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Development of inducible promoter-based biosensors for high throughput screening of lignin degrading enzyme library

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Lignin is a potential renewable raw material for synthesis of various value-added chemicals that can substitute fossil-derived consumer products. Huge amounts 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. Inspite 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. Seven 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.

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Analyte sensing for health and fitness

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Detection and measurement of physiological relevant analytes is an essential step for diagnosis and monitoring of health and fitness conditions. The integration of lithography and microfabrication has allowed the miniaturization of medical devices, biosensors and applications in the fields of biological, environmental science, sports and fitness through clinical medicine. The miniaturization of biosensors has enabled new discoveries, diagnoses and treatments by creating novel devices, systems, and analyses. Biosensors are biophysical devices which can detect the presence of specific analytes (e.g. sugars, proteins, hormones, pollutants, toxins). They are also capable of measuring the quantities of these specific substances in the environment and human body. For example, diabetes is a health condition where biosensors have made a significant contribution. According to the National Report from the Center of Disease Control and Prevention, diabetes affects more than $29 million people in the USA alone. The total medical cost for diabetics is around $245 billion dollars a year. Glucose biosensors are a great tool helping diabetic patients to monitor and manage their disease more efficiently and effectively. New advances in these devices such as integrating redundancy and alternative sensing, algorithms and data analytics has allowed for better and more accurate monitoring and treatment of the disease.

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