Acetobacter pasteurianus DSM 3509 produces cobalamin

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A strain of acetic acid bacteria was found to have the ability to synthesize cobalamin. A preliminary genetic study of the gene of uroporphyrinogen-III synthase and a survival test indicated the ability to synthesize cobalamin. By a modified microbiological assay based on *Lactobacillus delbrueckii* spp*. lactis* DSM 20355, 4.57 ng/mL of real cobalamin and 0.75 ng/mL of analogues were detected. The product extracted and isolated in its cyanide form had the similar UV spectrum as standard cyanocobalamin and as cobalamin prodcuced by *Lactobacillus reuteri* DSM 20016. No cobalamin was detected in the fermentation broth containing 1% acetate, and less cobalamin was obtained when acetate started to be consumed.

Mikropartikelunterstützte Kultivierung MPEC verbessert die Produktion von Aromastoffen mit filamentösen Pilzen

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Höhere Pilze spielen eine bedeutende Rolle bei der biotechnologische Produktion von technischen Enzymen, Grund- und Feinchemikalien sowie pharmazeutischen Wirkstoffen. Viele industriell relevante Stämme neigen jedoch zur Bildung von Zellagglomeraten bis hin zu zentimetergroßen Pellets, was häufig ihren technischen Einsatz erschwert und die Produktivität verringert. Die MPEC (microparticle enhanced cultivation)-Technik erlaubt es die Morphologie der Pilze zu beeinflussen. Die Zugabe von Mikropartikeln ermöglicht es die Größe der Pellets zu steuern bis hin zur Ausbildung von freiem Mycel. Bisher wurde ein positiver Einfluss der Technik auf die Produktion von Enzymen wie Chloroperoxidase durch *Caldariomyces fumago* [1] und Fructofuranosidase durch *Aspergillus niger* [2] nachgewiesen.
MPEC unterstützt jedoch auch die Bildung von Sekundärmetaboliten. Der rosenartige Aromastoff 2-Phenylethanol wird aus L-Phenylalanin gebildet und seine Produktion wurde bisher überwiegend an Hefen untersucht. Es konnte jedoch nachgewiesen werden, dass *Aspergillus niger* DSM 821 ebenfalls das Potential dazu besitzt. Mit diesem Stamm konnten nahezu doppelt so hohe Produktkonzentrationen (700 mg/L) erreicht werden wie mit bisher beschriebene *Aspergilli.* Der Einsatz von MPEC erhöht die Konzentration um weitere 30%.

[1] Kaup, B.A., Ehrich, K., Pescheck, M., Schrader, J. 2008. Microparticle-enhanced cultivation of filamentous microorganisms: Increased chloroperoxidase formation by *Caldariomyces fumago* as an example. *Biotechnol Bioeng*, **99**(3), 491-498.
[2] Driouch, H., Sommer, B., Wittmann, C. 2010. Morphology engineering of Aspergillus niger for improved enzyme production. *Biotechnol Bioeng*, **105** (6), 1058-1068.

Antifungal activity in seed coat extracts of *Theobroma cacao* L.

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Abstract

Seed coat is an important tissue for the regulation of imbibition and maintenance of the integrity of seed, and it is also the first seed barrier encountered by pests and pathogens. Seed cotyledons contain an array of proteins that may be involved in the protection of quiescent seeds against fungi. Now we know that seed coat from *Theobroma cacao* L. seeds contains an antifungal activity. In the present study inhibition tests of seed coat extracts against microorganisms isolated from cocoa bean fermentations were performed. Seed coat was extracted using surface-sterilization and filter-sterilization. To determine antifungal activity in seed coat extract agar diffusion and broth microdilution tests were used. Seed coat extract can inhibit growth of fungi (e.g. *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Mortierella isabellina, Penicillium citrinum, Penicillium purpurogenum, Penicillium roquefortii*). and yeasts (e.g. *Candida krusei, Candida lipolytica, Candida guilliermondii, Cryptococcus laurentii, Rhodotorula mucilaginosa, Rhodotorula rubra, Saccharomyces cerevisiae, Schizosaccharomyces pombe*). No inhibition effect could be detected against gram positive bacteria (e.g. *Bacillus subtilis, Staphylococcus aureus, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus reuteri, Propionibacterium freudenreichii*) and gram negative bacteria (e.g. *Escherichia coli, Acetobacter pasteurianus, Acetobacter tropicalis, Acetobacter pomorum, Gluconobacter frateurii, Acetobacter orientalis*).

The minimum inhibitory concentration (MIC) of seed coat extract was determined. 25 mg/mL of seed coat extract can inhibit growth of fungi (e.g. *Penicillium citrinum, Aspergillus niger, Penicillium purpurogenum*), and 10 mg/mL seed coad extract can inhibit growth of yeasts (e.g. *Saccharomyces cerevisiae, Rhodotorula rubra, Candida lipolytica*).

Nisin production in tofu by fermentation

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Tofu has a high nutritional content but on the other hand it can easily be spoiled. In Indonesia tofu production takes place mostly in small factories without an opportunity for pasteurization. Nisin production in tofu by fermentation with *Lactococcus lactis* ssp. *lactis* DSM 20729 may be a possibility to preserve tofu. The fermentation of tofu cubes (2*2 cm) submerged in water was performed for two days. Two methods of detection of nisin in fermented tofu were tested and compared by using inhibition test (modified method of Pongtharangkul and Demirci 2004), and a liquid chromatography electrospray ionization tandem mass spectrometry method (LC-ESI-MS/MS), based on ISO/TS 27106:2009. The detection limit was significantly lower by using the inhibition test. The nisin content was 0.19 mg/kg in tofu and 0.06 mg/L in liquid substrate. In comparison to this the detection limit for nisin using LC-ESI-MS/MS was 0.34 mg/kg in tofu. Matrix calibration of the liquid substrate could not be carried out by LC-ESI-MS/MS, because the background noise of the matrix was too high. Furthermore the production and distribution of nisin in tofu cubes was investigated. The nisin concentration on the surface of tofu was 2.64 mg/kg and so it was nine times higher compared to the interior of tofu. The optimum storage condition for fermented tofu was a combination of low temperature and low pH-value. An alternative to preserve tofu may be fermentation of tofu with *L*. *latis* ssp*. lactis*. Nisin concentration of complete tofu cubes could be successfully determined by using both methods. The inhibition test proved to be more sensitive for nisin detection in tofu.

Pongtharangkul T, Demirci A (2004) Evaluation of agar diffusion bioassay for nisin quantification. Appl Microbiol Biotechnol 65: 268-272

International Organization for Standardization: ISO/TS 27106 (2009) Cheese-determination of nisin A content by LC-MS and LC-MS/MS. Geneva, Switzerland