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Endophytic Bacteria of *Ceratoides ewersmanniana* and their Importance

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A B S T R A C T

This article describes the results of experiments aimed at isolation and identification of endophytic bacteria from the *Ceratoides ewersmanniana* plant, common in arid regions of Uzbekistan, determining the effect of isolated isolates on the fertility of wheat seeds, and selecting promising strains recommended for practical use. These data are preliminary results of research on the endophytic bacteria of *C.ewersmanniana*, which are of scientific and practical importance.

Introduction

Ceratoides ewersmanniana is a plant belonging to the *Amaranthaceae* family, which mainly grows in Russia, Ukraine, Kazakhstan, European countries and arid and saline areas of North-South America, Asia and North-South Africa. adapted to the swelling. Ceratoides ewersmanniana plant lives up to 17-23 years, has a strong root system, penetrates to a depth of 1-3 m in the first year and to a depth of 6 m at the age of 10. This semi-shrub bears yellow flowers in July and August, and long hairy seeds ripen in September and October (Gintzburger *et al.*, 2020). *C.ewersmanniana* is distributed in the sub-mountainous, semi-desert region of Uzbekistan and is a high source of food for livestock. The areas where this species is distributed are mainly arid desert areas, and in these unfavorable conditions, in order to obtain a high yield from agricultural crops, in the experience of developed countries of the world, the use of biopreparations created on the basis of the community of microorganisms that control plant growth and development, from diseases protection mechanisms have been created (Jayakumar *et al.*, 2020). Many endophytic bacteria carry out the synthesis of various active phytohormones (indole-3-acetic acid, ACC deaminase) in the plant itself to ensure the growth and development and viability of the plant under various adverse conditions (Hassan *et al.*, 2017; Alikulov *et al.*, 2022; Shurugin *et al.*, 2022). To date, researches on endophytic microorganisms of *C.ewersmanniana* are almost rare and as a result of the effect of endophytic bacteria on plant growth promoting properties, it is necessary to isolate endophytic bacteria, identify bacteria, spread and diversity of bacteria in plant organs in order to increase salinity tolerance and productivity of agricultural crops. Substantiation of scientific solutions in areas such as evaluation of diversity,

analysis of the properties of endophytic bacteria that stimulate the development of cultivated plants is of great scientific and practical importance.

For this reason, the separation and identification of endophytic bacteria *C.ewersmanniana*, the assessment of the distribution and diversity of bacteria in plant organs, the analysis of the growth-promoting properties of endophytic bacteria in agricultural crops, and the justification of scientific solutions are of great scientific and practical importance.

Materials and Methods

Plant samples were formed on the basis of segments isolated from the roots, stems and leaves of the *C.ewersmanniana* plant, which is widespread in the arid regions of Uzbekistan. Samples were taken from plants growing at a distance of not less than 10 meters in the spring of 2021 and cleaned of microbes and soil particles by surface sterilization method.

Bacterial isolation and cultivation were performed according to the method of Coombs *et al.*, (Coombs *et al.*, 2003). The following experiment was carried out to study the possibilities of using endophytic bacterial strains in wheat production: The isolated endophytic bacteria were grown in nutrient broth at 30°C for 96 hours and the cell concentration was reached to 108 KOE/ml. Wheat seeds were inoculated with bacteria by keeping them in a 20% (200 ml/liter) solution of bacterial suspension for 12 hours. 20 seeds were grown in thermostatically sterilized petri dishes at 25°C, which is considered optimal for wheat seeds, and the germination rate of the seeds was determined on the 4th and 7th day. Experiments were performed based on three replications.

The identification of the selected promising isolates was carried out by the method of Dashti *et al.*, (2009). Extracted DNA was tested for 16SrRNA gene analysis by polymerase chain reaction (PCR) using the following primers: 27F 5'-GAGTTTGATCCTGGCTCAG-3' (Sigma-Aldrich, St. Louis, MO) and 1492R 5'-GAAAGGAAGTG ATCCAGCC-3' (Sigma-Aldrich, St. Louis, MO). PCR products were checked by electrophoresis using GelRed. ABI PRISM BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) was used for sequencing. The obtained sequences were compared with the sequences of the most closely related species of the National Center for Biotechnology Information (NCBI) Genbank (http://www.ncbi.nlm.nih.gov).

Results and Discussion

In our research, more than 300 segments of the stem, leaves and roots of the *C.ewersmanniana* plant, which is widespread in the arid regions of Uzbekistan, were studied. 35 isolates of endophytic bacteria (CrEw1001-CrEw1035) grown on the surface of the nutrient medium were isolated from the samples brought from these regions.

In order to select promising strains from isolated isolates, the effect of treatment with isolates on wheat seed germination under optimal conditions was evaluated (results are presented in Figures 1 and 2).

Studies have shown that treatment with isolates of endophytic bacteria *C.ewersmanniana* has a certain effect on wheat seed germination. On the 4th day of the research, the level of wheat seed germination in the experimental variants was 35% to 90% in different isolates, while in the control variant it was equal to 55%.

It was found that isolates of *C.ewersmanniana* endophytic bacteria CrEw 1021 (90%), CrEw 1004, CrEw 1018 (80%), CrEw 1015, CrEw 1024, CrEw 1029 (70%) had a high effect on seed germination. On the 7th day of research, the germination rate of wheat seeds in experimental variants was 65% to 100% in different isolates, while in the control variant it was equal to 65%.

During this period, it was found that 4 isolates of

C.ewersmanniana endophytic bacteria, including CrEw 1004, CrEw 1015, CrEw 1018 and CrEw 1021 isolates, had a high effect on the fertility of wheat seeds. In these experimental variants, the seeds germinated 100%.

In the course of our research, selected promising isolates were identified and species composition was determined.

The identification of the selected isolates was carried out by 16SrRNA gene analysis (Table 1) and the obtained results were registered in the National Center for Biotechnology Information (NCBI) numbers ON567360-ON567363 (http://www.ncbi.nlm.nih.gov/).

The analyzes showed that the 16S rRNA gene similarity of the 4 strains with the highest plant growth promotion among *C.ewersmanniana* isolates and the closely related species in Genbank ranged from 99.58% to 99.73%. Isolates isolated from *C.ewersmanniana* belong to 2 classes (Bacilli – 3 isolates, Proteobacteria – 1 isolate), 2 genera (Bacillales (3) and Pseudomonadales (1)) and 3 genera (Bacillus (CrEw1018, CrEw1021), Priestia (CrEw1004), Pseudomonas (CrEw1015)) were identified (Table 1).

Thus, the research conducted by us proved that endophytic bacteria of *C.ewersmanniana* plant can be used as stimulants of agricultural crops. As a result of the experiments, *Priestia megaterium* CrEw1004, *Pseudomonas putida* CrEw1015, *Bacillus subtilis* CrEw1018 and *Brevibacillus parabrevis* CrEw1021 strains were selected for future practical use.

Treatment with *C.ewersmanniana* plant endophytic bacteria isolates has a certain effect on wheat seed germination. It is recommended to research promising strains of *C.ewersmanniana* endophytic bacteria *Priestia megaterium* CrEw1004, *Pseudomonas putida* CrEw1015, *Bacillus subtilis* CrEw1018 and *Brevibacillus parabrevis* CrEw1021 as a starting source for creating microbial preparations.

Table.1 The effective plant growth promoting endophytes isolated from *C.ewersmanniana* and their closest relatives from GenBank

Isolated strains deposited to GenBank			**Closest match (16S rRNA genes) (GenBank)**		
Strain	**Length (bp)**	**Accession number**	**Reference strains**	**Accession number**	**Percent identity**
CrEw1004	1432	ON567363	*Priestia megaterium*	MZ379521.1	99.58
CrEw1015	1475	ON567362	*Pseudomonas putida*	AB621834.1	99.66
CrEw1018	1504	ON567361	*Bacillus subtilis*	LC178546.1	99.73
CrEw1021	1455	ON567360	*Brevibacillus parabrevis*	MN204065.1	99.59

Fig.1 Effect of *C.ewersmanniana* endophytic bacterial isolates on wheat seed germination (isolates with 100% seed germination on day 7 were marked separately) (n=3)

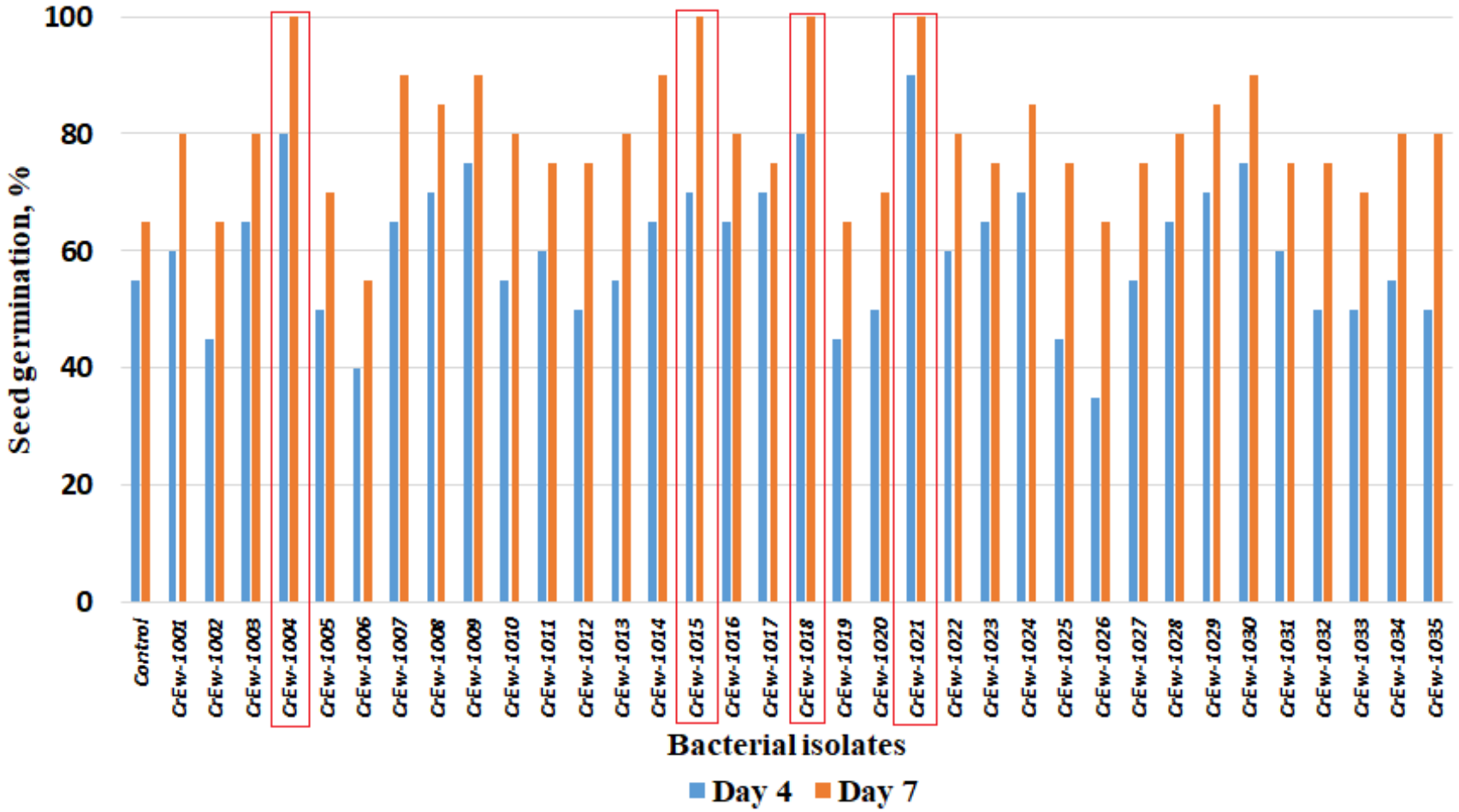


Fig.2 Effect of *C.ewersmanniana* endophytic bacterial isolates on wheat seed germination

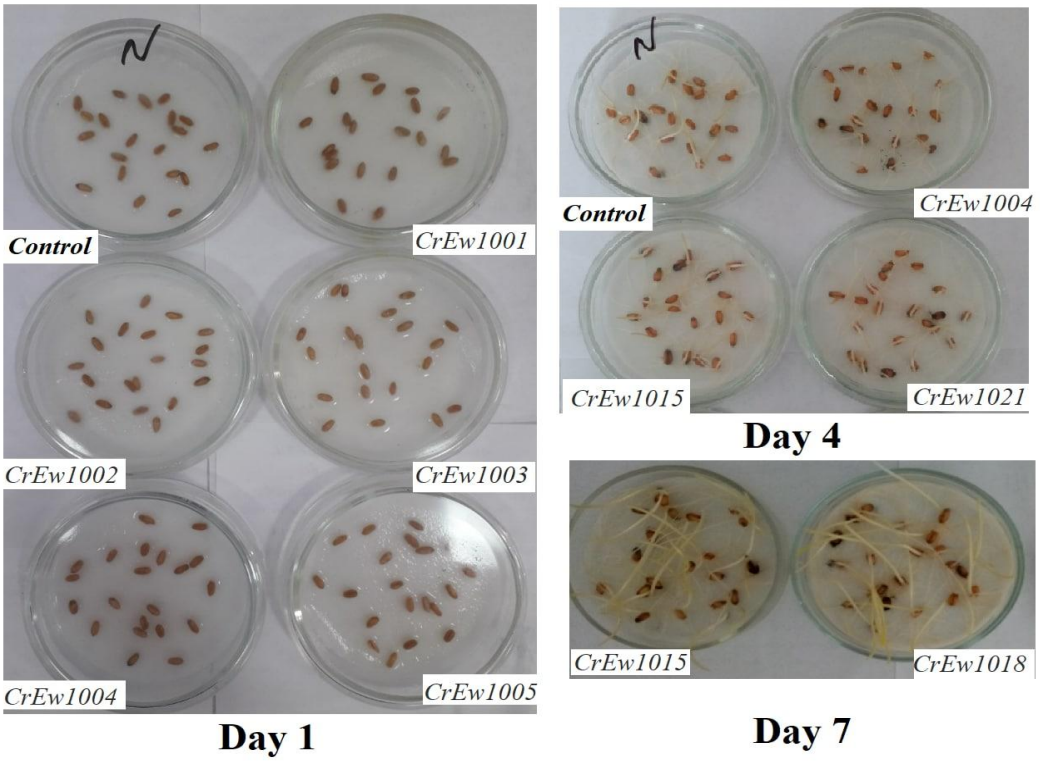
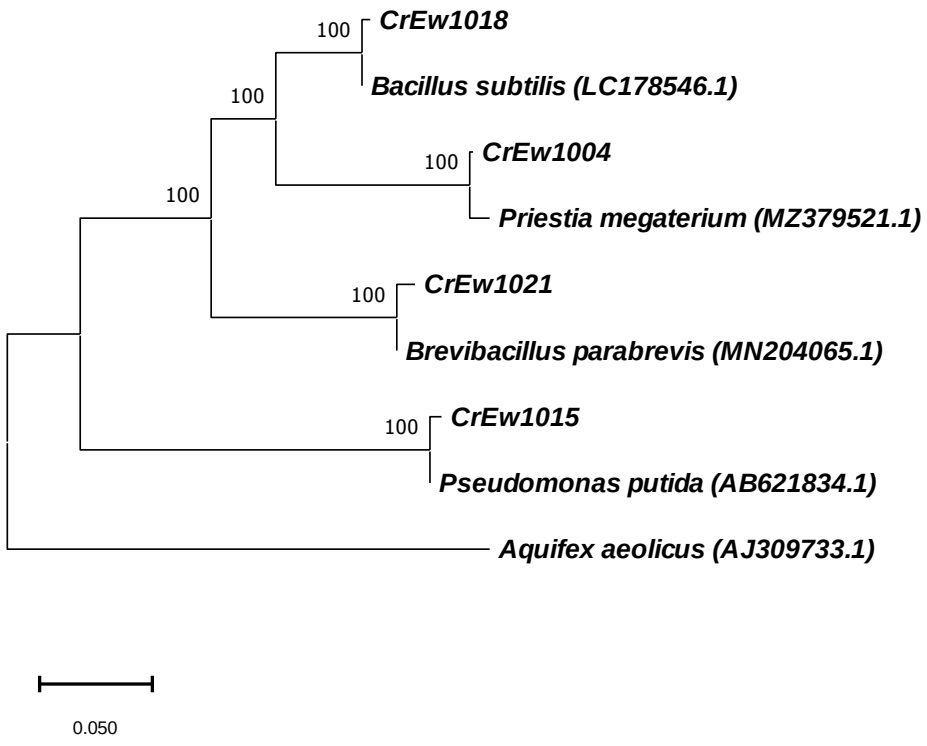


Fig.3 Phylogenetic tree of endophytic bacteria from *C.ewersmanniana* with the closest relatives registered in GenBank



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