ELICITOR CONTROLLED PRODUCT RELEASE IN AN IMMobilIZED BACTERIAL FLOW SYSTEM

IDEA

Generic drugs used today are generally given in periodic doses. Yet it is known that the most efficient action impossible when the drug is in the right place at the right time. Such a delivery system would have to be capable of detecting a need to deliver and be capable of delivering it at the right time. Even though various pumps exist which can perform the function of direct injection into the blood stream, these are not sensitive to the need of the body. We attempt to develop a skeletal system that utilizes recombinant bacteria to release controllable amounts of a peptidal drug into a continuous stream by detecting the levels.

CHALLENGES TACKLED

1. In order to cater to the problem of peptide secretion outside the cell we decided to incorporate the alpha-hemolysin system into E. coli. Here is a brief description about it—

THE ALPHA-HEMOLYSIN SYSTEM IN E. COLI

The alpha-hemolysin system is a type I secretion system found in wild type E.coli which enable them to secrete a natural toxin HlyA protein or hemolysin. This system in natural bacteria causes an antigentic response in the human body.

FUNCTIONING

The hemolysin system originates from a series of hly genes. These genes are present in the series hly-A - hly-B - hly-H - hly-D. Of these, HlyA codes for the protein responsible for the infection. HlyB and D are membrane proteins that work in coordination for the secretion of HlyA. The synthesis, activation and secretion of HlyA in E. coli is determined by the hlyCAB operon. This operon is either located on the chromosome or on a transmissible plasmid.

We decided to develop a continuous flow stirred reactor, perfusion analogous, with engineered E. coli cells immobilized in it. Rough schematic of the flow process is shown below.

2. We decided to attempt to control, by production of renin inhibitor, the Renin Angiotensin Aldosterone System (RAAS).

RENNIN ANGIOTENSIN ALDOSTERONE SYSTEM

The Renin Angiotensin Aldosterone System is a hormone system in the human body that is responsible for the regulation of blood pressure and fluid balance.

FUNCTIONING

A decrease in renal perfusion causes the release of the enzyme renin which subsequently cleaves angiotensinogen to angiotensin I. This is further converted to angiotensin II by angiotensin converting enzyme (ACE). The bioactive product, angiotensin II, causes the constriction of blood vessels, leading to an increased blood pressure. It is also responsible for the secretion of aldosterone by the adrenal cortex, which acts by increasing the re-absorption of sodium and water into the blood. Hence, the fluid volume as well as the blood pressure is maintained in the body.

RESULTS AND FUTURE DIRECTION

The chimeric PCRs hlyA - gfp and HlyB and d genes together respectively. We successfully made one bio-brick by the integration of the hlyA-gfp into the desired backbone. However the location of HlyB and d into the desired backbone was confronted with several problems. We shall attempt to obtain the NlyB and d plasmid as well. Only then we could get the desired E. coli, which we need to immobilise in order to study its flow characteristics. The flow analysis and optimisation is yet to be done but we have decided upon the intricate details of the flow system.

METAETHOD AND EXPERIMENTS

The required culture with the incorporated haemolysin systemplasmids were obtained from Karolinska Institute, Stockholm, Sweden in the form of a paper culture growth as shown.

The elicitor in general condition could be a variety of different stimuli. Thus we assumed the conversion of these stimuli to a safely recognizable chemical signal. So, we used a chemical signal such as PTD (topoerytho-beta-thioleptopseudomono) or ATP, (Adenosine monophosphate) as elicitors to induce appropriate amounts of protein. For easy detection and verification, we planned to use Green fluorescent protein (gfp). This was also acceptable since it has been found that the level of protein secretion is not only dependent on the protein itself.

For this we introduced two vectors into E. coli as shown below.

The first vector which contained the gfp(Ipl)-HlyA chimeric sequence with pLac promoter was used to produce the chimera GFP protein which contains 6x amino acidd residues from the C-terminus of hlyA protein, a prerequisite for the secretion of the protein outside the cell body. Failing a chimera did not in any way change the structure or function of the protein.

The vector contains the HlyB and HlyD sequences with Tet promoter sequence. These two proteins will be constitutively expressed. These proteins are present in the membrane and provide for an extracellular domain.

The result vector containing the gfp(Ipl)-hlyA chimeric sequence with Tet promoter was grown on media containing one mg Litmus media.

After we were successful in obtaining GFP-HlyA chimera outside the cell body, our next task was to immobilize these cells in a flow system and characterize the flow expression profile. We wanted to keep the cells in 60 phase so as to have continuous expression of the protein without death.

We also worked towards minimizing the toxins and other products so that such a system can ultimately be thought of to be incorporated and the extracellular system in our body.

FLOW SYSTEM STUDY

INTRODUCTION

The flow system study focuses on a set of parameters that are important in various aspects of the system. These parameters tend to be different for various purposes that this device can be used for. Thus the characterization needs to be done at these various conditions keeping in view of the robustness of the biological system and the possible effects on the production of the protein.

PLAN

The preliminary requirement was to get the bacteria into a productive phase without considerable loss in the productivity of the system. Once this step has been achieved with the cells, then the immobilization technique will then be optimized for the particular proteins in mind. The next part of the plan varies according to the use to which this system is put. The flow system then has to be optimized for various things such as sugar concentration, pressure drops and flow rates. Further the elicitor flow characteristics need to be set, and the interaction between the bacteria and the elicitor needs to be looked into. Once these have been carried out the final characterization of the bacterial system that needs to be used will be optimized for final product delivery.