MIRZO ULUG‘BEK NOMIDAGI
O‘ZBEKISTON MILLIY UNIVERSITETI
JIZZAX FILIALI



"BIOTEXNOLOGIYANING RIVOJLANISH ISTIQBOLLARI VA MUAMMOLARI"

MAVZUSIDAGI RESPUBLIKA MIQYOSIDAGI ILMIY-TEXNIK ANJUMAN TO‘PLAMI





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МИНИСТЕРСТВО ВЫСШЕГО ОБРАЗОВАНИЯ, НАУКИ И ИННОВАЦИЙ РЕСПУБЛИКИ УЗБЕКИСТАН

ДЖИЗАКСКИЙ ФИЛИАЛ НАЦИОНАЛЬНОГО УНИВЕРСИТЕТА УЗБЕКИСТАНА ИМЕНИ МИРЗО УЛУГБЕКА

СБОРНИК ТЕЗИСОВ РЕСПУБЛИКАНСКОЙ НАУЧНОЙ КОНФЕРЕНЦИИ

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DATA MINING ON PHOSPHATASES FROM THE REFERENCE GENOM OF RAHNELLA AQUATILIS BACTERIA

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Due to the annual population growth in our country, it is necessary to obtain high-quality plant production yields and enhance soil fertility to ensure the population is supplied with food and agricultural products. One of the key conditions for increasing agricultural productivity is the level of phosphorus availability to plants. Phosphorus, along with nitrogen, is an essential nutrient for plants. It is involved in almost all major metabolic processes in plants, including nucleic acid synthesis, photosynthesis and respiration, energy metabolism and storage, molecular biosynthesis and signal transduction, as well as nitrogen fixation in legumes.

During the decomposition of phosphorus-containing organic matter, phosphoric acid is formed, which immediately binds with soil bases and turns into insoluble salts of calcium, magnesium, and iron. These salts are converted into soluble phosphoric acid salts through the action of bacteria that produce organic acids [1].

A promising direction for improving phosphorus nutrition in crops is the biological mobilization of phosphates using soil microorganisms, which facilitates the conversion of poorly soluble phosphorus compounds from soil and fertilizers into forms accessible to higher plants [2].

The mineralization of organic phosphorus occurs under the action of various phosphatase enzymes that catalyze the hydrolysis of phosphoric acid esters [3,4].

The aim of this research is to identify the phosphatase genes of *Rahnella aquatilis* strain 16 based on data mining of phosphatase genes from the reference genome CIP 78.65. *Rahnella* belongs to the Yersiniaceae family and is a gram-negative, rod-shaped, fast-moving, microaerophilic bacterium found in soil, the plant phyllosphere, and other habitats [5].

Results. Previously, we isolated 25 rhizosphere bacteria from the surface of wheat roots grown in the Tashkent and Kashkadarya regions of Uzbekistan. Based on studies of molecular nitrogen fixation, phosphorus, and potassium mobilization, three active wheat root rhizobacteria were selected. As a result of molecular-genetic taxonomy, these bacterial strains were identified as *R. aquatilis*.

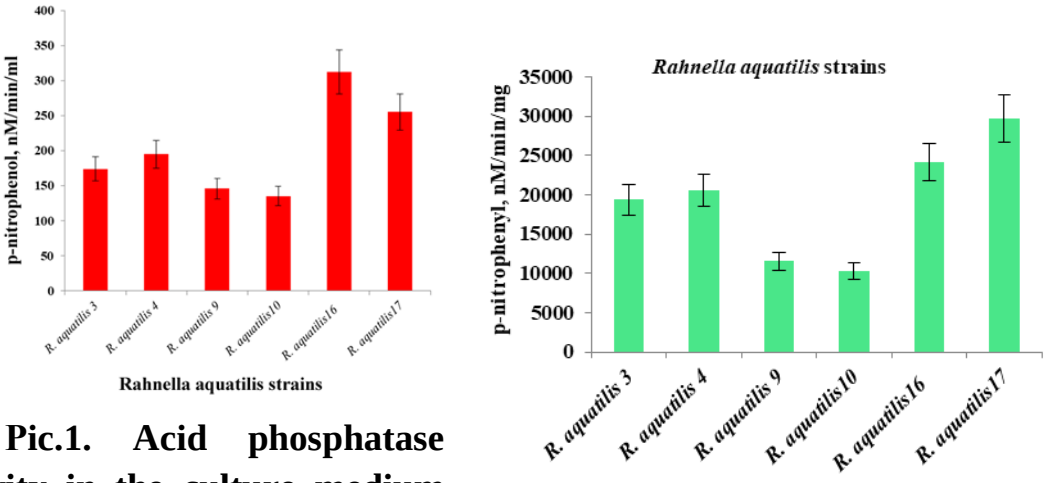
Since the complete nucleotide sequence of the *Rahnella aquatilis* CIP 78.65 genome has been determined, this genome was used as a reference genome [6]. This genome contains 4,970 protein-coding genes. Information on all proteins of the *Rahnella aquatilis* CIP 78.65 strain was obtained from the NCBI database. The search for phosphatase proteins in the reference genome identified 154 proteins, from which we selected five amino acid sequences of phosphatases as potential candidates for phosphorus-mobilizing activity. These enzymes included acid phosphatase, histidine acid phosphatase, alkaline phosphatase, myo-inositol monophosphatase, and exopolyphosphatase.

Using the BLAST program, the amino acid sequences of phosphatase proteins were converted into their corresponding nucleotide sequences. Primer pairs were then selected for each gene for PCR analysis. Genomic DNA was extracted from rhizobacteria strains *Rahnella aquatilis* 16 and 17 using a modified Marmur method [7]. DNA purity was determined using gel electrophoresis in a 0.9% agarose gel and an Implen NanoPhotometer nanospectrophotometer. The PCR reaction was performed using selected primer pairs for acid phosphatase, histidine acid phosphatase, alkaline phosphatase, and exopolyphosphatase.

Using genomic DNA from local strains of *Rahnella aquatilis*, nucleotide sequence fragments of acid phosphatase, histidine acid phosphatase, alkaline phosphatase, and exopolyphosphatase were amplified. Analysis of PCR products of the aforementioned phosphatases in 2% agarose gel electrophoresis confirmed the presence of phosphatase genes in local strains.

Additionally, the study of enzymatic activity of acid phosphatase, histidine acid phosphatase, and alkaline phosphatase in culture media confirmed the presence of these enzymes in *Rahnella aquatilis* bacteria.

Picture 1 presents data on acid phosphatase activity in culture media and cell-free supernatants of various *Rahnella aquatilis* strains. The results indicate that the highest activity in rhizobacteria culture media was observed in strains No. 16 and No. 17 (317.75 and 255.33 nM/min/mg).



Pic.1. Acid phosphatase activity in the culture medium (a) and cell-free supernatant (b) of Rahnella aquatilis bacterial strains.

The acid phosphatase activity of the remaining strains ranged from 42% to 84% of the activity of the strains with the highest catalytic activity. The phosphatase activity in the cell-free supernatant of rhizobacteria was 10 times higher than in the culture medium, with maximum activity observed in strains No. 17 and No. 16—29,665.30 and 24,100.68 nM/min/mL, respectively.

Using scientific literature, a pair of primers was designed for the acid phosphatase gene of *Rahnella aquatilis*. Based on these primers, a PCR analysis of the genomic DNA of *Rahnella aquatilis* strain 16 was conducted.

The study of PCR products using agarose gel electrophoresis revealed a single band with a PCR product size of 195 bp for acid phosphatase.

The obtained data were the first to confirm the presence of both the gene and the acid phosphatase protein in the genome of *Rahnella aquatilis* strain 16.

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TUPROQ MIKROBBIOTEXNOLOGIYASI

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Annotatsiya: Tuproq unumdorligini oshirishda mikroorganizmlarning rolini o‘rganishga bag‘ishlangan. Mikroorganizmlar tuproqning biologik jarayonlarini boshqarishda muhim ahamiyatga ega. Ularning faolligini oshirish qishloq xo‘jalik ekinlari hosildorligini yaxshilaydi.

Kalit so‘zlari: Tuproq, unumdorlik, mikroblar, hosil, tarkib.

Tuproq unumdorligini ta’minlashda biologik omillarning roli katta bo‘lib, bu masalaga V.V. Dokuchaev, P.A. Kostychev, V.R. Vil’yams kabi olimlar katta e’tibor qaratgan. Keyinchalik S.N. Vinogradskiy, E.N. Mishustin, D.G. Zvyagintsev va boshqalar bu g‘oyani yanada rivojlantirib, mikroorganizmlarning tuproq unumdorligidagi beqiyos o‘rnini isbotladilar. Bugungi kunda mikroorganizmlar tuproq unumdorligini belgilovchi asosiy omillardan biri sifatida e’tirof etilmoqda. Shuning uchun qishloq xo‘jaligida tuproq unumdorligini oshirish mikrobiologik jarayonlarni boshqarish bilan uzviy bog‘liqdir [2].