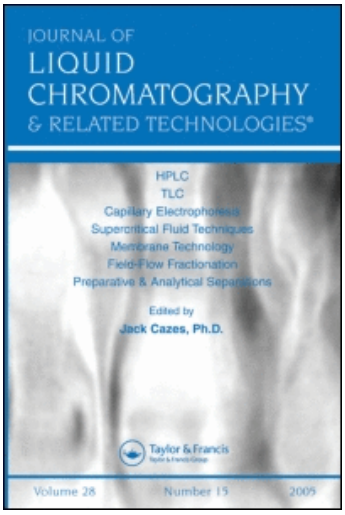

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Presumptive Identification of the Bacterium Escherichia Coli by High Performance Liquid Chromatography

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PRESUMPTIVE IDENTIFICATION OF THE BACTERIUM ESCHERICHIA COLI BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The potential of high performance liquid chromatography (HPLC) for bacterial identification has been explored employing Escherichia coli (E. coli) as an example.

E. coli produced a distinct pattern of changes in the media, different from several other organisms. These changes are time dependent and inversely related to media concentration and can be detected after one hour incubation. Some of these changes have been subjected to chemical analysis and it was found that E. coli rapidly degraded tryptophan and produced indole. The ratio of tryptophan to phenylalanine in the media served as a good index for bacterial growth. Different isolates of E. coli showed the same metabolic pattern. Although other bacteria are capable of degrading tryptophan, they produced a different pattern.

HPLC appears to have a potential for rapidly identifying bacterial isolates and may provide a useful tool for use in microbiology laboratories.

INTRODUCTION

The gas chromatograph equipped with an electron capture has been shown to be a sensitive tool for bacterial identification

(1,2). However, the instrument is limited to analysis of compounds which can be rendered volatile directly or indirectly by derivitization. In addition to that, the instrument does not lend itself easily to the routine lab.

On the other hand, the high performance liquid chromatograph (HPLC) is more suited for separation of non-volatile compounds and for application in the routine lab. In most instances, time-consuming steps such as extraction and derivitization, are not necessary. The instrument has not been applied for bacterial identification.

In this work we will illustrate the potential of the HPLC for rapid and simple bacterial identification employing E. coli as an example based on the disappearance of a certain characteristic compound in the media.

MATERIALS AND METHODS

E. coli and other organisms were grown for 1-4 hours on peptone broth media at 37°C. At the end of the incubation time, the bacteria were removed by filtration and an aliquot of 10-20 µl of the filtered media was injected directly on the liquid chromatograph.

The instrument consisted of an Altex model 110 A pump (Altex Scientific, Inc. 1780 Fourth St., Berkeley, CA 94710) adjusted to deliver the solvent at 1.5 ml/min through a C18 µ-

Bondapak column (Waters Associates, Milford, Mass. 01757). Column effluent was monitored with micromeritics variable wavelength detector model 785 (Micromeritics Instrument Corporation, 5680 Goshen Springs Rd., Norcross, GA 30093). The samples were introduced through Model U6K injector (Waters Associate). The signal was recorded on a recorder model A 25 (Varian Aerograph, 2700 Mitchell Dr., Walnut Creek, CA 94598).

RESULTS AND DISCUSSION

The culture media such as peptone and tryptic soya digest is composed chiefly of water soluble molecules such as amino acids, peptides and nucleotides most of which are quite suitable for analysis by the HPLC. Figure 1 illustrates that the peptone culture media contain few compounds which are separable by the HPLC. When E. coli is grown for a short period of time (1-4 hours) on this media some peaks decrease or completely disappear,in particular compound T, while other compounds such as compound P do not change. The more the media is dilute, the lower is T/P ratio (Fig. 2.) This observation is analogous to the enzymatic assay of substrates, where the substrate has to be present in a very low concentration relative to the enzyme. During this short period of incubation, bacterial growth is at minimum and the changes which are seen are probably mainly due to the activities of the present enzymes in the

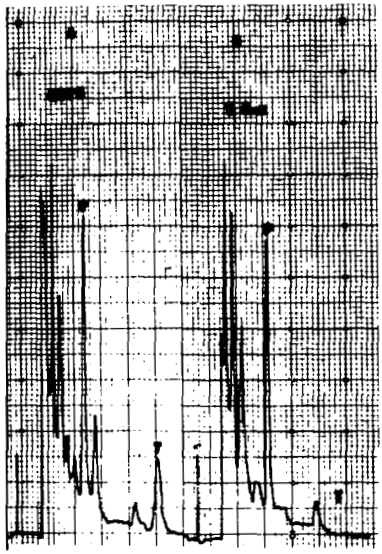

Figure 1. A. Chromatograph of peptone media before E. coli growth. Solvent 10% methanol in phosphate buffer 0.01 mole/liter, pH 2.6. Detector wavelength 200 nm. (The time from injection to peak T is 10 min).

B. Chromatograph of peptone media after E. coli growth for 2 hours. Conditions are the same as in A.

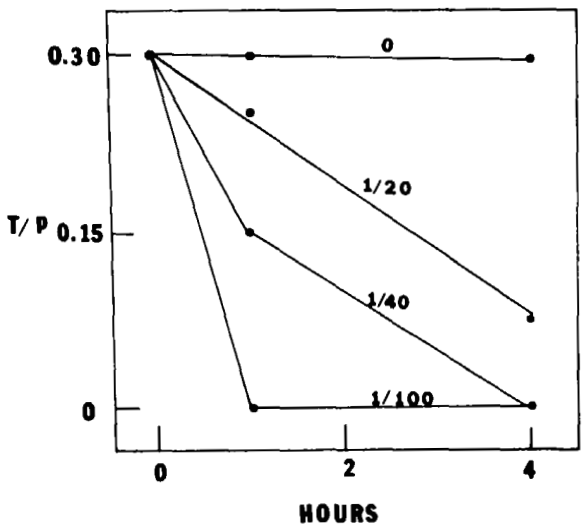

Figure 2. The ratio of Tryptophan (T) to phenylalanine (P) after E. coli growth on peptone media diluted 1/20, 1/40 or 1/100 with water at 0, 1, and 4 hours of incubation.

inoculum. A 1/50 dilution of the commercial peptone media was found most suitable for subsequent experiments.

The disappearance of compound T was accompanied by an increase of a lesser polar compound which required a 50% methanol for elution (compound I) (Fig. 3). Compounds T, I, and P were characterized from their chromatographic behaviour

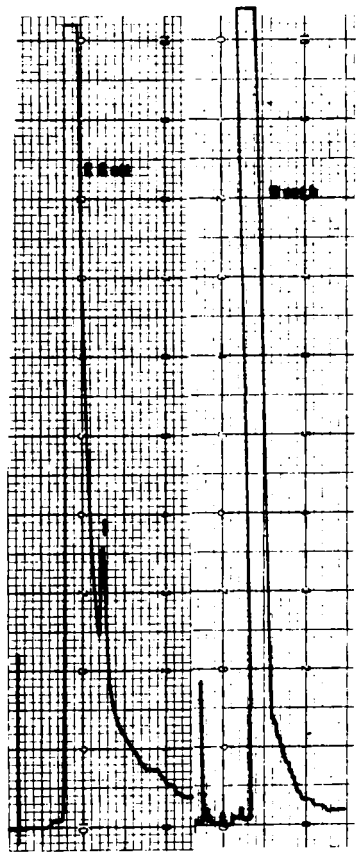

Figure 3. Indole (I) production after E. coli growth on peptone broth;and before growth (Broth).

as Tryptophan, Indole, and Phenylalanine, respectively. This was further confirmed by incubating E. coli in pure tryptophan and monitoring the indole production (Fig. 4) and by reacting the indole with Ehrlich's reagent. This is also consistent with the known metabolic pathways of this organism. Six different isolates of

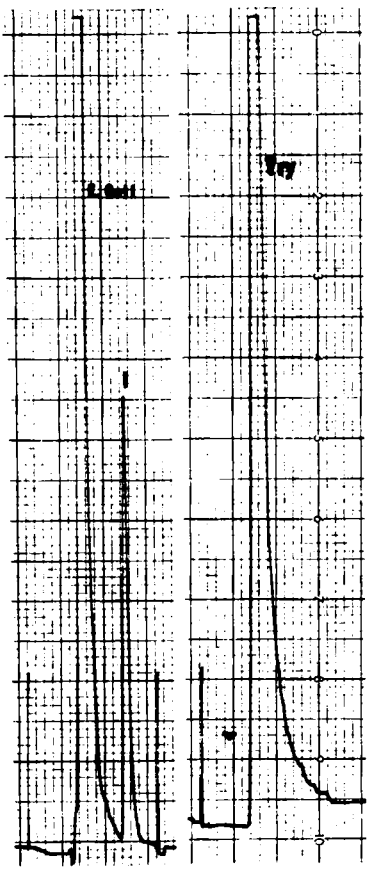

Figure 4. Indole (I) production after E. coli growth on tryptophan; and before growth(Try). Solvent was 50% methanol in phosphate buffer 0.01 mole/liter, pH 2.6. Detector wavelength was 214 nm.

E. coli consistently showed this pattern, indicating the reproducibility of this system.

Other indole-producing organisms did decrease tryptophan to some extent similar to E. coli (Fig. 1). However these organisms were different from E. coli by producing changes in the more polar compounds which required only phosphate buffer for elution (Fig. 5a and b).

The effect of the initial number of organisms in the inoculum on the disappearance of tryptophan from the media after 2 hours of incubation of E. coli is illustrated in Figure 6.

These preliminary experiments demonstrate the feasibility and the potential of the high performance liquid chromatograph for bacterial identification. Most of the work with bacterial identification by the gas chromatograph has been aimed at studying the appearance of certain characteristic volatile acids and alcohols. On the other hand, this work with the HPLC is aimed at measuring the disappearance of key compounds from the media in addition to monitoring the appearance of new products. This system has the advantages of simplicity, the avoidance of extraction or derivitization, and great speed.

Although a commercial media has been used for this work, the advantages of a synthetic media designed for the HPLC is

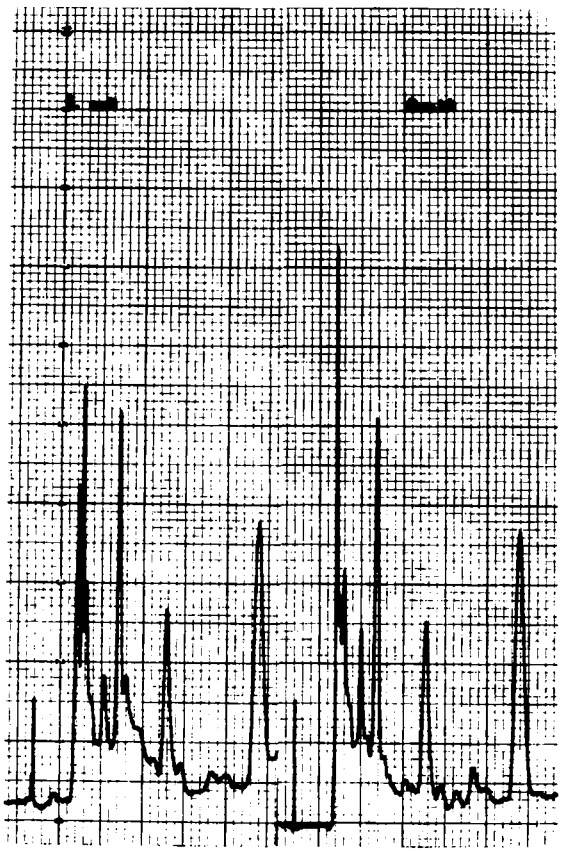

Figure 5. A. Separation of peptone broth before and after E. coli growth for 2 hours. Solvent was 0.01 mole/liter phosphate buffer, pH 2.6. Detector wavelength was 195 nm.

quite obvious and is under development. Many other factors which affect this system, such as pH, temperature of the incubation, the effect of mixed organisms, are under study. The

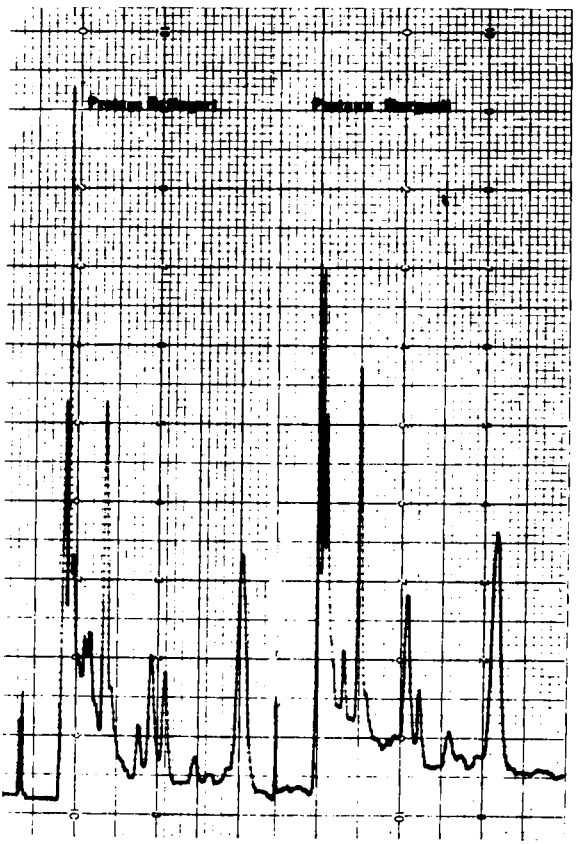

Figure 5. B. Separation of peptone broth after Proteus rettgeri and Proteus morganii growth for 2 hours. Conditions are the same as in A.

derivitization of the media with fluorescent reagents expands the potential of this method further.

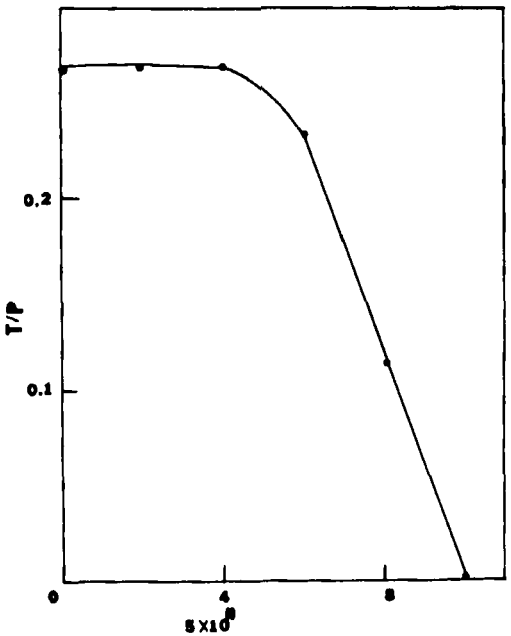

Figure 6. The effect of number of E. coli in the inoculum on the ratio of Tryptophan to phenylalanine (T/P).

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