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Acid-Phosphorus Activity of Wheat Varieties Rhizobacteria of Uzbekistan

Zair Saatovich Shakirov[1], Iskandar Takhirovich Yakubov[2], Karomat Suvanqulovna Mamanazarova[1,3*], Mardanov Ikrom Xasan Ugli[1], Xayrullaev Abdumalik[1]

[1]Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan
[2]Institute of Botany, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan
[3]Institute of Botany, National University of Uzbekistan Named after Mirzo Ulugbek, Tashkent, Uzbekistan
Email: *karomat.3005@mail.ru

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Abstract

In this work, local strains of phosphate-solubilizing microorganisms were isolated and identified from the wheat rhizosphere and exogenous acid phosphatase enzymes of locally active phosphate- and potassium-mobilizing rhizobacteria belonging to the genera *Escherichia*, *Rahnella*, *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Pantoea* were studied. The efficiency of the physiological properties of rhizobacteria is determined by the production of soluble phosphorus, and the amount of phosphorus depends on the activity and biomass of bacteria that secrete phosphorus. This is done by phosphate solubilizing bacteria, and the habitat ecosystem is enriched with beneficial micronutrients. In these studies, active rhizobacteria activity of acid phosphatase in nutrient liquid was studied at different temperatures. Optimum pH activity index and temperature variability of enzymes were determined. It should be noted that in the most active phosphate-solubilizing strains the maximum enzymatic activity was observed in the culture fluid of *R. aquatilis* strain 17, which produced 1.086 μmol p-nitrophenol μmol/min/ml. P. agglomerans 22, P. agglomerans 20 and Ps. kilonensis 32 cultures phosphatase activity was 0.143 - 0.680 p-nitrophenol μmol/min/ml. It should be noted that the phosphatase activity of bacteria belonging to the same genus and species was very different from each other. That is, the enzyme activity of *Rahnella aquatilis* strain 17 was 9 times higher than the enzyme activity of *Rahnella aquatilis* strain 9. The pH optimum of sour phosphatase enzymes in Rahnella aquatilis strain 16 was 6.0. The optimum temperature of acid phosphatase activity was 45˚C and 50˚C. The reason for this may be that the strains were isolated in different soil and climate conditions. When the acid phosphatase activity of *R. aquatilis* 3, 9, *E. cloacae* 8 and *P. agglomerans* 22 cultures was determined

at a temperature of 45˚C, it was observed that the enzyme activity increased by 2 - 4 times. *Es. hermannii* 1, *Ps. kilonensis* 26 and *B. simplex* 28 bacteria acid phosphatase activity was not significantly affected by temperature rise.

Keywords

Rhizobacteria, Phosphate Mobilization, Nitrogen Fixation, Acid Phosphatase, Wheat, Strains

1. Introduction

Today, the productivity of agricultural crops is indescribably dependent on chemical fertilizers. However, most of the applied phosphorus fertilizers are immobilized in the soil particles and cannot reach the plants. The large amount of high-quality phosphorite rock is required for the synthesis of manufactured chemical phosphorus fertilizers, which leads to a significant decrease in global phosphorus reserves [1]. Unfortunately, the excess amount of chemical phosphorus fertilizers compensates for the lack of phosphorus in the soil, which negatively affects human health and causes many serious environmental consequences [2] [3] [4]. Accordingly, it should be reduced in favor of finding alternative sustainable phosphorus resources to increase crop production and help solve environmental problems [5]. In this case, an alternative to the existing chemical methods of obtaining phosphorus is the possibility of direct microbiological extraction of phosphates from insoluble raw materials through bacteria. This, in turn, is based on the production of organic acids by bacteria and the use of phosphatase enzyme activity. However, the lack of understanding of microbiological and chemical processes can present many challenges, including the enzyme activity of bacteria and especially rhizobacteria, their optimal levels, changes in temperature and pH.

It should be noted that the mineralization of organic phosphorus occurs under the influence of various phosphatases, including phosphomonoesterase, phosphodiesterase and phosphotriesterase enzymes, which catalyze the hydrolysis of phosphoric acid esters [6]. It has been reported that phosphate solubilization and mineralization can occur with the same bacterial strain [7]. In addition to supplying plants with phosphorus, phosphate-dissolving bacteria activate the process of nitrogen fixation and also provide plants with trace elements (Co, Mo, Fe, Zn) [8]. The ability of rhizosphere bacteria to solubilize phosphate is specific to a particular plant or soil type. Calcareous soils contain a large amount of soluble phosphorus [9].

Soil microorganisms enhance the uptake of nutrients by plants. They participate in a wide range of biological processes, including the transformation of non-resolvable food particles in the soil [10]. This biological process plays an important role in phosphorus cycling. Different groups of enzymes are involved

in this. The first groups of enzymes are those that dephosphorylate the phosphor-ester or phosphoanhydride bond of organic compounds. They are non-specific acid phosphatases (HCaP's). The most studied among these HCaP's enzymes released by PSM, are the phosphomonoesterases also referred to as phosphatases [11]. Phosphorus solubilizing bacteria may also aid the growth of plants by stimulating the efficiency of biological nitrogen fixation, synthesizing phytohormones and enhancing the availability of some trace elements such as zinc and iron [12].

Phosphorus (P) is the second-most important nutrient after nitrogen in terms of plant growth and development [13] [14] [15] [16].

The phosphatase enzyme is present in all types of flora and fauna, only its activity has been observed to be different in different plant and animal tissues, as well as in microorganisms. Phosphatases are phosphodiesterase enzymes that break phosphodiester bonds (C-O-P) in organic substances. As a result of phosphatase activity, a phosphorus ion and a free hydroxyl group are formed due to the breaking of monoether bonds of an organic substrate containing phosphorus. Phosphatase enzymes can be acidic or alkaline phosphatases depending on their optimal pH value [17]. Bacterial acid phosphatases are often released from the cell as exoenzymes or stored as membrane-bound proteins [17] [18]. Also, acid phosphatases act in the phosphorus supply system of bacteria and play an important role in survival in the host's organism [19]. A special classification for phosphatases was proposed in order to study the role and function of non-specific types of heterogeneous phosphatases and to compare them with each other [20]. Through this system, phosphatases were classified into subgroups based on their resistance to certain chemicals such as sodium tartrate, sodium molybdate, and EDTA [21], as well as their molecular weight and tetramer, dimer, and monomer form. Many procarotid pathogens have been found to produce acid phosphatase and purple acid phosphatase, such as *Mycobacterium tuberculosis*, *Mycobacterium Shigella sp*, *Morganella sp*, *leprae*, *Salmonella sp*, *Providencia sp*, *Francisella tularensis* and *Cyanobacterium Synechocystis sp*. [22]. Studies show that the enzyme acid phosphatase plays an important role in the survival of bacteria in the host plant [23]. Acid phosphatase enzyme of bacteria is contained in cell wall and cell surface polysaccharides [24]. Many sour phosphatase-producing bacteria have been found to have plant growth-stimulating, antagonistic and phosphate-mobilizing properties against plant pathogens [25] [26].

2. Materials and Methods

Active phosphate-mobilizing magal rhizobacteria belonging to the genera *Escherichia*, *Rahnella*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Pantoea* were grown aerobically in MPB nutrient medium at 28˚C for 3 days. Then, the bacterial suspension was centrifuged at 6000 g for 20 min to extract the culture fluid. $(NH_4)_2SO_4$ was added to the culture liquid up to 70% and left in a refrigerator at 4˚C for 24 hours. The precipitate formed in the culture liquid was separated by

centrifugation at 12,000 g for 20 min. The separated precipitate was dissolved in 500 mM phosphate buffer (pH 4.5) and dialyzed. The semi-purely isolated bacterial enzyme extract serves as a material for the determination of sour phosphatase activity and for the pure isolation of the phosphatase enzyme. The reaction mixture for the determination of acid phosphatase activity consisted of: 3.0 ml of sodium acetate buffer (500 mM, pH 5.0), 30 µl of paranitrophenyl phosphate (65 mM), 0.1 ml of $MgCl_2$ (10 mM). 1.0 ml to the enzymatic reaction mixture begins with the addition of enzyme extract. The reaction mixture was incubated at 37˚C for 15 min. The reaction was quenched by adding 0.5 mL of 1 M NaOH, and the p-nitrophenyl formed was measured at 405 nm. The reaction mixture without enzyme was used as a control. Acid phosphatase activity was determined by para-nitrophenol calibration curve [27].

The protein content of acid phosphatase enzyme was determined by the Lowry method [28]. To determine the optimal temperature of the acid phosphatase enzyme of bacteria, the reaction mixture was heated for 20 min at temperatures of 25˚C, 30˚C, 35˚C, 40˚C, 45˚C, 50˚C and 60˚C after incubation in medium, acid phosphatase activity was determined.

3. Results

Determination of acid phosphatase enzyme activity of rhizobacteria showed that different strains showed different activity at 38˚C temperature (**Figure 1**). Maximum enzymatic activity was observed in *Rahnella aquatilis* strain 16 and was found to produce 1.086 p-nitrophenol nanomol/min/ml (product).

The phosphatase activity of *Pantoea agglomerans* 21, *Pantoea agglomerans* 19 and *Pseudomonas kilonensis* 32 cultures was 0.279 - 0.680 p-nitrophenol nanomol/min/ml. Acid phosphatase activity of *Escherichia hermannii* 1, *Enterobacter*

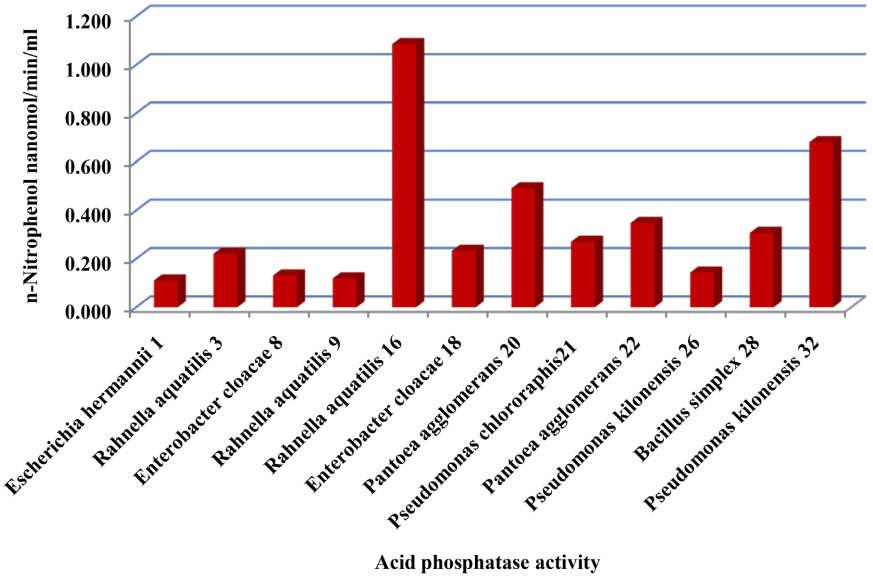

Figure 1. Activity of sour phosphatase enzyme of local rhizobacteria at temperatures of 38˚C.

cloacae 8, *Rahnella aquatilis* 9 and *Pseudomonas kilonensis* 26 strains was 0.143 - 0.221 p-nitrophenol nanomol/min/ml.

The maximum enzymatic activity was observed in the culture fluid of *R. aquatilis* strain 16 and produced 1086 μmol/min. *P. agglomerans* 22, *P. agglomerans* 20 and *Ps. kilonensis* 32 cultures phosphatase activity was 0.143 - 0.680 p-nitrophenol nanomol/min/ml. It should be noted that the phosphatase activity of bacteria belonging to the same genus and species is very different from each other.

When the phosphatase activity of *Rahnella aquatilis* 3, *Enterobacter cloacae* 8, *Rahnella aquatilis* 9 and *Pantoea agglomerans* 21 strains was observed at a temperature of 45˚C, it was found that the enzyme activity increases by 2 - 4 times. However, acid phosphatase activity of *Escherichia hermannii* 1, *Pseudomonas kilonensis* 26 and *Bacillus simplex* 28 bacteria was not significantly affected by temperature (45˚C) increase. Taking into account that acid phosphatase enzymes of bacterial strains belonging to the genus *Rahnella* have been poorly studied at the moment, their acid phosphatases were partially purified and some catalytic properties were studied. Among rhizobacteria, *Rahnella aquatilis* strains 16 and 17 were noted to have high acid phosphatase activity (118 and 95 μM/min/mg). The rest of the strains had 38% - 83% of the above-mentioned activity. Acid phosphatase activity in nutrient fluids of different strains is presented below (**Figure 2**).

From the obtained results, we can see that the AF activities of the strains were 27% - 63% compared to the catalytic activity of the highest strains. *Rahnella aquatilis* 16 was used for partial purification of acid phosphatase enzyme from nutrient fluids. After this step, the protein concentration in *Rahnella aquatilis* 16 enzyme preparations was 0.99 mg/ml. After this step, the protein concentration in Rahnella aquatilis 16 enzyme preparations was 0.99 mg/ml. At this stage, the relative activity of sour phosphatase enzyme is 118 μM/min/mg increased to 666 μM/min/mg. At the same time, 54.2 percent of the total activity of the enzyme was recovered (**Table 1**).

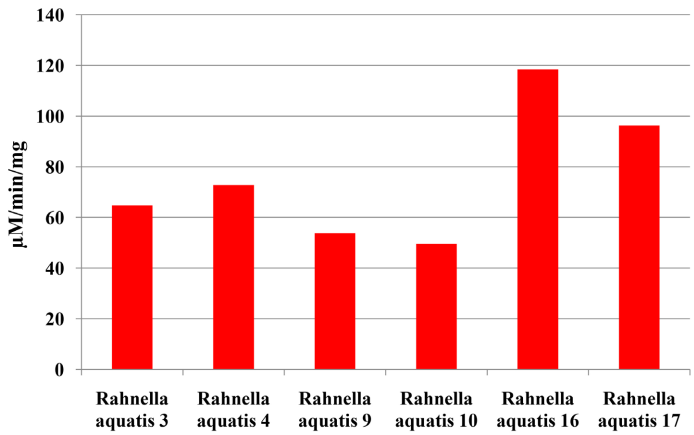

Figure 2. Acid phosphatase activity in nutrient fluids of 3 day-old *Rahnella aquatilis* strains.

Table 1. Sour produced by *Rahnella aquatilis* strain 16 partial purification of phosphatase.

Stage	Protein, mg	General activity, U	Relative activity, U/mg	Cleaning time	Results, %
Culture fluid	10.3	1215	118	1	100
Ammonium sulfate	0.99	659	666	5.64	54.2

A partially purified enzyme preparation of *Rahnella aquatilis* strain 16 was used to study their catalytic properties. The study of the phosphatase enzyme activity of Rahnella aquatilis strain 16 depending on the pH medium showed its activity in the pH range of 4.5 - 6.0. The highest activity was recorded for Rahnella aquatilis 16 enzyme at pH 5.5 - 6.0 (**Figure 3**). Acid phosphatase enzymes showed almost no activity at pH values of 4, 7 and 8 of the reaction medium.

Study of the activity of sour phosphatase preparations of *Rahnella aquatilis* 16 and strains depending on the temperature of the reaction medium. Good results were noted when the optimal temperature for *Rahnella aquatilis* 16 enzyme was in the range of 40˚C - 45˚C (**Figure 4**).

Also, when the acid phosphatase activity of *R. aquatilis* 3, *R. aquatilis* 9, *E. cloacae* 8 and *P. agglomerans* 22 cultures was determined at a temperature of 45˚C, it was observed that the enzyme activity increased by 2 - 4 times. *Es. hermannii* 1, *Ps. kilonensis* 26 and *B. simplex* 28 did not significantly affect the activity of sour phosphatase by increasing the temperature.

To determine the thermostability of acid phosphatases, 0.2 ml of enzyme solutions were incubated at different temperatures for 10 minutes, and then the relative activity of enzymes was determined (**Figure 5**).

In the obtained results, it was observed that the enzyme of *Rahnella aquatilis* strain 16 retains 15% - 20% activity at a temperature of 80˚C.

During the research, it was found that the enzyme activity of *Rahnella aquatilis* 17 strain is 9 times higher than the enzyme activity of *Rahnella aquatilis* 9 *Enterobacter cloacae* 18 strains. The pH optimum of *Rahnella aquatilis* 16 phosphatase enzymes was 6.0. The optimal temperature of acid phosphatase activity was 45˚C - 50˚C, which may be due to the fact that the strains were isolated from different soil and climate conditions.

4. Discussion

Based on the obtained results, it can be concluded that rhizobacterial acid phosphatases have high catalytic activity in culture fluids and partially purified enzyme preparations. They have also been found to be temperature resistant.

Using Rahnella aquatilis 16 for partial purification of the acid phosphatase enzyme from nutrient fluids with ammonium sulfate salt, 0.99 mg of protein was isolated from 100 ml of culture fluid. During the reaction, the relative activity of acid phosphatase increased from 118 μM/min/mg to 666 μM/min/mg. At the same time, 54.2 percent of the total enzyme activity was restored.

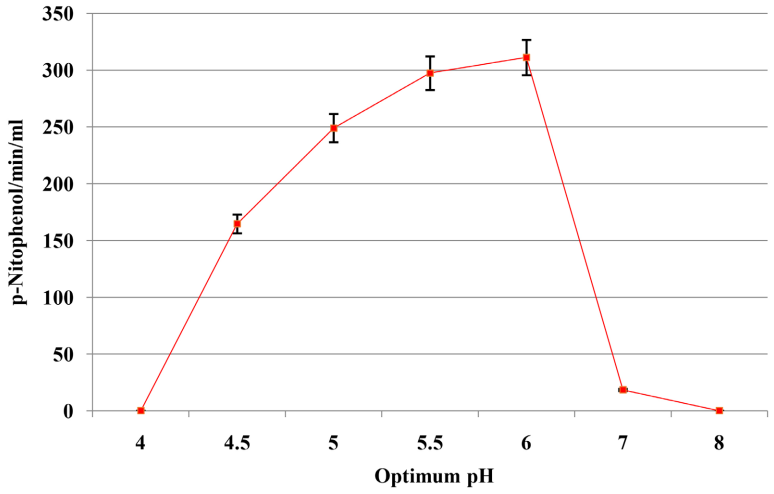

Figure 3. Dependence of acid phosphatase activity of *Rahnella aquatis* 16 strains on environmental pH.

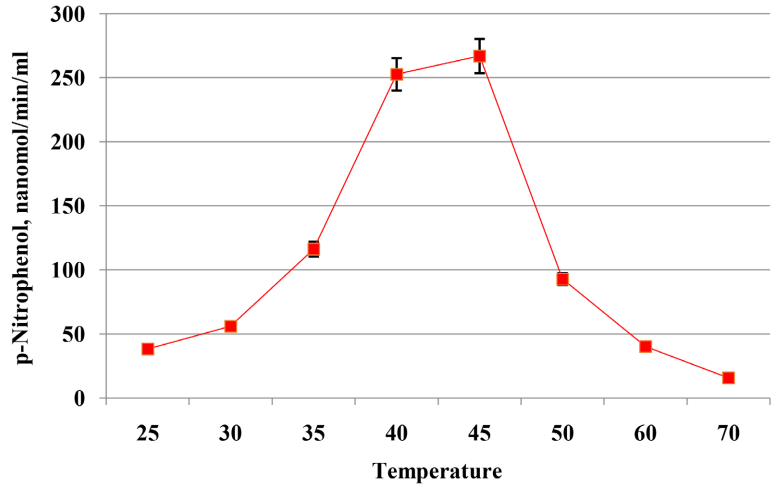

Figure 4. Dependence of acid phosphatase activity of *Rahnella aquatis* strain 16 on ambient temperature.

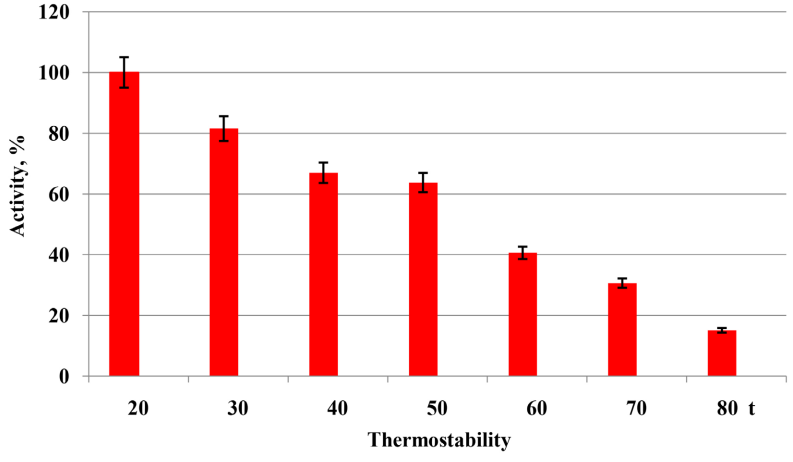

Figure 5. Effect of temperature on acid phosphatase activity of strain *Rahnella aquatis* 16.

When carrying out the enzymatic activity of the phosphatase of the strain Rahnella aquatilis 16, depending on the pH of the medium, it showed that at pH 4.5 - 6.0. Its activity is in the range. The highest activity was registered for the enzyme Rahnella aquatilis 16 at pH 5.5 - 6.0. Acid phosphatase enzymes showed almost no activity at pH 4, 7, and 8 of the reaction medium.

In further scientific research, it is important to study the effect of different nutrients on the acid phosphatase activity of rhizobacteria and the regulation of its synthesis, to isolate the acid phosphatase enzyme in pure form, and to study its physicochemical properties.

Acid phosphatase activity in nutrient liquid of active rhizobacteria was 0.119 - 1.086 p-nitrophenol μmol/min/ml at 38˚C temperature. The optimal pH of acid phosphatases of *Rahnella aquatilis* 16 was 6.0. The maximum activity of enzymes was shown at 45˚C.

The use of phosphorus biofertilizers is a promising approach to improving food production through enhancing agricultural yield as it is better to use an environmentally friendly approach (that is, a paradigm that emphasizes the use of biological soil amendments in place of chemicals) to solve the problems of infertile soil [10].

The nonspecific enzyme acid phosphatase is synthesized in response to stress caused by a lack of carbon, nitrogen, and phosphorus in bacteria [19]. To study the role and function of various nonspecific phosphatases and compare them, a specific classification of phosphatases was proposed [20]. Within this system, phosphatases have been divided into subgroups based on their resistance to certain chemicals such as sodium tartrate, sodium molybdate, and EDTA [21], as well as their molecular weight and tetramer, dimer, and monomer form. Many procarotid pathogens have been found to produce acid phosphatase and purple acid phosphatase, such as Mycobacterium tuberculosis, Mycobacterium Shigella sp, Morganella sp, leprae, Salmonella sp, Providencia sp, Francisella tularensis, and Cyanobacterium Synechocystis sp. [22]. Studies show that the enzyme acid phosphatase plays an important role in the survival of bacteria in the host plant [21]. The enzyme bacterial acid phosphatase is present in cell walls and cell surface polysaccharides [24]. It was found that many acid phosphatase producing bacteria have plant growth stimulating, antagonistic and phosphate mobilizing properties against phytopathogens [25] [26].

Based on the above, our research has great scientific and practical significance in studying the exogenous enzyme acid phosphatase (AP) of indigenous rhizobacteria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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