Research Article Screening of Alkaline phosphatase activity in Bacteria

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ABSTRACT

The study was conducted in the pathological analysis department during the period from 13th. NOV. To 30th. MAR 2017. Specimens used were different species of bacterium, by applying ALP activity determination in the broth of the bacterium species. The study reflects a higher total activity of ALP in ***Proteus*** *species than the other species. While, specific activity was seen in* ***E.Coli****.*

Keywords: *ALP activity, Bacterium species, colorimetric Assay.*

I. INTRODUCTION

Microorganisms excrete a wide variety of proteolytic enzymes, which are also found in mammalian systems. They are molecules of relatively small size and are compact, spherical structures that catalyze the peptide bond cleavage in proteins They hydrolyze peptide bonds and therefore, lead to the disassembly of proteins. Commercially they are very important as more than 60% of the total enzyme market relies on proteases and phosphatases, isolated from plants, animals, bacteria and fungi. Proteases and phosphatases are (physiologically) necessary for living organisms; they are ubiquitous, found in a wide diversity of sources.

Alkaline phosphatase (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment; it is sometimes used synonymously as **basic phosphatase**.

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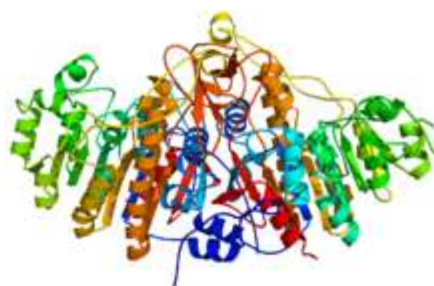

N-terminus = blue, C-terminus = red) of the dimeric structure of bacterial alkaline phosphatase.

Fig – 1: Three dimensional structure of ALP protein.

Alkaline phosphatase is elevated in undifferentiated pluripotent stem cells,[3] and the primordial germ cells which produce spermatozoa and oocytes.

In Gram-negative bacteria, alkaline phosphatase is located in the periplasmic space, external to the cell membrane. Since this space is much more subject to environmental variation than the actual interior of the cell, bacterial alkaline phosphatase is resistant to inactivation, denaturation, and degradation, and contains a higher rate of activity. The permeable outer membrane have porins which form channels that allow phosphorylated compounds to enter the cell. Although the purpose of the enzyme is not fully resolved, the simple hypothesis is that it serves to cleave phosphate groups from phosphorylated compounds facilitating transport across membranes and providing the cell with a source of inorganic phosphate at times of phosphate starvation. The main purpose of dephosphorylation by alkaline phosphatase is to increase the rate of diffusion of the molecules into the cells and inhibit them from diffusing out. However, other possibilities exist. For instance, the presence of phosphate groups usually prevents organic molecules from passing through the membrane; therefore, dephosphorylating them may be important for bacterial uptake of organic compounds.

Alkaline phosphatase is a zinc-containing dimeric enzyme with the MW: 86,000 Da, each subunit containing 429 amino acids with four cysteine residues linking the two subunits. Alkaline phosphatase contains four Zn ions and two Mg ions, with Zn occupying active sites A and B, and Mg occupying site C, so the fully active native alkaline phosphatase is referred to as $(Zn_AZn_BMg_C)_2$ enzyme. The mechanism of action of alkaline phosphatase involves the geometric coordination of the substrate between the Zn ions in the active sites, whereas the Mg site doesn't appear to be close enough to directly partake in the hydrolysis mechanism, however, it may contribute to the shape of the electrostatic potential around the active center.

Alkaline phosphatase is heat stable and its function is to remove phosphate groups from phosphorylated compounds facilitating transport across membranes and providing the cell with a source of inorganic phosphate. Alkaline phosphatase in *E. coli* is located in the periplasmic space and can thus be released using techniques that weaken the cell wall and release the protein. Due to the location of the enzyme, and the protein layout of the enzyme the enzyme is in solution with a small amount of proteins than there are in another portion of the cell. Some complexities of

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bacterial regulation and metabolism suggest that other, more subtle, purposes for the enzyme may also play a role for the cell. In the laboratory, however, mutant *Escherichia coli* lacking alkaline phosphatase survive quite well, as do mutants unable to shut off alkaline phosphatase production.

The optimal pH for the activity of the *E. coli* enzyme is 8.0 while the bovine enzyme optimum pH is slightly higher at 8.5

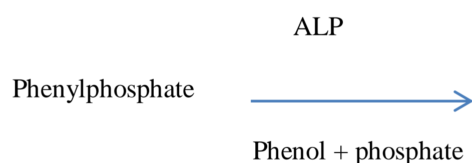
II. MATERIAL AND METHODS

Determination of ALP Activity:

A colorimetric determination of ALP activity was proved using a BioMerieux Kit

Principle:

Colorimetric determination of ALP activity according to the following reaction:



The liberated phenol is measured in the presence of 4 – aminoantipyrine and potassium ferricyanide.

The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Reagent used:

Reagent used were described in the below table:

R1 (substrate buffer)	Disodium phenylphosphate 5mmol/L Carbonate-bicarbonate buffer pH 10 50mmol/L Sodium merthiolate 0.1 g/L
R2 (Standerd)	Phenol equal to 20 King U
R3 (bloking reagent)	4-aminoantipyrine 60 mmol/L Sodium arsenate 75 g/L
R4 (color reagent	Potassium ferricyanide 150mmolL

Specimens:

Different species of Bacterial isolates were used. These were as follows:

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Klibsella

E.Coli

Proteus

Pseudomonas

Salmonilla

S.aureus

Procedure:

The bellow table of addition was used:

	Broth sample	Blank	Standerd	Reagent blank
R1	2 ml	2 ml	2 ml	2 ml
Incubate for 5 min at 37 C				
R2	50 µl		50 µl	
Incubate for exactly 15 min at 37C				
R3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably votrex				
R4 Broth H_2O	0.5 ml	0.5 ml 50 µl	0.5 ml	0.5 ml 50 µl
Mix let stand for 10 min in the dark then measure.				

The color intensity is stable for 45 min.

$$\text{Calculation of ALP activity} = \frac{\text{OD sample - OD blank}}{\text{OD standerd}} \times n$$

King U/100 ml: n = 20 [one king unit is the amount of enzyme which,

In the given conditions, liberate 1 mg of phenol

In 15 min at 37 C]

U/l : n = 142

III. RESULTS

Activities of ALP enzyme were determined in different types of bacteria species on using a colorimetric assay protocol. The data obtained were reflected variation in ALP activity depending type of the bacteria species. These activities of ALP were measured in the extracted broth of the different species of bacteria. Table (1) represents the values of the ALP activities determined.

Table-1: ALP activities obtained from broth of different bacteria species

Bacteria Species	Absorbance	ALP activity U/L
Klibsella	0.088	27
E.Coli	0.092	28
Proteus	0.153	47
Pseudomonas	0.063	19
Salmonilla	0.084	25
S.aureus	0.052	15

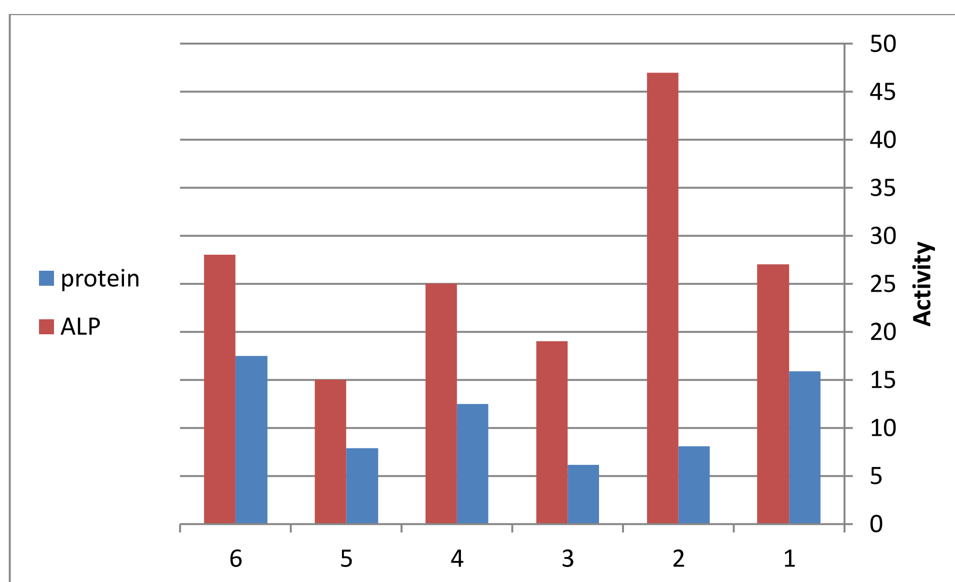
Table (2), represents the total amount of proteins and the specific activities of ALP enzymes obtained.

Table – 2: Specific activities of ALP found in bacterial protein.

Bacteria Species	Total protein g/dl	Specific Activity U/mg
Klibsella	1.7	15.9
E.Coli	1.6	17.5
Proteus	5.8	8.1
Pseudomonas	3.1	6.13
Salmonilla	2.0	12.5

S.aureus	1.9	7.9

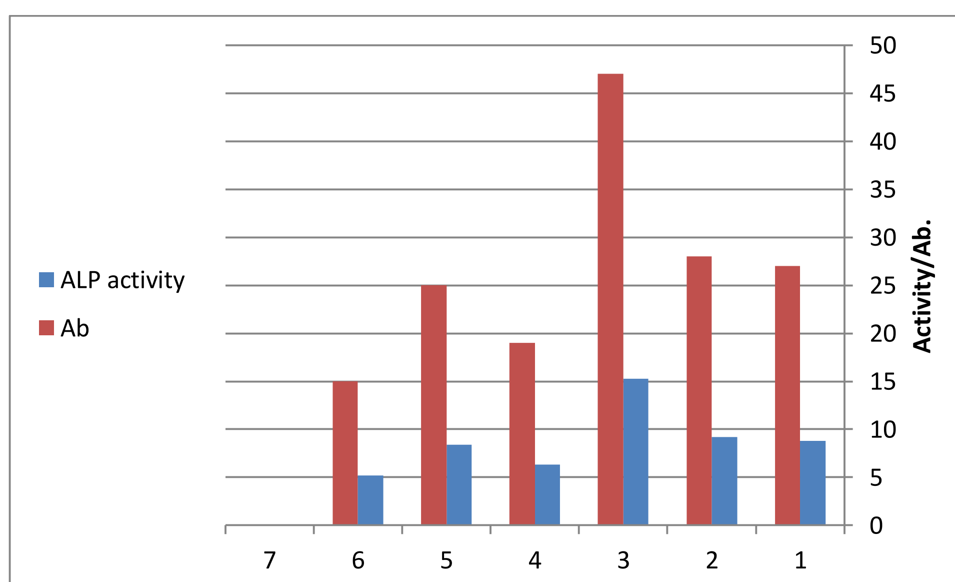
Figure (2), reflects the amount of ALP activities found in according to the total proteins obtained.



E.coli S.aureus Salmonilla Pseudomonas Proteus Klibsella

Fig – 2 : ALP activities determined in total proteins.

Figure (3), represent the specific activities of ALP in comparison with their total activities.



E.coli S.aureus Salmonilla Pseudomonas Proteus Klibsella

Fig – 3 : Comparison of ALP specific activity with total activity in Different bacteria species.

IV. DISCUSSION

Microbial alkaline phosphatases are produced on large scale in an economical way within limited space and in short time. Alkaline phosphatases are crucial in phosphate metabolism. The enzyme with its wide specificity and activity is potential in bioprocessing. An emphasis is placed on the diverse applications of microbial alkaline phosphatases.

Most of the microbial alkaline phosphatases of significant application in diagnostic studies are obtained from bacteria. Alkaline phosphatase based biosensors play an essential role in environmental monitoring. Microbial alkaline phosphatases have a major application as biofertilizer. They are also useful for the evaluation of the soil quality and the perturbation occurring in agricultural fields. The assessment of alkaline phosphatase activity is used as a marker for milk pasteurization in dairy industries. Alkaline phosphatase is an important biochemical tool in limnological studies. A yelowish rose color developed on the bacterial growth when alkaline phosphatase was produced by the bacteria; subsequently, the entire solution became rosee. The principle of the reaction is that alkaline phosphatase hydrolyzes the substrate, and highly soluble color is formed at the site of the enzyme . All bacteria species LP tests were give the same color.

Other organisms that produced a positive result were Escherichia, Klebsiella, Proteus, and some other enterobacteria; the yellowish -rose color developed within 15 min, Pseudomonas, did also produce alkaline phosphatase .

This method (involving phoshoantipyrine as a substrate for detecting bacterial alkaline phosphatase formation, which is simple, rapid, and direct.

Use of this procedure to detect alkaline phosphatase activity, to differentiate between different bacterial species. All species were reflect spechfic activity, and E.Colo species was the highest one. The Proteus species resulted in a highest total activity compared with the other species.

V. CONCLUSSION

It seems that as ALP activity was as known to be a marker enzyme released from bone marrow, but the investigation protocol was reflect an obvious secretion of the enzyme in different bacterial species.

VI. AKNOWLEDGMENT

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