# Reference

Jin, Y., Xue, X.Y., Liu, Y.F., Xiao, Y.S., Zhang, J., Shi, H., Zhang, F.F., Liang, X.M., 2008. A novel method of prediction and optimization for preparative high-performance liquid chromatography separation. J. Chromatogr. A 1183 (1–2), 76–86.

doi:10.1016/j.jbiotec.2008.07.202

# I3-P-044

# The photosensitive effect of chlorophyll derivatives on bacteria

Lei Tang\*, Lei Xu

Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China

# E-mail address: ltang@jiangnan.edu.cn (L. Tang).

The extensive use of antibiotics has resulted in an increase of antibiotic resistant strains of bacteria. Photodynamic chemotherapy is one of the alternative methods to solve the problem (Wainwright, 1998). Chlorophyll (Chl) and its degradation compounds are one of the most abundant pigments on earth (Takamiya et al., 2000). Although the accumulation of even small amounts of visible light absorbing Chl metabolites is extremely phototoxic in plant cells (Chung et al., 2006), the photodynamic effect on bacteria has not yet been studied in detail. In the present work, the alkali, acidic and enzymatic hydrolysis products of Chl were prepared, and their photosensitive activities towards bacteria were compared. The results showed that the structures of the Chl derivatives, the types of bacteria, and the binding ability of the derivatives to the bacterial cells were three main factors of photosensitive activity. The removal of the phytol group and the opening of the V ring of Chl enhanced the phototoxicity of the compound. The Gram positive bacteria were more sensitive to the Chl derivatives than Gram negative ones. The bacterial cells which bound to the derivatives effectively were killed by the photodynamic effect. As the Chl and its degradation intermediates are widely distributed in plants, they may provide cheaper resources of photosensitisers.

#### References

Chung, D.W., Pružinská, A., Hörtensteiner, S., Ort, D.R., 2006. The role of pheophorbide a oxygenase expression and activity in the canola green seed problem. Plant Physiol. 142, 88–97.

- Takamiya, K., Tsuchiya, T., Ohta, H., 2000. Degradation pathway(s) of chlorophyll: what has gene cloning revealed? Trends Plant Sci. 5, 426–443.
- Wainwright, M., 1998. Photodynamic antimicrobial chemotherapy (PACT). J. Antimicrob. Chemother. 42, 13–28.

## doi:10.1016/j.jbiotec.2008.07.203

#### I3-P-045

### Antifungal protein from a medicinal plant, Curcuma caesia Roxb.

Krishnaraj Mannangatti\*, Mathivanan Narayanasamy

Biocontrol and Microbial Metabolites Laboratory, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600025, India

E-mail address: prabhamathi@yahoo.com (K. Mannangatti).

To determine the antifungal activity of the protein fraction of a medicinal plant, *Curcuma caesia* against drug resistant *Candida albicans*. The rhizomes of *Curcuma caesia* Roxb. were obtained from the virgin forest of Dandakaranya, Orissa, India. The fungal human pathogen, *C. albicans* was provided by the Infectious Disease Laboratory, Voluntary Health Services, Chennai, India. The protein was

extracted from the mature rhizomes (100 g) were frozen in liquid nitrogen, ground into fine powder and the resulted flour was suspended in five volume of 20 mM phosphate buffer, pH 6.2 contained 50 mM KCl, 5 mM EDTA, 1 mM aprotinin and 10 mM thiourea. The suspension was incubated at 4°C overnight with constant stirring. The slurry was filtered through three layers of gauze cloth and subsequently centrifuged at 32000 × g for 35 min at 4 °C (Xingyong et al., 2006). The supernatant was precipitated by 85% ammonium sulphate, dialyzed against phosphate buffer. The protein fraction was lyophilized and stored at -20 °C until further use. The antifungal assay against C. albicans was performed in vitro condition, a lawn of pathogen was made and subsequently 5 mm wells were prepared and filled with 100 µl of crude protein and autoclaved protein. Control wells received phosphate buffer only (Xingyong et al., 2007). The crude protein extract of C. caesia showed strong antifungal activity against C. albicans with the minimum inhibitory concentration of 55  $\mu$ g/ml. The protein sample had shown the inhibition zone of 12 mm as compared to 6 mm caused by the autoclaved protein fractions. The inhibitory activity of C. caesia protein extract was found to be fungicidal in nature. The present study showed the potentials of isolating peptide antifungal compound from C. caesia for the control of C. albicans.

# References

Xingyong, Y., Jun, L., Xianbi, L., Rong, S., Yan, P., 2006. Isolation and characterization of a novel thermostable nonspecific lipid transfer protein like antimicrobial protein from motherwort (*Leonurus japonicus* Houtt) seeds. Peptides 27, 3122–3128.

Xingyong, Y., Jun, L., Xiaowen, W., Weiguo, F., Michael, J.B., Rong, S., Yuehua, X., Yan, P., 2007. Psc-AFP, an antifungal protein with trypsin inhibitor activity from *Psoralea* corylifolia seeds. Peptides 27, 1726–1731.

# doi:10.1016/j.jbiotec.2008.07.204

#### I3-P-046

## Organic solvent effect in skin whitening agent screening system

Chi Ho Choi\*, Sung Won Choi, Jae Dong Shin, Jung-Hyun Kim, Hyang-Bok Lee, Hae Jong Kim, Eun-Ki Kim

Department of Biological Engineering, Inha University, Incheon, Republic of Korea

## E-mail address: ekkim@inha.ac.kr (C.H. Choi).

Skin color is determined by the amount of melanin and tyrosinase is a key enzyme of melanin synthesis. MITF (microphthalmiaassociated transcription factor) belongs to the basic helix-loophelix-zip family of transcription factors and is the major regulator of tyrosinase and the related enzymes (TRPs). To screen depigmenting agents by HTS (high-throughput screening) system, a protein chip containing recombinant MITF protein was constructed. Since many compounds have low solubility in water, they need to be dissolved in an organic solvent. However, effect on the MITF protein chip and the toxicity of organic solvents used in bioassays had not yet been fully documentated. Therefore, seven solvents (MeOH, EtOH, acetone, acetic acid, iso propyl alcohol, tetrahydrofuran, DMSO) were chosen and tested for their effects on bioassays. To search for the best solvent among them, a cell toxicity assay was performed by MTT. The effect of organic solvents on melanin synthesis was investigated. Finally effect of organic solvent was checked in the MITF protein chip. In conclusion, MeOH and DMSO were found to be the best solvents which can be used in protein chip and bioassays. Based on the computer-simulated stricter, 27 chemicals were selected and were investigated as a MITF-DNA binding inhibitor. Twenty-seven chemicals were dissolved in DMSO and tested by bioassay and MITF protein chip. Chemical number 18 was shown to