Chinese Chemical Letters Vol. 10, No. 10, pp. 849–850, 1999

A new enzymatic method for determination of *E. coli*

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Abstract: β-Galactosidase, a kind of endoenzyme in *E. coli* cells, can be released by the pore-forming action of colicin E1, and *E. coli* can be detected rapidly by enzymatic method.

Keywords: *E.coli*, colicin E1, enzymatic method

In this paper, a rapid, sensitive and simple enzymatic method for detecting the *E. coli* cells has been established. β -Galactosidase is a kind of endoenzyme existing in the *E. coli* cells. It can be released from the cells by the pore-forming action of colicin E1, and can be detected rapidly by enzymatic method[1]. Colicins are plasmid-encoded proteins, bind specifically to particular receptors on the outer membrane of sensitive cells and act mainly through two pathways[2]. Because they can form pores in the membrane, colicins have been widely used as tools to study the biological and physiological role of the receptors and bioanalysis of living cells. In this paper, we studied the kinetics of the interaction between colicin E1 and membrane of the *E. coli* cells by enzymatic method. Also, the character of specific killing and channel-forming of colicin E1 is the basis of detecting *E. coli* by enzymatic method.

The principle of this experiment is that the β-galactosidase leaks from the cells of *E. coli* as colicin E1 forms channels in the membranes and reacts with its uncolored substrate o-nitrophenyl β -D-galactopyranoside (ONPG). This enzymatic reaction produces yellow o-nitrophenol and can be detected by spectrophotometry. The formula of the enzymatic reaction[1] is :

$$\textbf{ONPG (subject)} + H_2O \xrightarrow{\textbf{β-galactosidase}} \textbf{o-nitrophenol} + \textbf{D-galactose}$$

pH 7.5 is the best pH value for this reaction with very high speed of hydrolysis which is measured at 420 nm, the wave length of maximum absorbance. In parallel, at the same time of the measurement of the absorbance of experimental release, the absorbency of the spontaneous release was also measured (**Figure 1**). From **Figure 1**, we can see that compared with the absorbance of experimental release induced by colicin E1, the absorbance of the spontaneous release can be neglected. The result can be interpreted by that colicin E1 caused more β-D-galactopyranosidase to be released from *E. coli* cells. According to reference[3], we can infer that colicin E1 formed channels in the membrane of *E. coli* cells. Also, the absorbance result of the time course shows that the absorbance had a great increase within 30 minutes. It means that the interaction of colicin E1 with *E. coli* cells proceed in several steps. This result can be interpreted by the model in references 2 and 3. Because the reaction of colicin E1 with *E. coli* cells is that of receptor with ligand, one *E. coli* cell can only accept a certain amount of colicin E1. So under enough concentration of colicin E1, there exists a linear relationship between the absorbance caused by ONPG and the concentration of *E. coli* cells, which can be used to determine *E. coli* quantitatively (**Figure 2**). But the linear relationship does not exist in a large interval of concentration of cells. For example, from 10^5 ml^{-1} to 10^7 ml^{-1} of *E. coli* the divergence happened; from 10^5 ml^{-1} to $10^6 ml^{-1}$, the linear relationship was excellent.

We found that colicin E1 should surpass a certain valve value, for example, 1 μg ml^{-1} colicin E1 for 10^5 ml^{-1} to $10^6 ml^{-1}$ of the cells.

Figure 1. Kinetic curve of interaction of colicin E1 with E. coli characterized by enzymatic reaction at pH 6.5 [ONPG]=1.0 mg ml^{-1}. 1: spontaneous release; 2:

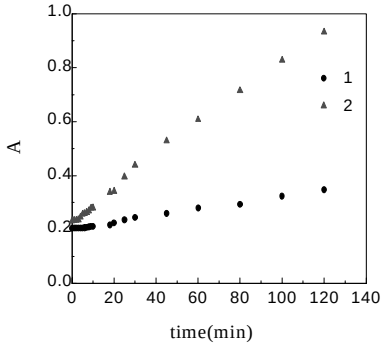
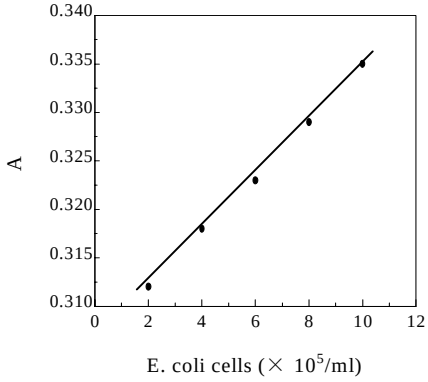


Figure 2. Detecting the quantity of E. coli cells, pH 6.5 [ONPG]=1.0 mg ml^{-1} experimental release;



Colicin E1 characterizes by its specifically killing *E. coli* cells. So the enzymatic reaction induced by colicin E1 can be used to detect *E. coli.* Traditionally, the detection of *E. coli* in environment or in food was by the colony counting method which was time-consuming. Apart from the inducement of β-D-galactopyranosidase by galactoside, the enzymatic method only needs half an hour. However, this method still needs to be improved to get high sensitivity. For example, the amplification of the enzymatic reaction should be made in order to produce obvious response of color rapidly and the inducement by galactoside is not needed. Furthermore, an artificial membrane bound or adsorbed by the colicin E1 and the substrate of β-D-galactopyranosidase attaches on food contaminated by *E. coli*, then the enzymatic reaction indicates the existence of *E. coli* because of the release of β-D-galactopyranosidase.

Acknowledgment

This work was supported by the National Natural Science Foundation of China.

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Received 24 April 1998