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Inducible promoter-based biosensor: A potential tool for high throughput screening of lignin degrading enzyme library

Barindra Sana[1], Balamurugan Ramalingam[2], Sarada Raghavan[1], Chia Kuan Hui Burton[3], Niranjan Nagarajan[3], Jayasree Seayad[2] and Farid J Ghadessy[1]
[1]Agency for Science Technology and Research (A*STAR), Singapore
[2]Institute of Chemical and Engineering Sciences, Singapore
[3]Genome Institute of Singapore, Singapore

Lignin is a potential renewable raw material for synthesis of various value-added chemicals that can substitute fossil-derived consumer products. A huge amount of lignin is produced as a by-product of paper industry while cellulosic components of plant biomass are utilized for the production of paper pulp. In spite of vast potential, lignin remains the least exploited component of plant biomass due to its extremely complex cross-linked three dimensional structures. Nature has provided a few enzymes known to degrade lignin biomass; however, till date there are no efficient processes available for enzymatic degradation of these extremely complex molecules. Development of effective lignin degrading enzymes may be possible by amending activity of some currently available enzymes, using protein engineering techniques. Directed evolution is one such protein engineering tool that could be used for this purpose but application of this technique for improving efficiency of potential lignin degrading enzymes is limited due to lack of an effective high throughput screening method. With an objective of detecting the Lignin Degradation Products (LDPs), we identified *E. coli* promoters that are up-regulated by vanillin and a few other potential lignin degradation products. 7 potential promoters were identified by RNA-Seq analysis of *E. coli* BL21 cells pre-exposed to a sub-lethal dose of vanillin for different exposure times. A 'Very Green Fluorescence Protein' (vGFP) gene was recombinantly placed under control of these promoters within a customized plasmid and transformed in *E. coli* BL21 cells to generate the whole cell biosensors. Fluorescence of two biosensors enhanced significantly while grown in the presence of the lignin degradation products (e.g. vanillin, acetovanillone and guaiacol), which was detected by Fluorescence-Activated Cell Sorting (FACS) analysis. The sensors did not show any increase of fluorescence by the presence of lignin, lignin model compounds or non-specific chemicals. The fluorescence change by the presence of LDPs was dose-dependent; one sensor can detect vanillin at the concentration as low as 0.5mm.

Biography

Barindra Sana is currently working as a Research Scientist at the Agency for Science Technology and Research (A*STAR), Singapore. He has completed his Master's degree in Biotechnology and PhD from Jadavpur University, India. He pursued his Post-doctoral research at Nanyang Technological University, Singapore. He has research interests in multiple area of industrial biotechnology including microbial bioprospecting, molecular microbiology, enzyme engineering, biomass conversion, fermentation and downstream processing. Currently, he is working on microbial/enzymatic conversion of biomass to biofuel or value-added chemicals. He has published several research articles in internationally reputed journals.

bsana@p53Lab.a-star.edu.sg

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