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Transformed *E. coli* JM109 as a Biosensor for Penicillin.

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A variety of approaches were explored towards the development of a biosensor for analysis of a most important drug class, penicillins. This biosensor for penicillin was developed by immobilization of penicillinase on the bulb of a pH meter. Transformed cells of *Escherichia coli* JM109 produced the enzyme penicillinase. This transformation was carried out with plasmid pGEM, which attributes penicillinase production, by JM109. The transformed JM109 were immobilized on an ion selective electrode (pH meter) using acrylamide. This unit, the biosensor gave response to varying concentration from 10^{-1} to 10^{-4} M of penicillin prepared at pH 6.8. The initial response time for biosensor was 20 to 30 seconds.

Biosensor, though being quite an old concept, it is only recently that the implementation of research in this arena has taken pace[1]. A biosensor employs a biologically derived material, immobilized onto a suitable transducing system that converts the biological reaction into a quantifiable and readily processed signal. The key advantages of using biological components in sensors are their exquisite specificity and sensitivity for a particular substance. Consequently, biosensors are particularly suitable for applications in the pharmaceutical industry, medical diagnostics, food and drug testing and environmental monitoring[1].

Research and development on biosensors is proceeding rapidly as a result of advances in microsensor technologies, developments in recombinant DNA technology and in the use of monoclonal antibodies[2]. Biosensors can be characterized depending upon the biological moiety used for sensing like the microbial or enzymatic biosensors[3]. The enzymatic biosensors have superior specificity, sensitivity and response time in comparison to microbial biosensors but to overcome some of the inherent disadvantages of enzymatic biosensors microbial biosensors prove much beneficial[3]. For example microbial biosensors are less sensitive to inhibition by solutes and more tolerant to sub optimal pH and temperature values than enzymatic biosensors. Microbial biosensors also tend to have a longer lifetime and are cheaper because an active enzyme does not have to be isolated. However, microbial cells contain many enzymes and care has to be taken to ensure selectivity, for example, by optimizing storage conditions or by additions of specific enzymes or transport inhibitors to stop undesirable enzyme reactions. Mutant microorganisms lacking enzymes can also be used. The microbial biosensors too have some disadvantages, for example some have a longer response time than enzymatic biosensors and take longer time to return to the base-line signal after use. The advantages of using a microbial biosensor must therefore, be balanced against the known disadvantages[3-5].

Analysis of penicillin class of drugs has always been a problem for all pharmaceutical concerns; hence an accurate, precise and viable methodology is targeted in this design of a biological sensor for penicillin. Ion-selective biosensors have been successfully developed for a variety of inorganic cations and anions; however, there has been limited success in the fabrication and testing of selective electrodes for the analysis of organic species by use of biosensors[1]. This biosensor for penicillin has been developed utilizing transformed cells of *E. coli* JM109 producing penicillinase (β-lactamase) responsive to intact penicillin. The biosensor is assembled by immobilizing these trans-

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formed cells into a thin membrane of polyacrylamide gel molded around the bulb of a pH electrode. *E. coli* JM109 is transformed using a plasmid pGEM for production of enzyme penicillinase. When this biosensor is exposed to an aqueous solution of penicillin adjusted to a pH 6.8, the immobilized cells hydrolyze intact penicillin to produce penicilloic acid[5]. The increase in hydrogen ion concentration from the penicilloic acid is sensed by pH electrode and the potentiometric response is noted. This potentiometric response is directly proportional to amount of penicillin present in the sample[5].

MATERIALS AND METHODS

Stock solutions of (1 M) sodium benzyl penicillin and sodium ampicillin (Glaxo) were prepared on the day of use. In order to obtain the concentrations of penicillin desired, the stock solutions were diluted with distilled water to 10^{-1} to 10^{-4} M. pH was adjusted to 6.8 with dilute hydrochloric acid and dilute sodium hydroxide. Plasmid pGEM-7Zf (+/-), *E. coli* JM109 (Promega), microbial media requirements (Himedia), pH electrode (Elico) were utilized for the preparation of biosensor.

Competent cell preparation:

Cells were made competent using $CaCl_2$ uptake method[6]. The culture *E. coli* JM109, grown on Luria Burtenii (LB) medium was inoculated in 100 ml LB broth and then kept on a shaker overnight at room temperature. This culture was then grown in LB broth at 37° on a shaker. The cell suspension was centrifuged at 5000 g for 15 min at 4°, after the optical density reaches to a value of 1.0 (600 nm). The supernatant was discarded and the tubes were placed immediately in an ice bath. Ten milliliters of chilled freshly prepared 50 mM $CaCl_2$ was added and the pellet was dispersed by vortexing. The pellet obtained was responded in 1.0 ml chilled 50 mM $CaCl_2$ and centrifuged at 5000 g for 15 min at 4°. This tube is kept in ice after brief vortexing to disperse the pellet. Now the cells were competent for transformation[6].

Transformation of competent cells:

All the operations were carried out in chilled conditions using ice bath. The competent cells were transformed using the plasmid pGEM-7Zf (+/-). Two Eppendorf tubes were placed in ice bath, one of the tubes contained 10 ml of plasmid DNA and 50 ml of 50 mM $CaCl_2$ while the other contained only 50 ml of 50 mM $CaCl_2$ solution. Now 100 ml of competent cells were added in both the tubes and kept in ice bath for 10 min. The tubes were then placed in water bath at 42° for 2 min and subsequently were transferred again to an ice bath for 10 min. Finally 1.0 ml of LB was added to both the tubes and incubated at 37° for 1 h. Then 0.1 ml and 0.9 ml were plated on two plates (The 0.9 ml sample was concentrated to less than 0.1 ml volume before plating). The above plating was carried out using LB medium with ampicillin and without ampicillin. The transformed cells were then plated on LB plates with penicillin and without penicillin. The cells resistant to penicillin were studied for their colony characteristics and were selectively proliferated by inoculating in 500 ml (LB broth with 100 mg/ml of penicillin) and then incubating at room temperature for 24 h on a shaker. These transformed cells were then separated by centrifugation at 5000 g for 15 min. The cells were resuspended in 100 ml saline and recentrifuged. This final pellet was then suspended in 10 ml saline[6].

Biosensor assembly:

The pH electrode was washed well with distilled water, wiped dry with tissue paper and mounted upside down. A piece of one square inch nylon cloth was placed over the glass bulb of the electrode and held with a thin nylon thread, preferably just below the glass bulb. Three grams of acrylamide and 0.58 g of N'N' methylenebisacrylamide were dissolved in 25 ml of 0.1 M Tris buffer at pH 7. Three milligrams each of riboflavin and ammonium persulfate were used to catalyze polymerization. To above solution, 1 ml culture was added. This cell solution was added drop wise on the electrode; normally only 10-15 drops were needed. After the addition of the cell solution for approximately 30 min the polymerization was allowed to take place. A second piece of nylon cloth was placed over the gel layer and held in the place with a thread. The electrode was finally equilibrated in pH 7 Tris buffer. The biosensor was stored in at 4° to preserve enzyme activity[5].

The Biosensor was then assembled by immobilization of transformed cells in a polyacrylamide mixture and placed around the bulb of pH electrode. Two different derivatives of penicillins; sodium benzyl penicillin and sodium ampicillin were used for conducting the assays. Varying molar concentrations of these penicillins were prepared and a potential response of the biosensor was checked. The effect of pH on and the response time of the electrode were also noted.

RESULTS AND DISCUSSION

The biosensor senses the hydrogen ions produced at the membrane via the penicillinase catalyzed hydrolysis of the penicillin. It is clear from the fig.1, which shows that the

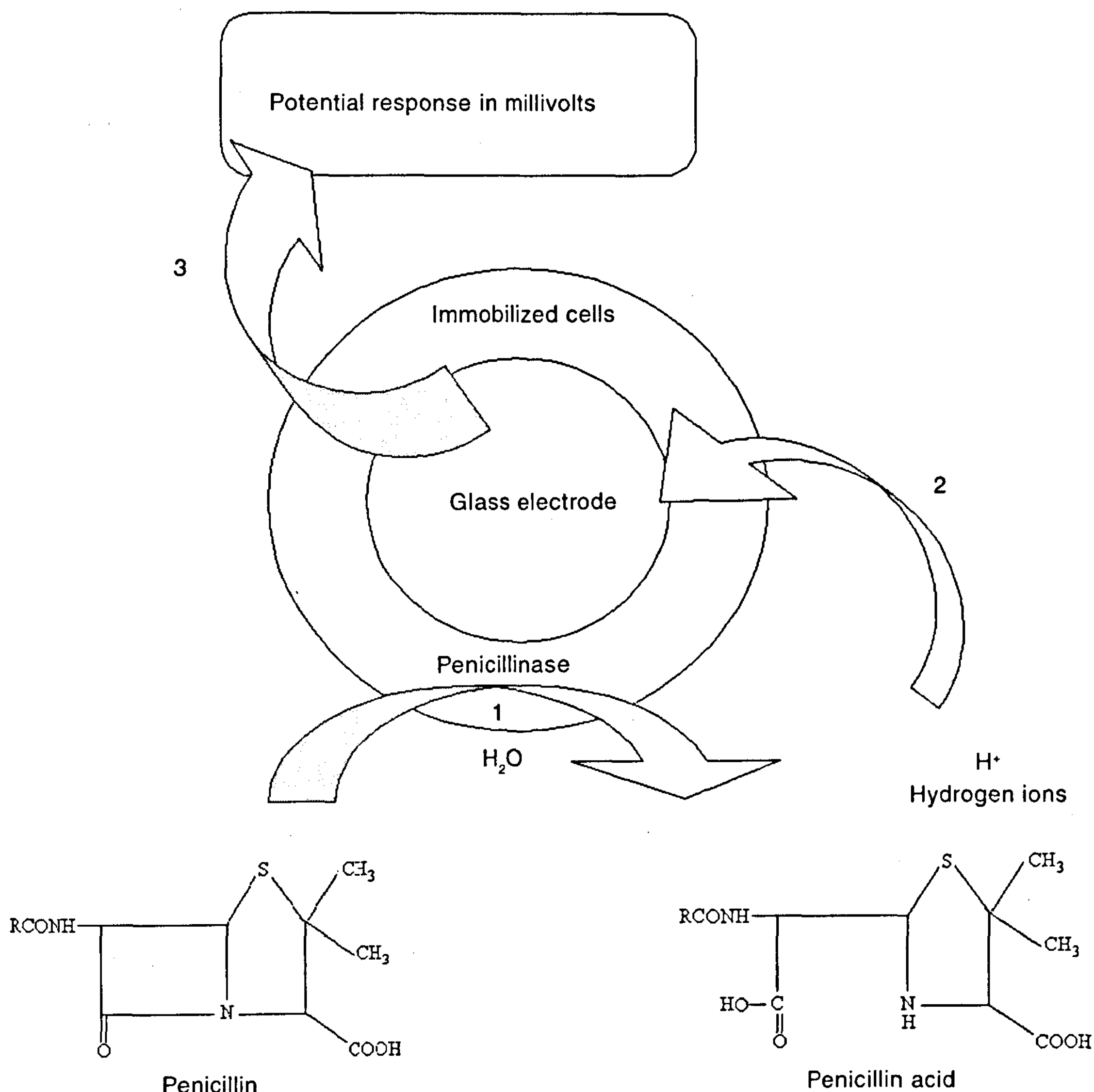

Fig. 1: Diagramatic representation of the Biosensor for penicillin.

The sequential stages of biosensor's response to penicillin in following steps: Step 1- the enzyme penicillinase acts on penicillin to produce penicilloic acid and H^+ ions; Step 2- the detection of H^+ ions by pH electrode and in Step 3- the dispay of potential response of electrode in millivolts.

potential of the electrode can be related to the concentration of penicillin directly.

$E = E° + 2.3\ RT/F \log a_H+$....(1) and $E = E° + 2.3\ RT/F \log$ [penicillin].....(2).

Equation 2 predicts a slope of 59 mV per decade change in penicillin concentration when log [penicillin] is plotted vs E. However, it has been determined that the response of the electrode is not completely Nernstian.

A slope of 51 mV of sodium benzyl penicillin and 53 mV for sodium ampicillin were obtained on a given day at ambient room temperature. Fig. 2 illustrates the type of response obtained for these penicillins; it can be observed that the electrode is analytically useful in the penicillin concentration range 10^{-1} to 10^{-4} M. Bleadel *et al.*[7] has attempted to explain in a rigorous mathematical manner, the kinetic behavior for such differences in Nernstian response. However, following conclusions can be drawn from the experi-

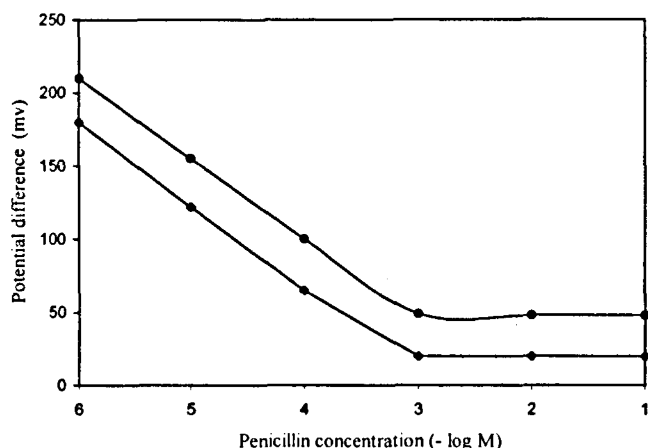

Fig. 2: Calibration curves for penicillins.

The response of Biosensor of varying concentration of sodium benzylpencillin (●) and sodium ampicillin (■) in mV.

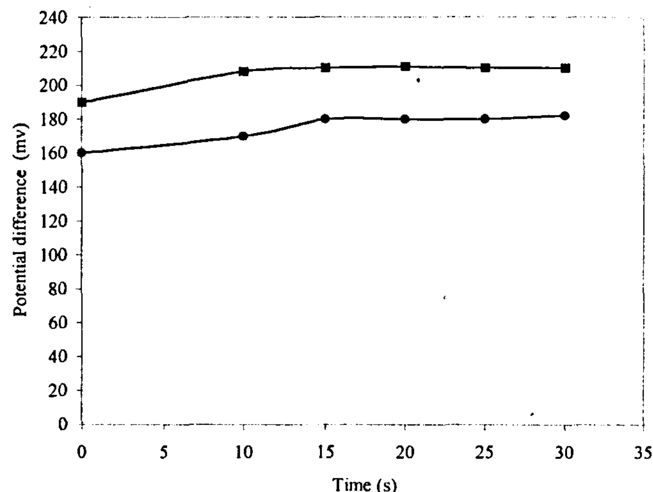

Fig. 3: Response time of Biosensor for penicillins.

The response time of Biosensor to sodium benzylpenicillin (●) and sodium ampicillin (■) in mV.

mental procedure utilized. In the fabrication of the biosensor, the thickness of the gel layer is not precisely controlled and therefore, variations in this gel layer lead to differences in slope. The age and frequency of use of the biosensor also affects the slope. The recent approaches have been targeted to tackle such problems of inadequate hydrogen ion permeation through membrane of electrode. Konchi *et al* has focused on the benefits of ionophores for increasing the sensitivity of biosensor[8]. This Biosensor exhibits a response time of 15 to 30 s. Fig. 3 illustrates the type of response obtained for two different penicillins.

This biosensor prepared has an ability to differentiate between intact drug and its degraded products due to the specificity of penicillinase. The pH of the sample is adjusted to 6.8 prior to immersing the biosensor; consequently the penicilloic acid present prior to introduction of biosensor has no effect on change in potential recorded. Since the major rout of degradation of penicillin is through the penicilloic acid, the biosensor would be useful in stability studies where partially degraded systems are measured. A linear response for penicillin from 10^{-1} to 10^{-4} M makes this biosensor competitive in terms of its specificity and sensitivity with the most widely used chemical methodologies namely iodometric titration assays. The ease of using *E. coli* is due to the fact that they grow in the very wide range of conditions and have their entire genome sequenced, which makes the biosensor reaction more predictable. This approach to design a biosensor could be applied to probably all kinds of drugs using respective vectors (plasmid/virus) for transformation.

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