A Novel Assay to Quantitatively Detect Bacterial Endotoxin by Harnessing PAMP-Triggered Immunity of FRK1-LUC Arabidopsis thaliana

Krishnan, Aravind (School: Hillsborough High School)

Harvesting Limulus polyphemus (horseshoe crabs) to produce the Limulus amoebocyte lysate endotoxin assay for medical devices, pharmaceuticals, and drinking water is ravaging coastal ecosystems. This project develops a more sustainable and cost-effective quantitative endotoxin assay. The PAMP-Triggered Immunity response of Arabidopsis thaliana to pathogen-associated molecular patterns (PAMPs) was harnessed for quantitative determination of endotoxin presence based on induction of the FRK1 gene. Transgenic FRK1-LUC A. thaliana were used to express luciferase (LUC) upon activation of FRK1 by exposure to gram-negative bacteria. Luciferase enzyme control was first tested via plate reader, and luminescence produced by varying enzyme quantities was recorded. Next, E. coli ranging from 6*10^5 to 10^3 CFU/mL were infiltrated into the leaf apoplastic space of FRK1-LUC and wild-type plants. Luminescence of infiltrated leaf discs was measured after adding luciferin substrate to reconstitute functional luciferase. The results showed a direct relationship between bacteria concentration and luminescence. The FRK1-LUC luminescence versus endotoxin concentration results yielded a formula of $y = 1518e^{0.0196x}$. ($R^2 = 0.937$). Data suggest this assay achieves a sensitivity down to 18 endotoxin units/mL ($p < 0.001$, SEM = 1.76%). To determine specificity, the SeeSAR software was used to calculate binding affinities of endotoxin (LPS) and flg22 with several receptors. Results indicate high specificity in LORE-LPS binding, signifying the luminesce results were caused by LPS concentration. This method’s sensitivity and specificity combined with its elimination of environmental impacts and low cost make it a promising new bacterial endotoxin assay for pharmaceutical and drinking water testing.

Awards Won:

Third Award of $1,000

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