the world. Species descriptions published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) are of particular interest as a source for metadata. For over 1000 new species descriptions up to 150 different metadata-types were extracted manually, yielding 72,618 data points. This collection was complemented by a set of metadata for over 4000 IJSEM species descriptions, integrated from a phenotypic trait database published by Barberán et al. By publishing these data in Bac*Dive*, the metadata not only became accessible and searchable but are also linked to strain taxonomy, isolation source, cultivation condition, and molecular biology data. Thereby, Bac*Dive* enables a broad potential for new analytical approaches in biodiversity research.

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Phylogenetic distribution of RsbR orthologs and sequence conservation in stressosome proteins and their putative output modules

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Introduction: The stressosome is a 1.8 MDa multiprotein complex that senses and responds to stress ultimately controlling the activity of the alternative sigma factor, SigB, in *Bacillus subtilis*, the model organism for stressosome activity. The stressosome proteins, RsbR, RsbS and RsbT, are located within the genome as part of an operon with downstream encoded proteins, which act as a cognate output module to the stressosome"s sensory input.

Objective: We will present a census of stressosome gene clusters and co-occurring output modules and highlight potential functionally relevant regions of stressosome proteins.

Method: *RsbRST* gene clusters and downstream encoded regulatory proteins were identified within microbial and archaeal genomes using BLAST searches. Sequence alignments and phylogenetic trees were constructed to identify signature amino acids for each group of Rsb proteins. Available structural information was used to investigate localization of conserved amino acid residues in Rsb proteins.

Results: The *rsbRST* module is found within many bacterial species. Cognate downstream genetic modules show a diverse range of potential output systems, which are often predicted to control the level of second messengers such as c-di-GMP. Signature residues, which might play important roles in protein-protein interaction during stressosome complex formation were identified in alpha helix 3 and adjacent regions of the STAS domain of RsbR, RsbR paralogs and RsbS.

Conclusion: We used a combination of different bioinformatic tools to gain insight into the occurrence of stressosome gene clusters within the bacteria and archea and to identify regions within Rsb proteins likely important for structure and function of the stressosome complex.

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New members of the family Eggerthellaceae isolated from a human fecal sample: Rubneribacter badeniensis gen. nov., sp. nov., Enteroscipio rubneri, gen. nov., sp. nov.

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Introduction: The *Eggerthellaceae* is an upcoming family of the human gut microbiome; members are known as normal dwellers and are often isolated from feces. However, some species are opportunistic pathogens. In this study, two unknown anaerobic strains were isolated from a human fecal sample.

Materials & methods: Identification and classification was performed using a polyphasic approach including phenotypic (Gram-staining, morphology, catalase, oxidase), biochemical (fatty acids, quinones, polar lipids and API tests) and molecular biological methods (16S rRNA, WGS).

Results: Analysis of 16S rRNA gene sequences indicated that both strains represent distinct lineages within the *Eggerthellaceae*. *R. badeniensis* showed 92.3% similarity to the type strains of *Eggerthella* and *Gordonibacter*, while *E. rubneri* clustered together with *Paraeggerthella hongkongensis* and *Arabia massiliensis* (94.8%). Quinone analysis revealed that MK-5 and MK-7 are present, which are described for the *Eggerthellaceae* for the first time. Polar lipids in both strains consist of eight and six glycolipids, respectively. Both strains possess phospholipids, phosphatidylglycerol and diphosphatidylglycerol. The percentage of total branched fatty acids is relatively high for *R. badeniensis* (42%) and *E. rubneri* (50%) but comparable to the value of *G. pamelaeae*.

Conclusion: Our results indicate that the two unknown bacterial strains belong to the *Eggerthellaceae* and represent

both new genera and new species: *R. badeniensis* ResAG-85^T (= DSM 105129^T = JCM 32272^T) and *E. rubneri* ResAG-96^T (= DSM 105130^T = JCM 32273^T).

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Diversity study of *Ochrobactrum* spp. isolated from medicinal leeches

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The *Alphaproteobacteria* genus *Ochrobactrum* shares a high phylogenetic relatedness with the genus *Brucella*. *Ochrobactrum* spp. occur in diverse habitats, free-living in water or associated with plants, animals, and humans. *Ochrobactrum* spp. are well known as endosymbionts in medicinal leeches specifically colonizing leech nephridia and bladders (Nelson & Graf 2011). The function of *Ochrobactrum* spp. in leeches is not known so far.

The aim of this study was to isolate and study the diversity of leech-associated *Ochrobactrum* spp. to address the question of host specificity. In total, of 318 *Ochrobactrum* spp. isolates were obtained from 68 separately cultured leeches. Isolates were phylogenetically identified by 16S rRNA gene sequencing. From 143 isolates, 41% were closest related to the type strain of *Ochrobactrum pseudogrignonense* (99.6%) and 30% closest related to *Ochrobactrum lupini/anthropi*type strains (99.8%). In order to identify if genetically different isolates were among those two determined phylotypes, isolates were differentiated at the strain level (genotypes) by genomic fingerprinting using BOX-, (GTG)5-, and RAPD-PCR. Many leeches carried genetically identical, but also genetically slightly different *Ochrobactrum* spp. isolates. To determine if these genotypes are leech specific or not, currently all the collected isolates are compared to isolates collected from other sources by a Multi Locus Sequence Typing (MLST) approach in order to address the hypothesis of host specificity.

References:

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Different diversity of abundant heterotrophic and methylotrophic bacteria cultivated by dilution-to-extinction cultivation from the phyllosphere of *Arrhenatherum elatius* and *Galium album* plants, exposed to ambient and elevated atmospheric CO_2

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Global climate changes increase atmospheric CO_2 concentrations. This affects plants, bacterial communities colonizing the aerial part of plants (phyllosphere) and the interactions of plants and phyllosphere bacteria.Aim of this study was to determine the effect of elevated CO_2 on the concentration and diversity of heterotrophic and methylotrophic phyllosphere bacterial communities. Leaves of two abundant grassland plant species of the Giessen Free Air Carbon Dioxide Enrichment system (Gi-FACE) in Linden (Germany) were collected in August 2015 from three areas of three control (CC) and three rings exposed to +20% elevated CO_2 (CE). Leaf associated heterotrophs and methylotrophs were enriched by dilution-to-extinction cultivation. The concentration of cultivated bacteria was determined by the most probably numbers method (MPN). Bacterial assemblages enriched in the three highest positive dilutions were compared by community fingerprinting using 16S rRNA gene targeted PCR- DGGE analysis. Bacteria from highest positive dilutions were furthermore isolated and identified by 16S rRNA gene sequencing.Concentrations of cultured heterotrophs and methylotrophs were not affected by elevated CO_2, but non-metric multidimensional scaling (NMDS) of community patterns showed significant differences of the phylogenetic compositions of cultivated bacteria. *Sphingomonas* were isolated in a higher abundance from the phyllosphere of CC ring plants, while *Arthrobacter, Flavobacterium, Microbacterium,* and *Stenotrophomonas* mainly from the phyllosphere of CE ring plants. This study indicates an adaptation of specific phyllosphere taxa to elevated CO_2. The high number of representative isolates now enables the investigation of the community shifts in distinct plant microbe interaction studies.

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The evolution of phage resistance in *Vibrio alginolyticus* K01M1 in response to two different filamentous bacteriophages

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Experimental Evolution: An evolution experiment was performed using *Vibrio alginolyticus* K01M1, which has been isolated from a healthy pipefish *Syngnathus typhle*, and two different temperate phages, that differ in the lytic activity using six replicate populations per treatment. After 48 h of being challenged by the phages, the amount of resistant clones per population was about 75 %. Follow-up analyses revealed that in both treatments, lysogens, which were dominating during the first generations, were rapidly outcompeted by phage-resistant strains. The spread of non lysogenic mutants was significantly faster in bacterial populations with a highly lytic phenotype. Furthermore, lysogens revealing a weak lytic activity remained at low densities during the entire experiment, whereas highly lytic phenotypes became extinct after 30 generations.

Genomic Analysis: Analysis showed shared SNPs and INDEL events correlating to the different treatments. Lysogens revealed the existence of either complete phages or with regional deletions. In contrast the genomes of the mutant strains exhibited different deletions in a gene cluster encoding extra cellular pili structures.

Conclusion: In our experiment the reaction of a growing *Vibrio alginolyticus* culture exposed to temperate phages can be considered as a time scheduled multiple response. Two strategies of bacterial defense were seen where the immediate response results in lysogenic evolution and a