1900). This is the first report known so far that demonstrates the presence of methanotrophic bacteria within termite gut microflora.

FTP030

Utilisation of proteolytically active lactobacilli for liberation of bioactive peptides from milk proteins

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Lactic acid bacteria (LAB) are of great interest because of their expansive application for production of various dairy products. As a consequence of several amino acid auxotrophies, LAB depend on exogen nitrogen sources such as caseins (α_{S1}-, α_{S2}-, β- and κ-casein) -which are the major milk proteins- as well as on an efficient proteolytic system providing amino acids for synthesis of endogenous proteins.
The aim of our study was to isolate, identify and characterise proteolytically active LAB for subsequent deployment for milk protein hydrolysis and liberation of bioactive peptides. In consideration of their proteolytic activities, we found primarly thermophilic lactobacilli, which exhibited highest proteolytic and caseinolytic activities among all LAB genera. Thereafter, we performed 16S-rDNA sequencing for identification and detected *L. delbrueckii* subsp. *delbrueckii* habouring highest proteolytic activity. For characterisation of caseinolytic behaviour of *L. delbrueckii,* specificity of casein degradation was studied with whole cell suspensions. The results indicated that *L. delbrueckii* preferentially degrades α- and β-casein, whereas for κ-casein almost no hydrolysis occurred. Furthermore, acidification curves, maximum acidification rates as well as cell growth in 10% skim milk were investigated in comparison to other proteolytically active strains. We found no correlation between proteolytic activity, acidification ability and cell growth, respectively.
LAB possess several peptide transport systems which may lower the (bioactive) peptide yield during casein hydrolysis. Hence, we successfully released proteolytic activity by using two different buffer systems and examined the cell free supernatants regarding to enzyme activity and protein profiles (SDS-PAGE). For release of bioactive peptides from milk, we applied cell suspensions to 10% skim milk. Released peptides were analysed by HPLC and pooled fractions will be tested for bioactivity (e.g. anti-oxidative).

FTP031

ZupT, a member of the ZIP family of zinc/iron transporters in *Cupriavidus metallidurans*

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The well-studied metal-resistant bacterium *Cupriavidus metallidurans* harbors a network of metal efflux systems with overlapping substrate specificities, which allows survival in highly heavy metal-polluted environments. Counterparts of these efflux systems are the primary and secondary metal uptake systems. Interaction of import and export reactions create a flow equilibrium of the cytoplasmic and periplasmic metal concentrations, which are kind of a "backbone" of cellular metal ion homeostasis. The central uptake system for Zn(II) and other ions in *C. metallidurans* is the ZIP (ZRT/IRT protein family) ZupT (2). Members of the ZIP protein family are ubiquitous, occurring in all kinds of organisms from bacteria to man, and play a key role in zinc transport. Similar to the first characterized bacterial ZIP protein from *E. coli* (1), ZupT from *C. metallidurans* contains 8 predicted transmembrane a-helices. The protein from *C. metallidurans*, however, possesses large histidine-rich loops that are not present in the enterobacterial transporter. To investigate the substrate specificity and kinetic parameters for metal transport, *zupT* from *C. metallidurans* was cloned into a pET28 derivate to add a N-terminal *His-tag* and expressed in an *E. coli* Rosetta strain. The expression of ZupT had a toxic effect on growth of *E. coli* and resulted in a low growth yield of the respective strain. Nevertheless, ZupT assembled into the membrane and could be detected due to its *His-tag*. ZupT could also be solubilized with sodium dodecyl sulfate (SDS) from the membrane, but not with other detergents tested.

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FTP032

Identification of Amino Acids of the PHB Binding Domain in PhaZ7 Depolymerase of *Paucimonas lemoignei*

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PhaZ7 depolymerase is the only known extracellular depolymerase that has been described as being active towards amorphous PHB (nPHB) [1]. The structure of PhaZ7 was solved first at 1.9 Å [2] and recently at 1.4 Å [3]. The active site of PhaZ7 is buried, so conformational changes must take place upon substrate binding. Comparison with other α/β hydrolases revealed that the structure of PhaZ7 is very similar to *Bacillus subtilis* lipase A except for an additional domain at one side of the molecule that is absent in LipA. Interestingly, this additional part of the enzyme is highly enriched in tyrosine and other hydrophobic residues. We suggest that this additional part could be responsible for interaction of the enzyme with the hydrophobic polymer. To find experimental evidence for this assumption we performed site-directed mutagenesis of selected positions in PhaZ7 and investigated the effect of the mutation on activity and polymer binding ability of PhaZ7. Our results showed that mutations of Y105, Y176, Y189, Y190 and W207 to alanine or glutamate resulted in reduced nPHB depolymerase activity and in an occurrence of a lag-phase at the beginning of the depolymerase reaction. The results of the binding assay of PhaZ7 with nPHB showed that Y105, Y176, Y189, Y190 and W207 mutein have reduced binding ability and verified that Y105, Y176, Y189, Y190 and W207 are essential for efficient PHB binding. Recently, the crystal structure of inactive PhaZ7 S136A mutein with bound 3-hydroxybutyrate (3-HB) trimer was also determined. It showed that 3-HB trimer is bound to a groove surrounded by Y105, Y176, Y189 and Y190. This result is consistent with our mutagenesis results. Interestingly, the superposition of free und trimer-bound PhaZ7 SerAla136A showed that both structures slightly differ from each other. The main changes were in the 280-295 and 248-251 region. The loop 280-295 was missing in the bound structure, suggesting some flexibility of these regions and their possible involvement in nPHB granules binding. Hence, the present results confirmed the involvement of Y105, Y176, Y189, and Y190 on PHB degradation.

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FTP033

Detection and analysis of biogenic sulfuric acid corrosion in wastewater systems

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Biogenic sulfuric acid corrosion (BSA) is one of the most serious and costly problems affecting the world´s sewerage infrastructure (e.g. concrete sewer pipes) and wastewater treatment (e.g. digesters). A complex microbial ecosystem, comprising sulfate reducing and sulfur oxidizing bacteria (SRB and SOB, respectively), is involved in the BSA process. The bacterial activity in the wastewater systems creates a sulfur cycle which can lead to bacterial formation of sulfuric acid (H_2SO_4) and consequently to corrosion of concrete. 20% of the total damage of concrete structures in sewer systems seems to be caused by BSA leading to global repair costs of several billions of dollars per year. Besides optical checks and acid analysis, currently, no precise test procedure is available for the detection of BSA attacks. Therefore, the aim of this research project (funded by AiF Projekt GmbH) is the development of a standardized biochemical test system for the accurate determination of the BSA potential in wastewater systems, especially in digesters. As a result, quantified information shall be gained about the extent of damage so that further evaluations about the structure stability can be made.
This test system will include (i) detection and quantification of SRB and SOB in the digested sludge and biofilm growing on the concrete surface, respectively and (ii) determination of the concrete corrosion potential carried out in specific simulation chambers inoculated with SRB and SOB and concrete specimens. A combination of different conventional microbiological as well as molecular-biological techniques such as polymerase-chain-reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE), sequencing and phylogenetic sequence analysis, fluorescence *in situ* hybridization (FISH), and quantitative real