Toetab TÜ ja TTÜ doktorikool
"Funktsionaalsed materjalid ja tehnoloogiad" (FMTDK)

ESF projekt 1.2.0401.09-0079

NATURAL AND RECOMBINANT BIOLUMINESCENT BACTERIA FOR HIGH THROUGHPUT TOXICITY SCREENING

Imbi Kurvet[1,2], Angela Ivask[1], Mariliis Sihtmäe[1,2], Monika Mortimer[1,2] and Anne Kahru[1]

[1]*Laboratory of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia;*

[2]*Tallinn University of Technology, Tallinn, Estonia*

e-mail: imbi.kurvet@kbfi.ee

The aim of this study was to show weather recombinant luminescent bacteria can be used for toxicity testing, analogously to naturally luminescent *Vibrio fischeri*. We compared the performance of two recombinant *Escherichia coli* (with 100-fold different level of luminescence) and *Vibrio fischeri* (a gold standard). Both, heavy metals (Hg, Ag, Cd, Zn, Cu) and organic toxicants (3,5-dichlorophenol; 3,5-dichloroaniline, aniline) were used for evaluation. The performance (sensitivity, reproducibility) of these three bacteria was analyzed by varying exposure time (30 seconds till 2 hours) and test medium composition (addition of phosphates and amino acids to isotonic NaCl solution).

Both *E. coli* strains, although producing different level of luminescence, had equal sensitivity towards all chemicals analyzed. Comparison of *E. coli* and *V. fischeri* (in 0.45% and 2% NaCl, respectively) showed that *E. coli* strains were more sensitive to Zn, Cd, Ag, Hg and less sensitive to Cu. Regarding organic chemicals, *V. fischeri* was more sensitive to aniline. For all three bacterial strains, the time-dependent toxicity of heavy metal $CuSO_4$ and organic compounds (3,5-dichlorophenol, 3,5-dichloroaniline, aniline) was observed, toxicity increased from 30-s to 2-h exposure up to 4000-fold. The addition of phosphates and amino acids to test media reduced up to 1000-fold the apparent toxicity of metals, probably due to decreased bioavailability (complexation) as no such effect was observed for organic reference chemicals. We demonstrated that recombinant luminescent bacteria could be used for high-throughput toxicity screening of various toxicants (e.g. synthetic nanoparticles). However, special attention should be drawn on test media composition.