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Research Article

Identification of Stress Fingerprints Induced by Acrylamide, a Toxic Contaminant in Starchy Food Products, Using Bacterial Bioluminescence

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Abstract

The presence of acrylamide in food is recognized as a significant concern to human health based on its ability to induce cancer and heritable mutations in laboratory animals. This study aimed to characterize the cellular-level damage of acrylamide by bioluminescence stress fingerprinting. Five genetically engineered strains containing selected stress-responsive *E. coli* promoters fused to the lux CDABE reporter were employed. Results showed that Luminous *E. coli* DPD2222, having DNA damage responsive promoter, recA, yielded the highest response, followed by luminous *E. coli* DPD2234, which contained protein damage responsive promoter, grpE. Quantitative and fingerprint assessment of acrylamide damage could be achieved by optimizing bioluminescence cells constructed with different stress-responsive reporter plasmids.

Keywords: Acrylamide; Luminous Bacteria; Biosensor; *E. Coli*; Stress Damage

Introduction

Acrylamide is a Maillard reaction-derived chemical hazard, a potential carcinogen to humans and a neurotoxin [1-4]. One-third of daily consumed food by the United States and European population contains detectable levels of acrylamide [5]. High-performance liquid chromatography (HPLC) [6-8], gas chromatography (GC) [9-11], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12-14], and gas chromatography-mass spectrometry (GC-MS) [15] are standard methods for detecting acrylamide. Although these techniques have high accuracy and sensitivity, they are complex, tedious, time-consuming, and require trained personnel.

Given the need for real-time toxicity-monitoring, significant advances have been made in applying bacteria as test organisms. High sensitivity, low costs, sizeable homogenous test populations, and, most importantly, rapid responses are advantages of microbial toxicity testing [16-18]. Moreover, by sophisticated genetic manipulations, engineering bacteria helps generate an easily assayed signal in response to a specific class of compounds, a unique benefit of bacteria application as test organisms. One approach to using bacteria as a sensing element is to fuse two genetic elements: the first is the promoter that reacts to the presence of the toxicant and "turns on" the expression of the second element that acts as the reporter. Bioluminescence genes are excellent candidates for the reporting element since light emission is easy to monitor and quantify [19-21]. Thus, in recent years, biosensors based on whole-cell bioluminescence have been a sensitive substitute to conventional analytical methods in determining concentrations of various compounds with high sensitivity and specificity [22-26].

This study employed five genetically engineered strains containing selected stress-responsive *E.coli* promoters fused to the luxCDABE reporter. The five-gene *lux* reporter system allows facile monitoring of gene expression because all components necessary for light production are present in the cell. The responses were biologically appropriate when stressed by internal acidification, protein damage, DNA damage, super-stationary phase, and dependent stress.

Materials and Methods

Reagent and chemicals

Unless otherwise stated all reagents were purchased from Sigma-Aldrich.

Bioluminescent *E. coli* strains

The preparation of culture for measuring relative light unit of strains was adopted from [27] and modified in this study. Five bioluminescent *E. coli* strains used in this study were obtained from DuPont Genetics Lab (DuPont Company, Wilmington, DE). Each strain contained a different selected stress-responsive promoter fused to the Photorhabdus luminescence *luxCDABE* reporter. The panel strains were chosen to represent a range of stress responses to result in different internally induced gene expression patterns. Each strain responds respectively to internal acidification, DNA damage, protein damage, "super- stationary phase" and sigma S Stress (Table 1). The *E. coli* strains were maintained in a 50% glycerol suspension at -80 °C. Before the assay, the stock cultures are transferred to a petri dish containing Luria Bertani (Fisher Scientific) medium (pH7) and incubated for 17 hours at 37 °C on a model 1575 orbital shaking incubator (VWR Scientific, Cornelius, OR). Then one colony was transferred from each petri dish to a 250 ml flask with 100 ml sterilized LB broth and incubated for 17 hours at 300 rpm (OD: 600nm=0.15 approximately 107 cells/ ml). To ensure the stability of the plasmids containing *lux* genes, ampicillin (10 µl/ ml) was added to the growth media.

Stress Response	Regulatory Circuit	Promoter Fused to *lux*	Strain Name
Internal Acidification	Mar/Sox/Rob	*inaA*	DPD2240
DNA damage	SOS	*recA*	DPD2222
Protein damage	Heat shock (δ^{32})	*grpE*	DPD2234
"Super-stationary phase"		*o513*	DPD2232
Sigma S stress Response	Stationary phase (δ^{s})	*yciG*	DPD2233

Table 1: Stress-responsive *E. coli lux* fusion strains.

E. coli stress fingerprinting

The culture of each *E. coli* strain was diluted with sterile distilled water at a ratio of 1: 10. Then, 10 µl of culture solution was transferred to a 1.5 ml Eppendorf microtube. The luminescence from *E. coli* of each tube was measured by GloMax® 20/20 single tube luminometer (Promega; Madison, WI, USA) and the luminescence values were presented as the instrument's arbitrary relative light unit (RLU). RLU values were recorded for two samples, one without adding acrylamide (control) and another containing 5, 10, 20, 40, 80, 160, 320, 640, and 2560 µg/L acrylamide. The difference and the ratio of the two RLU values ("RLU without acrylamide" and "RLU with acrylamide") were both calculated to indicate the stress responses from the bioluminescent *E. coli* strains.

ΔRLU = «RLU with acrylamide» - «RLU without acrylamide»

RLU ratio = "RLU with acrylamide"/"RLU without acrylamide"

If RLU ratios are more significant than one and ΔRLUs are more considerable than zero (" light-on"), the lux gene fusion is expressed because of the acrylamide. If RLU ratios are less than one and ΔRLUs are less than zero (" light-off"), it suggests a dampening of bioluminescent *E. coli* strains, which means that cells could not repair damages, in acrylamide.

Statistical Analysis

Data are presented as mean± the standard errors of the mean (SEM). Statistical analysis was performed using analysis of variance (ANOVA) Tukey's Multiple Comparison test utilizing SPSS software version 20.

Results and Discussion

Results showed (Figure 1 and Figure 2) that the highest significant difference with a probability of 95% for all strains was observed at concentrations of 40, 320, and 1280 µg/L of acrylamide. The light emitting of both *grpE* and *recA -lux* fusion strains was significantly increased at 5 µg/L concentration of acrylamide since ΔRLU values and RLU Ratios of these two strains showed significant differences between 0 and 5 µg/L amount of acrylamide ($P < 0.05$). For strains containing *YciG* and *o513* promoters a considerable increase in ΔRLU values and RLU Ratios were observed at 40 µg/L of acrylamide. Still for *inaA-lux* fusion strain was observed at 20 µg/L concentration of acrylamide ($P < 0.05$). ΔRLU and RLU Ratio of none of the strains showed significant differences between 1280 and 2560 µg/L concentrations of acrylamide ($P > 0.05$). Among these five strains, a substantial increase in ΔRLU values and RLU ratios of *recA-lux* fusion strain dose-dependently in response to acrylamide ($P < 0.05$). In addition, for *grpE-lux* fusion significant differences were observed at all concentrations except 20 and 40 µg/L concentrations of acrylamide.

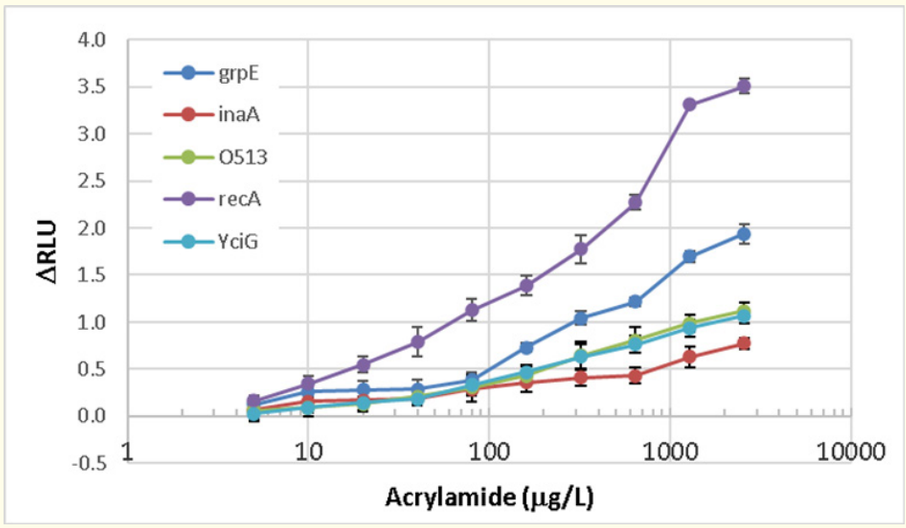

Figure 1: ΔRLU of five strains when adding different amount of acrylamide (µg/L). Shown are mean ± SEM, ANOVA Tukey's Multiple Comparison (n = 3).

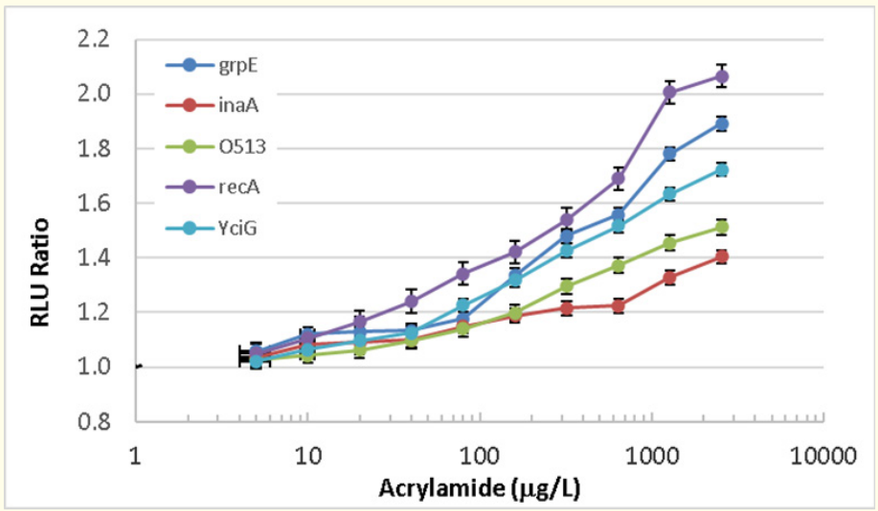

Figure 2: RLU ratio of five strains when adding different amount of acrylamide (µg/L). Shown are mean ± SEM, ANOVA Tukey's Multiple Comparison (n = 3).

The strains containing *recA*, *grpE*, and *yciG* promoters yielded high response ratios. The strain containing *recA-lux* fusion is in the SOS regulon and showed DNA damage in the presence of acrylamide. Although, to a lesser extent than to *recA-lux*, the strains containing *yciG-lux*, *grpE-lux*, *inaA-lux*, and *o5123-lux* fusion also responded to acrylamide stress. Expression of the *yciG* gene is under the control of SS. Thus, the *yciG-lux* fusion activated the SS-dependent stress response. While *grpE* is in the heat shock regulon controlled by *s32*, this response indicates that acrylamide also induced the protein-damage responsive regulon.

On the other hand, activation of *inaA* and *o5113* promoters showed internal acidification and super-stationary phase damage. Moreover, it was observed that increasing the amount of acrylamide light-emitting of bioluminescent *E. coli* strains increased significantly. In the entire range of examined acrylamide concentrations, ΔRLU values are positive. RLU ratios are more significant than one, meaning no cell dampening occurs up to adding, 2560 µg/ L concentration of acrylamide concentration.

In a previous study conducted by Van Dyk., *et al.* [28], these five strains were used for detecting a limited specific amount of other

toxicants, including H202, nalidixic acid, ethanol, sodium salicylate, methyl violgogen, dichlorophenoxyacetic acid, aluminum chloride, cadmium chloride, and aflatoxin B1. Comparing the results of this study with those obtained by [28], it was observed that in the presence of nalidixic acid and aflatoxin B1, similar to acrylamide the highest RLU ratio was related to *recA-lux* fusion strain, but no significant RLU ratio was observed in the presence of nalidixic acid for other strains. In addition, the presence of H202 *recA* and *grpE-lux* fusion strains showed elevated levels of RLU ratios. Moreover, ethanol, methyl viologen, and cadmium chloride could increase the RLU ratio of *grpE-lux* fusion compared to other strains. Still, different strains showed no response in the presence of ethanol. On the other hand, the *inaA-lux* fusion strain showed the highest response to sodium salicylate and dichlorophenoxyacetic acid, and the second place belonged to *grpE-lux* fusion. For aluminum chloride, the highest answer belonged to it, and the second level of the RLU ratio was related to *grpE* [26,28].

Moreover, in another study, Fukushima *et al*. showed that the highest RLU ratios in the presence of 0.3 mg/ml ephedrine and pseudoephedrine (two toxic compounds in traditional Chinese medicinal herb) were related to *recA-lux* fusion strain followed by *grpE-lux* fusion strain [27].

Therefore, *recA* and *grpE*-lux fusion strains highly responded to most toxicants, which contain acrylamide. As a result, it could be concluded that DNA and protein damage are the major types of damage induced by many toxicants.

Conclusion

We evaluated five genetically engineered strains of luminous *E. coli* as candidates to detect acrylamide. The approach presented here, has four unique attributes: i) luminous *E. coli* panel was capable of detecting the low amount of acrylamide, since acrylamide concentrations below 50µg/L were detected by *yciG-lux*, *inaA-lux*, and *o513-lux* fusion strains and even as low level as 5 µg/L acrylamide concentration was detected by *recA-lux* and *grpE-lux* fusion strains. ii) . This panel could detect a wide range of toxicants since the light-emitting of these five strains was measured in the presence of acrylamide concentrations ranging from 5 to 2560 µg/L. iii) this study revealed that light-emitting of these five luminous *E. coli's* increased accordingly by increasing amounts of acrylamide. IV) The highest light-emitting was related to the strains containing *recA* and *grpE* promoters and hence, the most severe damage to bacterial cells caused by acrylamide might be DNA and protein damage. Therefore, based on the highest and the most distinguished light-emitting of *recA-lux* and *grpE-lux* fusion strains in the presence of acrylamide, appeared to be proper candidates to study the effect of acrylamide on DNA damage.

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Author Contributions

ML conceptualization and supervision, MG data curation, writing original draft.

Conflict of Interest

Authors have no conflict of interest.

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