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ORIGINAL ARTICLE

An *in silico* structural, functional and phylogenetic analysis with three dimensional protein modeling of alkaline phosphatase enzyme of *Pseudomonas aeruginosa*

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Abstract Phosphorus is a primary macronutrient required for normal plant health, metabolism and survival. It is present in soil in compound insoluble form for which plant cannot uptake it directly from the soil. Some phosphate solubilizing bacteria possess some important enzymes for phosphate solubilization as well as mineralization. Alkaline (or basic) phosphatase (EC 3.1.3.1) is a type of zinc containing dimeric hydrolase enzyme responsible for removing the phosphate groups from various kinds of molecules including nucleotides, proteins, and alkaloids. Unlike acid phosphatases alkaline phosphatases are most effective in an alkaline environment. Alkaline phosphatases (ALPs) are of immense importance in various agricultural industries including dairy industries for testing successful pasteurization process. In this present study, *Pseudomonas aeruginosa* phosphatase was selected for a detailed computational investigation to exploit its physicochemical characteristics, structural properties including 3D model, model quality analysis, phylogenetic assessment and functional analysis using a number of available standard bioinformatics tools. The protein having average molecular weight about 51 kDa, was found thermostable and alkaline in nature belonging to metalloenzyme superfamily. Specifically, the thermostable behavior of the protein is suitable for the dairy industry. Moreover, this theoretical overview will help researchers to get an idea about the predicted protein structure and it may also help to design genetically engineered phosphate solubilizing bacteria by designing specific primers.

Abbreviations ALPs, Alkaline Phosphatases; PSB, Phosphate Solubilizing Bacteria

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1. Introduction

Different bacterial genera are involved in various activities to maintain the soil ecosystem. Phosphorus is a primary plant macronutrient which plays an important role in various plant metabolisms, growth and development. It is not only very important element for functioning as the key enzyme to regulate the different metabolic pathways but also it is the main important structural constituent of nucleic acid. It acts as a main constituent of membrane phospholipid and takes a role in membrane development and its function. It is one of the components of ATP, ADP, and AMP. ATP plays an important role in the cell in all energy requiring process and in metabolic pathways. In addition, phosphorus plays an important role in photosynthesis, respiration, energy storage and transfer, cell division, and cell enlargement. It helps plants to survive winter rigors as well as contributes to disease resistance in few plants [1–3].

A large amount of inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants [4] as plants can only absorb phosphate in soluble form. The transformation of inorganic phosphate into soluble form is carried out by soil microorganisms. Therefore, phosphate solubilizing bacteria (PSB) have crucial contributions in phosphate cycle, as it helps in both phosphate solubilization and mineralization [5]. One of the phosphate solubilizing enzymes is phosphatase which has been identified in many bacteria such as *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Aspergillus*, and *Penicillium* [1,6]. Phosphatase is an enzyme that removes a phosphate group from its substrate. It is a large group of protein which is present in bacteria, archaea, and eukarya. Mainly two classes of phosphatases are found i.e. acid and alkaline phosphatase. Alkaline phosphatase (Orthophosphoric monoester phosphohydrolase, E.C.3.1.3.1) is one type of hydrolase enzyme. It is a metalloenzyme, the active site of which contains two Zn^{2+} ions and one Mg^{2+} ion per monomer. It plays an important role in phosphate metabolism. The applications of microbial alkaline phosphatase in diverse areas such as immunology, molecular biology dairy technology and diagnostics have been well documented [7–10]. It also plays an essential role in environmental monitoring and it is an important biochemical tool in limnological studies. This enzyme is also very useful for the evaluation of the soil quality and the perturbation occurring in agricultural fields. The demand for enzymes is increasing globally at an Average Annual Growth Rate (AAGR) of 6.3 percent, reaching a value of nearly $7 billion by 2017 [11]. Alkaline phosphatase is having biggest share of $20 of $100 million in the world enzyme market.

Considering the above diverse application of microbial alkaline phosphatase particularly importance in agricultural field the present study is undertaken for its *in silico* analysis. *Pseudomonas aeruginosa* is one of the potent phosphate-solubilizing bacteria and used in agricultural field for better uptake of phosphate. It is a gram negative, rod shaped, monoflagellated bacterium used for phosphate solubilization and mineralization in agricultural soil. Complexity of its genome indicates an evolutionary adaptation permitting it to thrive in diverse environments. So, these bacteria have wide application in diversified soil of agricultural field.

In silico analysis of genes and proteins has been receiving greater attention with particular emphasis to find suitable biomarkers for rapid identification of different pathogenic genera [12,13], designing of drugs to combat the pathogenic microbes and superbugs [14,15], diagnosis of infectious diseases [16] and discovery of potent microbial representative useful for several agricultural and animal feed industries [17–19].

In the present study phosphatase enzyme (alkaline phosphatase) and gene sequences of *Pseudomonas aeruginosa* PAO1 were used for *in silico* analysis. Attempts were also made to study the physicochemical properties, predict secondary structure, modeling the 3-D protein, evaluate and analyze the alkaline phosphatase of the strain using different standard computational tools to help researchers get more acquainted with the protein structure.

2. Materials and methods

2.1. Retrieval of the sequences

The amino acid sequences of alkaline phosphatase and respective cDNA sequences of those amino acid sequences of 20 different strains of *P. aeruginosa* were retrieved from NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov) in FASTA format for computational analysis.

2.2. Phylogenetic tree construction

Phylogenetic tree is a branching diagram which helps to understand the evolutionary relationship among the biological species. Here MEGA6 [20–23] software was used to construct the phylogenetic tree. A total of two phylogenetic trees were constructed. One phylogenetic tree of amino acid sequences of alkaline phosphatase of different strains of *P. aeruginosa*. Another one constituted the cDNA of the protein of different strains of *P. aeruginosa* [20–23].

2.3. Primary sequence analysis

Any amino acid sequence contains a message which comes from transcription and translation of a gene. In addition, the amino acid sequence bears various important information such as amino acid composition, physiochemical properties such as isoelectric point (pI), molecular weight (Mw), extinction coefficient (EC – quantitative study of protein – protein and protein – ligand interactions), instability index (II – stability of proteins), aliphatic index (AI – relative volume of protein occupied by aliphatic side chains), and Grand Average of Hydropathicities (GRAVY – sum of all hydropathicity values of all amino acids divided by number of residues in a sequence). The physiochemical properties of amino acid sequences of alkaline phosphatase of different strains of *P. aeruginosa* were analyzed by Expasy protparam tool (http://web.expasy.org/protparam) [24]. Then the amino acid composition of protein of different strains of *P. aeruginosa* was graphically plotted and analyzed.

2.4. Secondary structure prediction

The secondary structure is related with protein folding. So, the helix, sheet, and turn of amino acid sequences of different