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Anaerobic biodegradation of cellulose-rich substrates

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Depletion of fossil fuels and increase of global climate changes demand the usage of renewable energy sources. Biogas forms anaerobically during the decomposition of different organic materials. In the process, three syntrophic groups of microbes work together. These are the polymer degraders, acetogens and methanogens. The main components of the produced biogas are methane (55-70%) and carbon-dioxide (30-45%). After upgrading, biogas could be injected into natural gas grid. For biogas production, many waste and raw material is suitable. Plant biomass is the largest amount of biomass on Earth. Plants can harvest solar energy during photosynthesis and convert it to plant tissues, therefore have vast energy potential. Plant tissues consist of lignocellulose as the major component. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose is a recalcitrant complex polymeric carbohydrate, cellulases are needed for its efficient decomposition. Cellulases are divided into three major groups: endoglucanases (EC 3.2.1.4), exoglucanases (3.2.1.91) and β-glucosidases (3.2.1.21). Endoglucanases cut at random internal sites into the cellulose polysaccharide chain, generating new chain ends. Exoglucanases act on the reducing or nonreducing ends of cellulose, liberating glucose or cellobiose units. β-glucosidases hydrolyze cellodextrins and cellobiose to glucose which can be used in metabolic pathways.

For the utilization of substrates having high cellulose content – without pretreatment - the biogas producing microbial community should contain a significant number of cellulose producing bacteria and they should break down cellulose to easily utilizable sugar monomers. An adaptation strategy to adapt the community to lignocellulosic substrate has been developed. The experiments were carried out under thermophilic conditions at 55 °C. α-cellulose was used as substrate for the adaptation and the control fermentors received glucose as carbon and energy source. The changes in the concentration of volatile fatty acids were followed by HPLC, the β-glucosidase enzyme activity was monitored regularly. From the adapted microbial community, cellulose degraders were isolated and were also used as inoculum in the next set of biogas experiments. The cellulose degrading microbes had positive effect, elevated the biogas and methane yield. DNA was purified from the cellulose degrading consortia and was undergone metagenome analysis. In the thermophilic cellulose degrading consortium, the main orders were Thermoanaerobacterales (70%) and Clostridiales (10%). *Thermoanaerobacterium thermosaccharolyticum*, *Caldanaerobacter subterraneus*, *Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum* were identified as dominant strains.

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Uniform or different? Heterogeneity of murine bone marrow mesenchymal stem cells in differentiation and immunosuppression

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Bone marrow mesenchymal stem (BMMSCs) are adherent, colony-forming cells and are defined as multipotent cells differentiating into several cell types (e.g. osteoblasts, chondrocytes and adipocytes). BMMSCs have been found therapeutically beneficent in models for numerous human diseases by multiple processes including enhancement of tissue regeneration, supporting angiogenesis, subduing inflammation and modulating the immune response at the site of tissue damage. Despite the incessantly increasing number of preclinical and clinical MSC studies, there are some basic issues about MSCs, which still remain unresolved. The heterogeneity in differentiation potential of MSCs was demonstrated decades ago. Up to this day, few and inconsistent data have been collected reporting uniform or different immunosuppressive properties of single MSC clones, even if it is highly relevant to the therapeutical effectivity of MSCs.

We aimed to examine the heterogeneity of murine BMMSC population through characterizing 6 single cell-derived MSC clones (MSC1-MSC6) in terms of differentiation potential, support of angiogenesis and immunmodulation.

To examine whether MSC clones maintain the multipotency of BMMSC population, MSC clones were induced to differentiate *in vitro* into adipocytes and osteoblasts. While MSC2-6 differentiated into both lineages, MSC1 differentiated only into adipocytes.

Analysis of the ability of the MSC clones to support angiogenesis has been carried out using an *in vitro* model, the capillary mimicry assay. MSC clones were co-cultured with H5V endothelial cells and the capillary-like structures were evaluated. Whereas neither MSCs nor H5V formed capillary-like structures alone, all MSC clones supported similarly the development of these structures when co-cultured with H5V.

The *in vitro* immunmodulatory properties of BMMSC clones were compared in ConA-stimulated T cell proliferation assay. MSCs were co-cultured with T cells isolated from mouse lymph nodes in the presence of ConA and cell division of CFSE (a fluorescent dye used for proliferation assays) labeled T cells was followed by flow cytometry. All MSC clones inhibited significantly but not uniformly the T cell proliferation in the following order: MSC2>MSC4=MSC5>MSC1>MSC3>MSC6. Differences in the inhibition of T cell proliferation