VARIATION OF ALKALINE PHOSPHATASE ISOENZYMES IN *ESCHERICHIA COLI* AND *SERRATIA MARCESCENS*

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1. Introduction

Nutrients of growth medium have marked effects on the synthesis of certain enzymes. Glucose, a powerful repressor of bacterial enzymes [1], has no effect on *Escherichia coli* alkaline phosphatase [2]. Glycerol and glucose induced the synthesis of alkaline phosphatase in *Bacillus subtilis* while lactate suppressed its biosynthesis [3]. Lowest level of acid hexose phosphatase activity was observed in *E. coli* with glucose as a carbon source and highest levels resulted when the basal medium was supplemented with casamino acids [4]. *Salmonella typhimurium* gave high yields of alkaline phosphatase in lactate medium and low yields in glucose medium [5]. Variation in the relative yields of isoenzymes of *E. coli* alkaline phosphatase has also been associated with peptone concentration [6]. Similarly inorganic phosphate concentration has been found to have a marked effect on the synthesis of phosphatase in several organisms, *E. coli* [2, 7, 8]; *B. subtilis* [9]; *Neurospora crassa* [10]; *Aspergillus* [11, 12]; *Saccharomyces cerevisiae* [13] and *Pseudomonas fluorescence* [14].

It was found that certain strains of *Serratia marcescens* synthesize two electrophoretically distinct alkaline phosphatases and the synthesis of one of them was suppressed by high concentration of phosphate in the growth medium [15]. It is the purpose of this paper to report the effect of different nutrients in the growth medium with particular reference to the variation of isoenzyme patterns observed in *S. marcescens* and *E. coli*. This should be of significance in the purification and characterization of individual isoenzymes.

2. Experimental

Strain 211 of *S. marcescens* is a white spontaneous mutant from red strain 210. It was isolated in connection with pigmentation studies in the Department of Biochemistry carried out by Professor J.L. Still and his colleagues. A number of low phosphate media described by other workers were used as such. They have respective carbon and nitrogen sources as follow: Torriani's medium [2] contained citrate and lactate as carbon and $(NH_4)_2SO_4$ as nitrogen source; Brockman and Heppel's medium [16] contained glycerol as carbon while peptone and NH_4Cl were used as nitrogen sources; Sayer's medium [17] contained citric acid and glucose as carbon sources while NH_4Cl was the nitrogen source; Garen and Levinthal's medium [8] contained glucose as carbon whereas peptone and NH_4Cl provided nitrogen sources.

The strain 211 was cultivated in a series of experiments in 200 ml of each media in conical flasks at 30° on a rotary shaker. After about 18–20 hr of growth cultures were harvested separately and were washed with Tris-Mg buffer, pH 8.0 (10 mM Tris, 5 mM $MgSO_4$, pH was adjusted to 8.0 with HCl). Enzyme from the cells was extracted by butanol treatment [18]. Enzyme activity of cells and butanol extracts were determined as described elsewhere [15]. The starch gel electrophoresis was carried out in discontinuous borate buffer, pH 8.6 [19]. The gel was stained with calcium-α-naphthyl phosphate and Azoene Fast Violet B salt [20].

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3. Results and discussion

Strain 211 cultivated in five different growth media gave enzymatically active cells in all cases except in Sayer's medium [17]. The extracts from these cells, when analysed by starch gel electrophoresis gave variation in the isoenzyme pattern. The results are presented in fig. 1. Sample A shows three coli type isoenzymes. In this case cells were grown in the presence of glycerol as a carbon source [16]. Sample B represents four isoenzymes, one distinctly fast moving anodic isoenzyme and three slow moving (similar to sample A) coli type isoenzymes. In this case strain 211 was grown in the presence of lactate as a carbon source [2]. Sample D also gave four isoenzymes roughly similar to sample B, but due to low enzyme activity in the extract, isoenzymes are not clearly seen. Sample E showed only the fast moving isoenzyme. In this case strain 211 was grown in the high phosphate medium. It is further interesting to point out that there existed a marked variation among the three coli-type isoenzymes. If these isoenzymes are numbered 1, 2 and 3 in order of their decreasing mobility towards the anode, isoenzyme 3, (fig. 1, sample A) is relatively stronger in intensity than isoenzymes 2 and 1, respectively. On the other hand, predominance of isoenzyme 1 over isoenzymes 2 and 3 is clearly demonstrated in sample B (fig. 1). The predominance of isoenzyme 3 over other isoenzymes was consistently observed when peptone was present in the growth medium and vice versa in the absence of peptone. Similar type of variation in the three isoenzymes have been observed for *E. coli* (fig. 2). Our results showing the effect of peptone on the predominance of the electrophoretically slow moving isoenzyme alkaline phosphatase from *S. marcescens* and *E. coli* are consistent with earlier reports on *E. coli* isoenzymes [6, 21, 22]. It was observed in the earlier experiments, when cultures free of peptone were allowed to grow a long time, i.e. into stationary growth phase, extracts from these cells gave the same isoenzyme pattern as reported in the presence

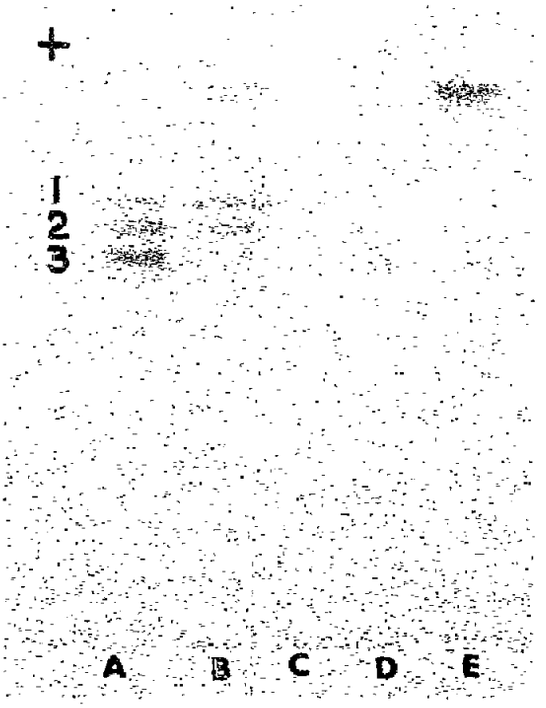

Fig. 1. A slice of starch gel after electrophoresis and stained for alkaline phosphatase activity. Samples A–D represent the isoenzyme patterns obtained from the extracts of *S. marcescens* strain 211 grown in four different low phosphate media [16, 2, 17 and 8]. Sample E represents the extracts from the cells cultivated in high phosphate media [2].

Fig. 2. A slice of starch gel stained for alkaline phosphatase activity after electrophoresis shows the variation of isoenzyme patterns from the extracts of butanol treated *E. coli* grown in low phosphate medium [16], which contain peptone as nitrogen source (sample A), and in low phosphate medium containing NH_4Cl as a nitrogen source [2] (sample B).

of peptone. Similar results were obtained from the extracts, when the exponentially growing cultures were allowed to sit in the cold room for a few days before harvesting. This phenomenon seems related to the presence of organic nitrogen in the growth medium and is independent of carbon source. In agreement with earlier reports on *E. coli* [2, 16] and *B. subtilis* [3], we have also found that glycerol and glucose do not affect the synthesis of alkaline phosphatase in *E. coli*. Unlike the *E. coli* enzyme [2] glucose seems to have an inhibitory effect on the synthesis of alkaline phosphatase in strain 211 of *S. marcescens* (fig. 1, sample C and D). Glycerol suppressed the biosynthesis of the fast isoenzyme but seems to have no such effect on the coli-type isoenzymes (fig. 1, sample A). Similarly Dovark et al. [4] have reported that acid phosphatase and acid hexose phosphatase of *E. coli* are subject to catabolic repression and their enzyme activities are significantly reduced by glycerol and glucose in the growth medium.

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