Biosensor bacteria

response of bacteria to environmental changes: presence of pollutants and changes in temperatures

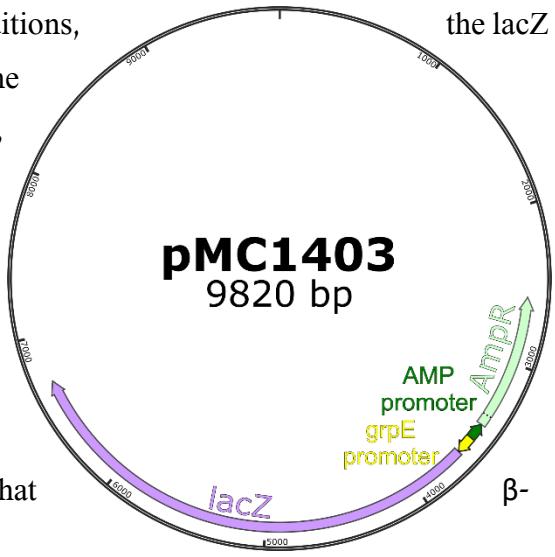
Introduction

The pollution levels worldwide are growing, and this is dangerous for human health and for the ecosystem. Hence, it is crucial to find sensitive, cheap and quick methods to detect and treat pollution.

Prof. Eliora Ron of Tel-Aviv University has been researching the response of bacteria to the environmental changes including pollutants and temperature changes. Her research led to better understanding of the molecular basis of the response of E. coli to these changes. Prof. Ron utilized this knowledge to recruit the bacteria to combat pollution: for example, engineered bacteria have been used to clean up oil-polluted sites.

Prof. Ron was able to do this because of her previous research done on the bacteria. She had discovered bacterial genes were respond to the environmental stress. By using the promoters of these genes, which are sensitive to the changes in the environment, and fusing the DNA sequence of a desired gene, we result a system which responds to the environment with a specific protein.

Prof. Ron fused the DNA sequence of the promoter of the grpE gene to the DNA of the reporter gene lacZ, which leads to the expression of β-galactosidase protein if the bacteria are exposed to high temperature or in the presence of alcohol. LacZ is a gene which is found naturally in E. coli as part of the lac operon you might be familiar with. In regular conditions, the lacZ gene encodes the β-galactosidase protein which cleaves the disaccharide lactose into glucose and galactose. However, the bacteria that Prof. Ron used lack this natural gene, so that any β-galactosidase in the bacteria were synthesized from the environmental-sensitive promoter. Except for cleaving lactose, the β-galactosidase enzyme can cleave an artificial substrate named x-gal. The x-gal is colorless, but after cleavage it changes color to blue. Hence, measuring the blue levels in the bacteria indicate that β-



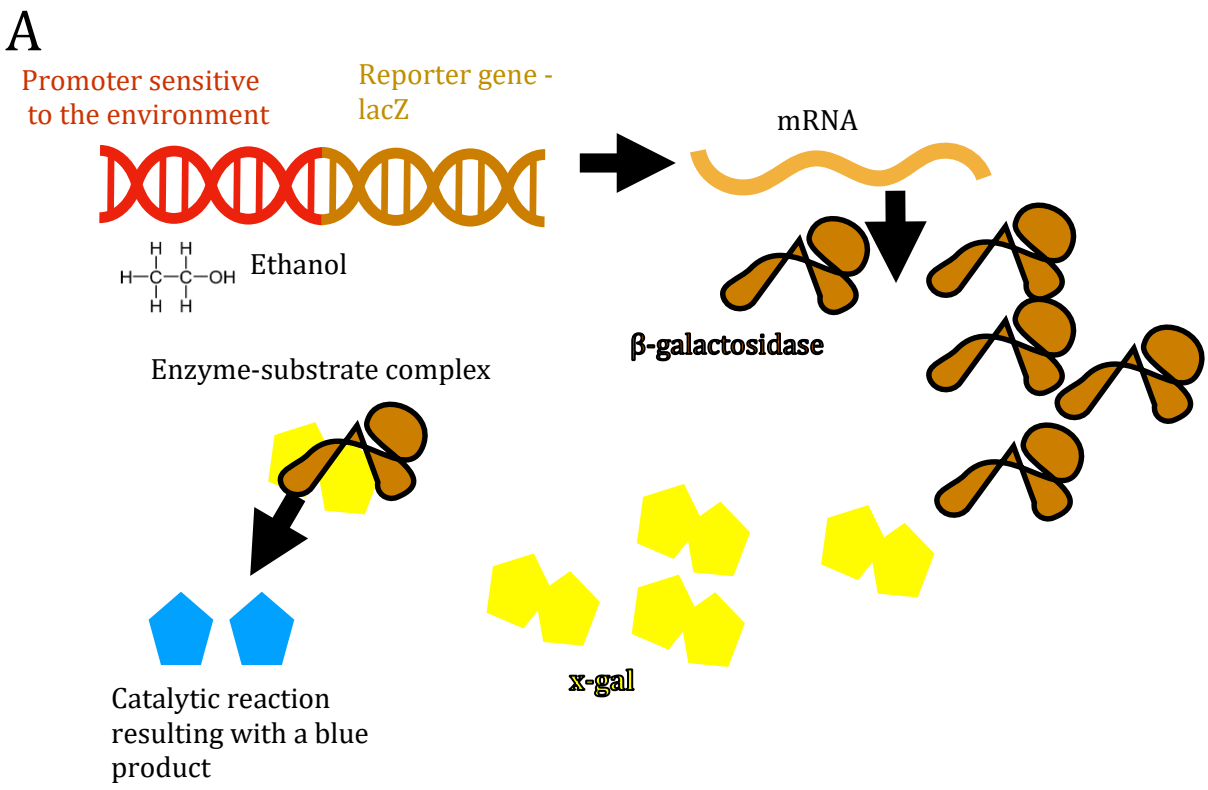
galactosidase was encoded in the engineered bacteria due to the regulation of the stress-responsive promoter sequence.

Steps to create the engineered biosensor bacteria:

1. Design a plasmid (circular DNA sequence), which includes the grpE promoter (marked yellow) fused to the lacZ gene (marked purple), and also resistance to antibiotics (marked green). This is used to select for bacteria that contain the plasmid.
2. Insert the plasmid to an E. coli strain which does not express the natural lacZ gene.
3. Grow the bacteria with antibiotics.

Steps to express the β-galactosidase in the engineered E .coli:

A – if the bacteria senses a stress factor (ethanol, for example), then the promoter is activated and the lacZ mRNA is transcribed and translated to the β-galactosidase enzyme, which cleaves the artificial substrate x-gal added by the scientist. This results in a blur product that could be measured.



B – if no stress factors exist in the environment, the lacZ gene is not expressed, so that even if the artificial substrate x-gal is added it is not cleaved.

Questions:

1. Why did the researches use bacteria that do not express the natural β-galactosidase?
2. Name other reagents that can cause damage to the DNA.
3. If these engineered bacteria will be exposed to gasoline, in the presence of x-gal a blue color can be observed. Describe the process that leads to the formation of the blue color.

You will now measure the effect of different concentrations of ethanol on the expression of β-galactosidase. To do so, you will receive a vessel with bacteria that was grown over night in LB growth media.

1. Plan the experiment: read the following instructions, and calculate the missing values in the tables.
2. Using a pipette, transfer 10 mL of this culture to a beaker. Set aside the rest of the culture (you will need some later).
3. Add 50 µ x-gal using a pipette.
4. Create a gradient of ethanol concentrations:
    a. Mark seven tubes with the numbers 1-7.
    b. To each tube, add the appropriate amounts of LB, ethanol 100% and bacteria you prepared in 3, according to the following table, to a final volume of 2 mL per tube. Do not forget to change the tip between different tubes.

Table 1. Preparing the experiment

Tube number	Volume of bacteria (µL)	Volume of ethanol (µL)	Volume of pure LB (µL)	Final concentration of ethanol (%)
1	1000	0	1000	
2	1000	100	900	
3	1000	200	800	
4	1000	400	600	
5	1000	600	400	
6	1000	800	200	
7	1000	1000	0	

5. Using the provided color scale, note the value observed after 0 minutes, 10 minutes and 30 minutes.

Table 2. Color observation time course				
Tube number	Color after 0 minutes	Color after 10 minutes	Color after 30 minutes	Final concentration of ethanol (%)
1				
2				
3				
4				
5				
6				
7				