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Metabolic engineering of Saccharomyces cerevisiae for optimizing 3HP production

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ical capabilities to thrive in extreme habitats, therefore marine habitat provides a magnificent opportunity to discover newer compounds such biosurfactants (BS) and bioemulsifiers (BE). The aim of this study was the screening of marine bacteria able to produce biosurfactants from cheap carbon sources. Fifty-eight bacterial isolates obtained from marine invertebrates were evaluated for biosurfactant/bioemulsifier production in liquid medium using glycerol, sucrose, mineral oil and soybean oil as carbon sources. The surfactant production was evaluated by the measurements of the surface tension of cell-free culture broth and the emulsifier ability by the drop-collapse technique. Sixteen isolates were preselected for their ability to produce BS/BE, three isolates showed surface tension of 35–40 mN/m and other three isolates showed the lowest surface tension values. The best results were obtained with an *Arthrobacter* sp. growing in soybean oil (ST 29.1 mN/m), *Brevibacterium luteolum* in mineral oil (ST 28.9 mN/m) and *Gordonia* sp. in soybean oil (ST 31.4 mN/m). The hydrophobic carbon sources were the preferred substrates for BS production by the marine bacteria.

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Poster 1.3.24

Protein encapsulation by biosilification catalyzed by glutathione S-transferase (GST)-silicatein

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A protein of sponge, called silicatein, can catalyze silica deposition in vitro under near neutral pH and ambient temperature condition. This biosilification process therefore has attracted much interest as a promising system to gain silica-based nanostructures or materials at environmentally benign condition. Since the yield of recombinant silicatein as a soluble form was low, we tried to improve that by employing *E. coli* based codon usage and fusion protein system. GST fusion proteins not only act as solubility enhancing partners but also offer an important biological assay for direct protein-to-protein interactions. The soluble expression of silicatein was enhanced by two folds when expressed as a GST fusion protein in *E. coli*. Furthermore, this fusion protein was immobilized on glutathione (GSH)-coated plate via GST-tag and remained active to form silica layer on surface by means of biosilification in the presence of tetraethyl orthosilicate as substrate at an ambient temperature and neutral pH. Simultaneously, green fluorescent protein or horseradish peroxidase enzyme was immobilized on silica surface by simple adding it during biosilification. Immobilized proteins retained their activity and were released gradually. This technique can be applied to form biocompatible silica coating for catalytic, diagnostic and sensing surface, and matrix for tissue cultures.

Keywords: Silicatein; GST-fusion protein; Biosilification; Enzyme immobilization

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Poster 1.3.25

Metabolic engineering of *Saccharomyces cerevisiae* for optimizing 3HP production

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The finite nature of fossil resources and the negative influence of CO_2 emissions on the global climate are key drivers in development of new biological processes. These are based on renewable resources such as sugar, starch, and biomass and aim at replacing chemical production from fossil fuels. Polyacrylates are a substantial part of the different plastic varieties found on the market. This kind of plastic is derived from acrylic acid, which is currently produced from propylene, a by-product of ethylene and gasoline production. Annually, more than one billion kilograms of acrylic acid is produced and the market for acrylate products exceeds USD 100 billion.

As an alternative to oil and gas derived acrylic acid, 3-hydroxypropionic (3HP) acid produced from renewable sources is highly desired, because 3HP can easily be converted into acrylic acid. We are setting out to produce 3HP in yeast *Saccharomyces cerevisiae*. One main reason for selecting Baker's yeast as host organism is that yeast has a high tolerance towards low pH in comparison to bacteria, e.g. *E. coli*. Hence, it lowers the consumption of base for neutralization of growth media when compared to bacteria. The preferred engineered pathway towards 3HP has a substantial need for NADPH equivalents. Consequently, a yeast host with elevated NADPH availability is preferred. We will redirect several of the glycolysis steps in order to increase the NADPH generation per glucose molecule and thereby increase 3HP production.

We believe this strain will be of high interest for other NADPH demanding biosynthetic routes.

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Poster 1.3.26

Peptide affinity to TiO_2 is necessary but not sufficient for prediction of its biomineralizing efficacy

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Using cyclic constrained TiO_2 binding peptides STB1 (CHKKPSKSC), RSTB1 (CHRRPSRSC) and linear peptide LSTB1 (AHKKPSKSA), it was shown that while affinity of the peptide to TiO_2 is essential to enable TiO_2 biomineralization, other factors such as biomineralization kinetics and peptide local structure need to be considered to predict biomineralization efficacy. Cyclic and linear TiO_2 binding peptides show significantly different