INTRODUCTION

Bisphenol A (BPA) is an organic compound primarily used in the production of polycarbonate plastics and epoxy resins. Despite its commercial use for the past five decades, BPA made recent headlines in April 2008 when Health Canada issued a mandatory recall of BPA-containing baby bottles and children’s toys. Concerns over human exposure to BPA have been fueled by in vivo studies demonstrating its interference with neonatal development (Kubo et al., 2003), its ability to predispose cells to cancer (Murray et al., 2007), and its role as an endocrine disruptor (Market et al., 2005). In contrast to the 1998 US Environmental and Protection Agency’s recommended exposure limit of 50µg/kg/day, these studies implicate that levels of BPA, 1000 times less than deemed toxicological levels, is sufficient to induce aberrant physiological changes. Thus, given society’s widespread exposure to BPA leached into our environment and food, Bisphenol A poses a significant health risk. Moreover, the commercialization of BPA, with pentafluorobenzolic acid, and bisphenols, have the potential to be of much benefit to poorer or third world nations, which may not be able to afford any standardized BioBrick to be expressed in plants, opening iGEM applications of this technology include a BPA detector system, whereby a ‘BPA-sentinel plant’ provides an early detection system to monitor soil in areas contaminated with BPA. Furthermore, the system can be used to activate two recently characterized genes for BPA metabolism (BisdA, BisdB) to help with bioremediation of contaminated areas.

Current methods for BPA detection rely on costly mass spectrometric analysis and HPLC. As an alternative, we propose the development of a biosensor capable of detecting Bisphenol A in the environment. Our project relies on BPA’s role as a xenometabolite, a compound able to mimic estrogen. When BPA is taken into the cell, it binds to cytoplasmic estrogen receptors and activates transcription of estrogen-related genes. Thus, pathways regulated by estrogen-binding are induced when estrogen is not present in the environment. By introducing estrogen receptor into E. coli, we are able to modulate the active transcription of reporter genes to allow for easy visual identification of environments contaminated with BPA. Furthermore, the system can be used to activate two recently characterized genes for BPA metabolism (BisdA, BisdB) to help with bioremediation of contaminated areas.

PROJECT OVERVIEW

Human estrogen receptor (hER) expressed in E. coli activates a reporter system in the presence of BPA. BPA diffuses through the cell and binds to free cytoplasmic estrogen receptor, causing the estrogen receptors to dimerize. An allosteric effect allows receptors to bind to the estrogen-responsive element (ERE). The ERE is placed immediately upstream of the promoter for a reporter. Binding of the receptor blocks the binding of RNA Polymerase and prevent the transcription of the repressor. When BPA is present in the environment, the repressor is repressed and the reporter gene is actively transcribed, giving us a visual way to determine the presence of BPA. BPA degrading genes from Sphingomonas bisphenolicum are then introduced to aid in the remediation of BPA contamination via degradation.

INSIGHT AND FUTURE PERSPECTIVES

The main goal for the University of Alberta 2008 iGEM project is to obtain proof-of-concept evidence that BPA-detection works in our E. coli system. Future applications of this technology include a BPA detector system, whereby a ‘BPA-sentinel plant’ provides an early detection system to monitor soil in areas contaminated with BPA. In line with this application, a second aspect of our project involves developing biological tools which would allow us to transform our project into plants. We also plan on developing a “cell free” version of our system to be turned on when estrogen is present.