Journal of Agricultural Sciences (Tarim Bilimleri Dergisi) 2023, 29 (1) : 26 - 37 DOI: 10.15832/ankutbd.887688

Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

JAS

Identification of Bacteria Obtained from *Dactylorhiza urvilleana* Rhizoid Region, Metal Tolerances, Bioremidant Characteristics and Effects on Maize Germination in Copper Presence

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ARTICLE INFO

Research Article

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Received: 02 March 2021 / Revised: 28 January 2022 / Accepted: 02 February 2022 / Online: 18 January 2023

Cite this article

ESERTAŞ ÜREYEN Ü Z, UZUNALİOĞLU E, BOZDEVECİ A, KARAOĞLU ALPAY Ş (2023). Identification of Bacteria Obtained from *Dactylorhiza urvilleana* Rhizoid Region, Metal Tolerances, Bioremidant Characteristics and Effects on Maize Germination in Copper Presence. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(1):26-37. DOI: 10.15832/ankutbd.887688

ABSTRACT

Rapidly increasing industrialization and technological developments cause hazardous wastes to spread to the environment at a high rate. In our study, three bacterial (5O1, 5O8, 112O1) strains isolated from the rhizoid region of the orchid plant (*Dactylorhiza urvilleana*) were characterized by conventional and molecular methods (nuclear 16S rDNA). In order to characterize the isolates, primarily macroscopic, microscopic, some biochemical and physical properties of the strains were investigated. The usability of the strains screened for their general properties as bioremediation strains, in the prevention of high copper accumulation in agricultural soils was investigated. With traditional and molecular studies, two of the strains were defined as species level (*Bacillus mycoides*, *B. popilliae*) and one at genus level. They were determined that strains were tolerant to the tested all metal salts (Fe, Zn, Cu, Pb and Ag salts in the 1-10 mM range) except for the 5O1 strain to Ag salt, and 112O1 strain to Zn salt. The highest copper tolerance was observed in 5O1, 112O1 and 5O8 strains, respectively. The strains were determined that the copper minimum inhibition concentration values were 12.5-25 mM and the minimum bactericidal concentration value was 50 mM. The examined in terms of properties such as Indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) Deaminase activities, and phosphate solubility, it was determined that they promoted plant germination and growth. When the germination success of maize seeds in the presence of copper was examined, it was concluded that positive results were obtained, there was no significant difference between strains and therefore strains could be used in copper bioremediation.

Keywords: *Bacillus*, Metal tolerance, Plant growth-promoting bacteria, *Zea mays*

1. Introduction

The use of biostimulant microorganisms, which can improve physiological processes in plants, increase nutrient uptake, and promote tolerance to stresses (abiotic and biotic), is important for meeting the demands in agriculture, increasing the use of fertilizers, and more sustainable agricultural activities. When heavy metal accumulation in water and soil reaches undesirable levels, it causes universal health problems to occur and threaten societies. For this reason, there is a need for alternative biological control methods and biological resources to prevent heavy metal pollution, especially in soils used for agriculture. Rapidly increasing industrialization and contamination of industrial wastewater with heavy metals are among the most increasing environmental problems (Cetinkaya Dönmez et al. 1999; Sahan et al. 2010). This is characterized by a series of problems that progress by causing accumulations in food chains and occur with many different health problems. Researchers who want to develop effective strategies in combating pollution caused by heavy metals have turned to microorganisms. Copper metal which used in the study is one of the basic trace nutrients. However, metal levels in all environments, including air, water and soil, rise to toxic levels with the addition of a wide variety of industrial and domestic sources (Tanzadeh & Shareghi 2017). Copper in particular is found in serious amounts in various industrial wastes (Ho et al. 2002; Özer et al. 2004). It has been reported that the presence of high copper in the soil, passing to water resources and indirectly to food sources can cause serious problems in terms of human health (Saha et al. 2010). Since the massive accumulation of environmental pollutants adversely affects life, potential methods are being investigated to control the release of pollutants and to break down existing pollutants or transform them into harmless forms with appropriate recovery techniques. Bioremediation refers to the biological processes applied to decontaminate ecological resources such as soil and especially groundwater (Gillespie & Philp 2013). Many microorganisms, as an important component of the environment, develop complex mechanisms against the toxic effects of metals and make significant contributions to the cleaning of contaminated areas (Nies 1999). Various microorganisms have a high affinity for heavy metals. Even if toxic metals remain in the soil, once they are attached to microorganisms, their uptake by plants or animals living in the soil is less. In this way, the presence of bioaugmentation and biostimulation involves the removal or reduction of heavy metals from the soil with bioremediation strategies (Das & Chandran 2011).

It is thought that obtaining new biostimulant strains that promote plant growth and increase resistance to biotic and abiotic stress will contribute significantly to the agricultural economy. In this research, we purposed to describe of physical, biochemical and plant growth promoting (PGPR) characteristics of 112O1, 5O8, 5O1 strains. Thus, 112O1, 5O8, 5O1 rhizoid bacteries isolated from *Dactyloriza urvilleana* on Ovit plateau, Rize, Turkey was identified with traditional (cultural, Gram staining and biochemical characteristics) and molecular methods (16S rRNA analysis). Heavy metal (Fe, Zn, Cu, Pb and Ag) tolerance and the ability to improve in different pH range in the presence of copper were investigated. The influence of bioremediation properties on the germination of maize plant was determined in the presence of copper.

2. Material and Methods

2.1. Conventional and molecular characterization of the isolates

For the isolation and conventional identification of bacteria, a set of morphological, physical and chemical test methods from Bergey's Systematic and Determinative Bacteriology Guide were used (Kandler & Weiss 1986; Holt et al. 1994). Mueller–Hinton (MH) agar and broth were used for the culture of the strain. Culture conditions were carried out under aerobic incubation conditions at 36 ºC for 24-48 hours. Total genomic DNA was performed according to the standard phenol and chloroform protocol (Sambrook et al. 1989). For the phylogenetic analysis and molecular characterization of bacterial isolates, specific primers 27F (5 'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used for nuclear 16S rDNA intragenic gene regions. The specific gene region was amplified by Polymerase Chain Reaction (PCR) and sequence analysis was performed. The DNA sequences obtained were identified by comparing their similarities with the BioEdit program and other DNA sequences in *National Center for Biotechnology Information* GenBank (Baker et al. 2003; Rivas et al. 2004).

2.2. Determination of pH and NaCl tolerance

To determine the pH and salt tolerance of the isolates, Mueller Hinton broth medium prepared at different pH (4.5, 5.0, 6.8 and 8.0) ranges and salt (10%, 15%) concentrations were used. McFarland 0.5 turbidity bacterial suspensions were prepared from overnight cultures of isolates in MHB medium and used. Chemical tests were carried out by adding 20 µL of culture to 200 µL MHB medium in ELISA plates and incubating for 24-48 hours at 36 °C. Growth was evaluated as positive in wells with turbidity. The number of repetitions for all samples was determined as three.

2.3. Determination of metal tolerance

Metal salts ($AgNO_3$, $CuSO_4.5H_2O$, $FeCl_3.6H_2O$, $ZnCl_2$ and $Pb(NO_3)_2$) at different concentrations (1, 2.5, 5 and 10 mM) were used to determine metal tolerance (Velásquez & Dussan 2009). First of all, MacFarland 0.5 turbidity 10 µL of concentration was added to MH agar media containing metal salts prepared at different concentrations from overnight cultures of 112O1, 5O1 and 5O8 isolates and incubated for 5 days at 36 °C. Minimum inhibition concentration (MIC) and minimal bactericidal concentration (MBC) tests were performed to determine the dose of copper salt inhibiting bacterial growth by microdilution methods (NCCLS 1999).

2.4. Growth curve in copper presence

After determining the optimum copper tolerance value of the strains, their ability to grow at this concentration (3 mM $CuSO_4$) and in liquid (MHB) medium at different pH (5.0, 5.5, 6.0, 6.5, 7 and 7.5) ranges were investigated. It was incubated in a Bioscreen C (Labsystems, Helsinki, Finland) incubator set to shake and take measurements (at 420-580 nm) every 30 minutes for 24 hours at 36 °C, the optimum growth temperature as culture conditions. Growth curves were calculated by transferring the measurement data to the excel file. Positive and negative controls were used for each pH concentration and experiments were performed in triplicate.

2.5. Plant growth-promoting features

Phosphate dissolution activity was carried out according to the Fürnkranz et al. (2009) and Aydoğan et al. (2013) methods. ACC (1-Aminocyclopropane-1-Carboxylate) deaminase activity was measured using the basic method of Dworken & Foster (1958). Chrome azurol S agar assay (Cas agar) was used to test the production of bacterial siderophore proteins (Dworken & Foster 1958; Alexander & Zuberer 1991). Indole Acetic Acid (IAA) test was performed to determine whether the strains produce indole acetic acid hormone, which is an important factor for plant growth (Fürnkranz et al. 2009). The experiments were done in 3 replicates and the mean values were used. Ammonium liquid medium (ALM) was used to measure bacterial ammonium production by colorimetric method. Color change from light yellow to red was evaluated as positive in the presence of ammonium (Aydoğan et al. 2013).

2.6. Germination test

The effect of Cu and bacterium on the success of germination of maize (May RX9292) was investigated. Maize seeds with equal weight were determined, disinfected using 70% ethanol and 3% sodium hypochlorite after then dried in the cabinet for 20 min. The bacterial suspensions (McFarland; 2) were prepared from overnight culture and 5 mL was added on maize seeds. A 10% gum arabic acid solution were added to the seed surface to allow the bacterium to adhere. The same amount of sterile water was added to the control group (Karunakaran et al. 2013). Maize seeds were shaken at 30 °C, 150 rpm for 2 hours. Ten maize seeds were placed in sterile petri dishes with filter paper. The experimental groups are listed below.

Control group

1. Maize (without Cu and bacteria).
2. Maize and 1.5 mM Cu (without bacteria).
3. Maize and 5O8, 5O1, 112O1 bacteria (without Cu).

Experimental group

Maize and Bacteria (each separately) and 1.5 mM Cu; 10 maize seeds separately for each bacterium were placed in three petri dishes and a solution of 1.5 mM Cu in 10 mL of water was added. All petri dishes were allowed to germinate at 26 °C for 16 hours of daylight and 8 hours of night in a climate cabinet with 70% humidity for a week.

3. Results and Discussion

Biochemical properties of the isolates (5O1, 5O8 and 112O1) used in the study are given in Table 1. It has been observed that the isolates are Gram positive, sporulated, Aerobic or facultative anaerobic and immobile. When physical and biochemical properties of isolates are examined; It has been observed that they can reproduce in a wide range of pH. Each has different biochemical properties, the 5O1 and 5O8 strains have a wide growth temperature range (10-45 °C), the 5O1 strain strong lecithinase activity and the ability to reduce 112O1 strain nitrate to nitrite. Microorganisms can be classified as psychrophiles, mesophiles, and thermophiles, according to the temperature range in which they can grow best. The growth temperature of microorganisms may vary according to environmental factors, but the most suitable temperature is known as the optimum growth temperature.

The metabolism of microorganisms is controlled by a wide variety of environmental factors, including pH, temperature, salinity, nutrient availability, and geographic locations (Lennon & Jones 2011; Amend et al. 2013). Among these environmental factors, pH plays an important role especially in the chemical activity of protons, redox reactions, mineral dissolution, and other geochemical reactions (Zhalnina et al. 2015). Environmental pH has the potential capability to shape interactions among microbial groups by promoting or inhibiting microbial redox reactions. The ability of our bacterial isolates to grow in a wide pH range shows that they have good adaptability to changing environmental conditions. In addition, it is thought that it can be used as growth-promoting microorganisms in plants that have the ability to grow in different pH environments.

The minimum and maximum growth temperatures of *Bacillus mycodies* 5O1 and *Bacillus popilliae* 5O8 were determined to be between 10 to 45 °C. It has been reported that heat stress tolerance can be increased in plants through growth-promoting thermotolerant endophytic bacteria and can be used as an alternative environmentally friendly way (Khan et al. 2020).

Table 1- Macroscopic, microscopic, some biochemical and plant growth promoting properties of bacterial isolates

Tests	*5O1*	*5O8*	*112O1*	*Tests*	*5O1*	*5O8*	*112O1*
Colony type	R	R	S	%10 NaCl	+	+	±
O_2 utilization	A	FA	FA	%15 NaCl	±	++	-
Oxidase	+	-	-	10 °C	+	++	-
Catalase	+	+	+	35 °C	+++	+++	+++
Motility	-	-	-	45 °C	+	+	-
Gram	+	+	+	pH 4.5	+	±	++
Spore	Cent.	Subter.	Cent.	pH 5.5	++	+++	++
Indole	-	-	-	pH 6.5	+++	+++	+++
Methyl red	+	-	+	pH 7.5	++	++	++
Citrate	-	-	-	pH 8.5	++	+	++
KIA	A/A	Al/Al	Al/Al	Glucose	ND	ND	+
H_2S	-	-	-	Lactose	ND	ND	+
Gas	-	-	-	Sucrose	ND	ND	+
Nitrate	-	-	+	Trehalose	ND	ND	+
Amylase	-	-	-	Maltose	ND	ND	-
Urease	-	-	-	Species	*Bacillus mycoides*	*Bacillus popilliae*	*Bacillus spp.*
Lecithinase	+++	-	-				

-: Negative, ±: weak positive, +: Positive, 2-3 +++: Strong Positive, A: Anaerobic, FA: Facultative anaerobic, KIA: Kligler Iron Agar, A: Acid, Al: Alkali, Cent: Central, Subter: Subterminal

The spore-forming property of *Bacillus* species provides an important advantage in the field of biological control in terms of their ability to withstand extreme environmental conditions. Therefore, developing cost-effective and stable sports-based products is an important consideration (Shafi et al. 2017). Among the many classes of biosurfactants, lipopeptides, usually produced by *Bacillus* strains (Ghojavand et al. 2008), are specifically interesting because of their remarkable surface properties (Afsharmanesh et al. 2013). Sinha & Mukherjee (2009) showed that especially Gram-positive spore-forming bacteria (*Bacillus* spp.) cell wall components with phosphate residues i.e. polysaccharides, teichoic and teichuronic acids, or phospholipid layers of the membranes can bind more heavy metals. *Bacillus* species contribute to the production of antibiotics and the emergence of plant systemic resistance against various plant pathogen diseases. It can also produce enzymes such as chitinase and β-1,3-glucanase, which have strong lytic activity. These lytic enzymes synthesized by *Bacillus* species have proven to be effective in breaking down the fungal cell wall (Leelasuphakul et al. 2006). Chitinases and glucanases are fungal cell wall hydrolytic enzymes produced by some *Bacillus* species. Many *B. subtilis* strains have been reported due to their chitinolytic activity (Das et al. 2010). Defense-related activities of enzymes have been proven against a variety of plant species and a variety of plant pathogens (Jayaraj et al. 2004). Most soils contain sufficient amounts of plant nutrients, but they are in an insoluble form, so plants cannot take up this undissolved nutrient from the soil (Francis et al. 2010). Beta-1,3-glucanases and chitinases play an active role in plant defense against various plant pathogens (Vidhyasekaran et al. 2001). Pathogenic and non-pathogenic microorganisms compete for both space and nutrients in the space around host plant roots. Nitrogen is the most essential nutrient for the development and productivity of crops. Bacteria located in the rhizosphere act as PGPR; They have the ability to improve atmospheric N fixation, hormone production, specific enzymatic activity, and plant and insect protection by producing antibiotics and other pathogen suppressants (Kamnev & Lelie 2000).

3.1. Plant growth promoting properties

Bacillus species, in addition to having a wide biocontrol ability, can multiply rapidly and are resistant to adverse environmental conditions. The most important bioactive molecules of the *Bacillus* genera are lipopeptides, polyketide compounds, bacteriocins, siderophores, and peptides not synthesized as ribosomes. Mostly, they have a broad spectrum of antagonistic activity against plant pathogenic bacteria, fungi, and viruses. *Bacillus* strains exhibit their biocontrol capacity through the induction of systemic resistance in plants and competition for ecological niches with plant pathogens, as well as inhibitory activity on the growth of plant pathogens. Apart from its antagonistic mechanism, *Bacillus* species have an important role in promoting plant growth by increasing the biosynthesis of plant hormones such as gibberellic acid (GA3) and indole 3 acetic acid (AA), which are closely related to plant nutrient availability (Chen et al. 2007). Plant growth-promoting microorganisms colonizing the host plant roots has direct and indirect strategies that affect plant growth and function by providing the plant with microbial compounds (ammonium production) and facilitating the uptake of nutrients. Many scientists have argued that *Bacillus subtilis* has the ability to increase plant growth and product yield. It also increases the production of plant hormones and nutrient uptake by reducing ethylene production in plant roots of *B. subtilis* (Chen et al. 2007; Idris et al. 2007). It was determined that our strains have strong siderophore and ammonium production ability and that 5O8 from our strains had strong IAA activity. In study results showed that the 112O1 isolate has the properties that support plant growth-promoting (such as IAA Activity, Ammonium and siderophore production, Phosphate solubility) (Table 2).

Table 2- Properties of bacterial isolates that support plant growth

Strain	*R/Z (mm)*		*ACC Deaminase*	*Ammonium Production*	*IAA Activity*
	Siderophore	*Phosphate*			
5O1	13/30	-	3/3	2+	-4.44±0.43
5O8	4/13	3/-	3/-	3+	28.68±2.33
112O1	20/43	4/8	3/3	+	13.53±0.31

R: Reproductive, Z: Zone, -: Negative, +: Positive, 2-3 +: Strong Positive

IAA has an important role in the formation and emergence of plant adventitious roots. It also increases shoot development by affecting cell expression, division and differentiation (Gardner 2009). These plant growth-promoting hormones increase the nutrient uptake ability of plants and help protect the plant against various biotic and abiotic stresses (Vessey 2003; Ghanashyam & Jain 2009).

3.2. Copper tolerance and determination of bacteria absorption ability

The surfaces of microbial cells contain various anionic structures and can be charged with a negative charge so that they can bind metal cations. This means that microorganisms have high affinity for metals. This feature, which also means heavy metal tolerance, is one of the basic conditions for a microorganism to be used in waste treatment processes (Jjemba 2004). Environmental heavy metal pollution has reached serious levels in many regions especially with industrialization. Heavy metals such as copper, lead, zinc, arsenic, chromium, mercury, manganese and cadmium, industrial, domestic and agricultural residues are contaminated into soils and waters in various ways (Jjemba 2004). The increase in environmental concentrations of metals such as the essential element Cu can cause them to become potentially dangerous. Since they cannot be metabolically degraded, they can accumulate in the food chain and reach dense amounts. When it is present in high amounts in food consumed by humans, it can lead to toxicity, cellular dysfunctions, long-term loss of workforce, disability and eventual death (Naja et al. 2010). In our study, the strains' tolerance to copper, lead, silver, iron and zinc salts were investigated in order to determine their bioremidant potential. It was observed that the strains were good growth in 10 mM iron concentrations, 5O1 and 112O1 strains showed very good reproductive potential at 10 mM Fe concentration, and the iron salt was not toxic at the concentrations tested (Table 3). In addition, the high siderophore activity of 5O1 and 112O1 strains parallels this result.

Table 3- The growth ability of bacteria in the presence of heavy metals (1, 2.5, 5 and 10 mM) in solid agar medium and determination of copper tolerance of isolates by agar-well dilution, MIC and MBC methods

Strains	*$AgNO_3$*	*$FeCl_3$*	*$CuSO_4$*	*$Pb(NO_3)_2$*	*$ZnCl_2$*
5O1	-	≤10/ + +	≤5/ ++ +	≤2.5/ +	≤2.5/ +
5O8	≤10/ +	≤10/+	≤1/ +	≤10/ +	≤2.5/ +
112O1	≤1 / +	≤10/ ++	≤2.5 / +	≤10/ +	-

	Inhibition of Copper (mM) by Agar Well Method (mm)						**Inhibition value (mM)**	
	100	**50**	**25**	**12.5**	**6.25**	**3.12**	**MIC**	**MBC**
5O1	20	10	6	-	-	-	12.5±0	50
5O8	16	12	8	6	6	-	12.5±12	50
112O1	13	11	8	6	-	-	25±0	50
B.subtilis W168*	16	14	14	10	6	-	6.3±0	50

-: No Reproductive, +: Reproductive, ++: Good Reproductive, +++: Very Good Reproductive, *: Used as a control.

It was determined that 5O1 did not grow in the presence of silver nitrate, 112O1 has a tolerance of up to 1 mM, but 5O8 was resistant to silver at 10 mM. It has been observed that 5O8 and 112O1 strains show high tolerance in the presence of lead nitrate, while 5O1 strain can reproductive at 2.5 mM. While the 112O1 strain did not reproductive in the presence of zinc chloride, it was observed that the 5O1 and 5O8 strains were only tolerant to 2.5 mM concentration. In general, it can be said that the strains have high metal tolerance. Iron is mostly in ferric form in oxidized and gaseous soils, insoluble in water at pH 7.4, and its concentration can be as low as 10^{-8} mol L^{-1}. This concentration is insufficient to support the growth and development of microorganisms. Microorganisms develop different ways to survive in these conditions, which enables their iron needs from the microenvironment (Shafi et al. 2017). Villegas et al. (2018) reported that eight of the 90 bacterial strains they isolated from the mined soil in Mogpog formed biofilm and tolerated Cu, Pb, Zn and Cd, and soil-based isolates had great potential in the treatment of wastewater contaminated with Cu. *Bacillus* species are known to be tolerant of high metal concentrations and are used to remove cation contaminants from wastewater (Cheung & Gu 2005). It has been observed that the tolerance of bacteria against copper sulfate is very good at 5O1 at 5 mM, whereas other strains are less. It was aimed to determine the Cu tolerance MIC and MBC values and it was observed that it was effective in the range of 25-100 mM when examined with the agar well inhibition test (Table 3). The fact that the 112O1 strain has 25 mM and the other strains have 12 mM MIC values and these values are higher than the control strain shows that these strains have strong copper bioremidantion potential. The fact that the growth-inhibiting Cu concentration value (MBC) was 50 mM in all strains, including the control strain, confirms these results.

Reproduction of microorganisms in the presence of metal; changes depending on factors such as time, metal concentration, and pH. It was aimed to determine the copper absorption / tolerance properties of strains at different pH values (pH range of 5.0-7.5) in liquid media to be used in metal studies. For this purpose, the growth ability of bacteria in liquid medium (Brain heart infusion broth) pH 5.0-7.5 in the presence (3 mM) and absence of copper was spectrophotometrically measured (Figure 1).

It was observed that *B. mycoides* 5O1 was generally able to grow between pH 5.0-7.5 in the presence and absence of copper. It was affected by low pH in the presence of copper and also the best growth in both environments was determined in pH 7.0-7.5. *B. popilliae* 5O8 strain showed good growth in the range of pH 5.0-7.5 in a copper-free environment. It was affected by low pH in the presence of copper, and the best growth was in the range of pH 6.5-7.5 (Figure 1). Compared according to Cu absorption properties at various pH ranges *Bacillus* sp. It was observed that 112O1 strain completed the logarithmic phase in 8 hours at all pHs in the absence of copper, while this period extended up to 18 hours in the presence of 3 mM Cu. The results also show that *Bacillus* sp 112O1 continued to grow in the pH range of 6.0-7.5 in the presence of metal after 24 h while transitioning to the stationary phase between pH 5.0-5.5. In a study by Esertaş et al. (2020), it was observed that the growth rate and density were affected at different pH values in the presence of metal, reaching the logarithmic phase in 8 hours in the presence of copper, while this period extends at all pHs (12 hours and above times) in the presence of copper. The results observed in *B. popilliae* 5O8 strain were found to be consistent with this study.

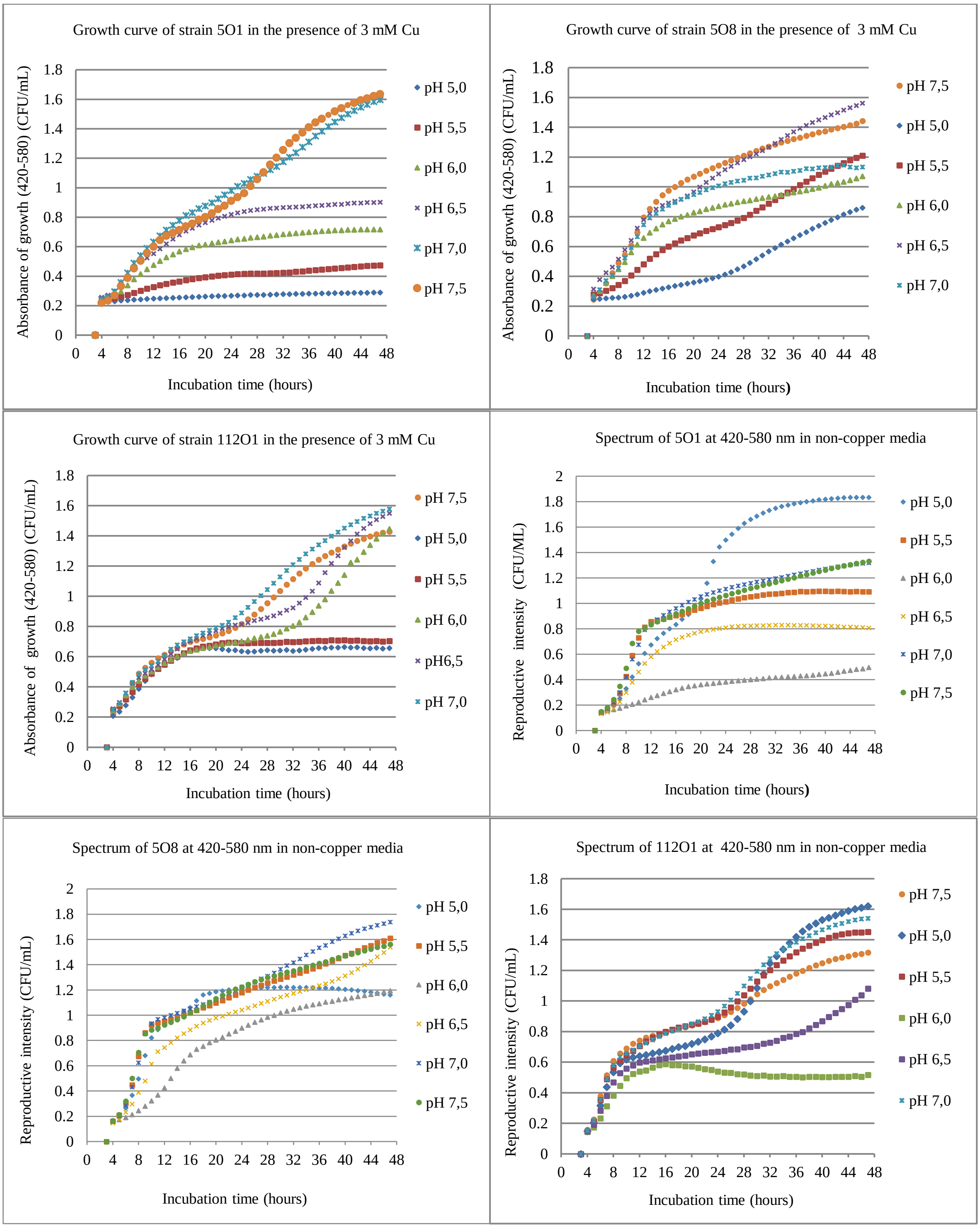

Figure 1- Growth graph of 5O1, 5O8, and 112O1 strains in BHIB medium with different pH, with and without Cu

3.3. The germination success of maize Seed in the presence of copper and bacteria

Some plants, such as *Secale cereale*, *Helianthus annuus*, *Brassica juncea*, and *Zea mays* which can absorb high concentrations of metal are called hyper accumulator plants (Visoottiviseth et al. 2002). In a study by Huang & Cunningham (1996), when the effect of the combination of maize and *Bacillus* spp. strains on Cu removal was tested, it was reported that maize accumulated Cu in its shoots and roots while reaching high biomass and growing well. In our study, the effect of metal-tolerant bacterial strains (in the presence of 1.5 mM Cu) with plant growth-promoting properties on the germination of maize, which is a hyperaccumulator plant, was investigated. For this purpose, germination success, stem, and root development parameters were measured by germinating a total of 30 (10 x 3) maize seeds for a week, 3 replicates for each group. The best germination success was determined for the 5O1 strain and the lowest for the 112O1 strain (Table 4, Figure 2). The germination success of 112O1, 5O1, and 5O8 strains, which have high plant growth-promoting properties, was determined in the range of 90-96.7% in the presence of bacteria on the seventh day. It was observed that not affected in the presence of Cu, and it was between 93.3% and 100% in the presence of Cu and bacteria. When the effectiveness of 3 different bacterial strains tested in the presence of copper on maize germination (stem and lateral root) was examined statistically (ANOVA), it was observed that it was significant (P<0.01) in terms of parameters other than stem weight (p= 0.117).

Table 4- Determination of bacteria and copper (1.5 mM) activity on maize germination

Groups	*Germination Percentage by Day*			*Coleoptile (Body)*		*Radicle (root)*		*Lateral Root*
	3.	*5.*	*7.*	*Weight(g)*	*Length(cm)*	*Weight(g)*	*Length(cm)*	*%*
Ma	87	100	100	0.199	3.63	0.199	10.04	3.6
501-Ma	70	83.3	90	0.146	4.64	0.250	8.68	8.0
5O8-Ma	80	86.7	90	0.148	4.95	0.323	10.42	8.9
112O1-Ma	76.7	83.3	96.7	0.161	4.48	0.195	7.30	5.2
Ma-Cu	96.7	100	100	0.120	2.60	0.085	1.69	8.3
501-Cu-Ma	83.3	96.7	100	0.113	2.92	0.407	1.10	7.9
5O8-Cu-Ma	80	96.7	96.7	0.132	3.10	0.041	1.18	6.8
112O1-Cu-Ma	80	90	93.3	0.108	2.63	0.062	1.94	5.7

Ma: Maize

Figure 2- Seventh day views of maize (*Zea mays* L.) germination test groups in the presence of Cu

It was observed that the length of the coleoptile in the presence of bacteria was better than all groups, and it was higher in the presence of Cu with bacteria compared to the Cu control. It was observed that the length and weight of the radicle in the presence of bacteria was better in the 5O8 strain compared to the negative control, while the other 2 strains were good in the copper-free environment, but were affected in the presence of copper (Figure 3).

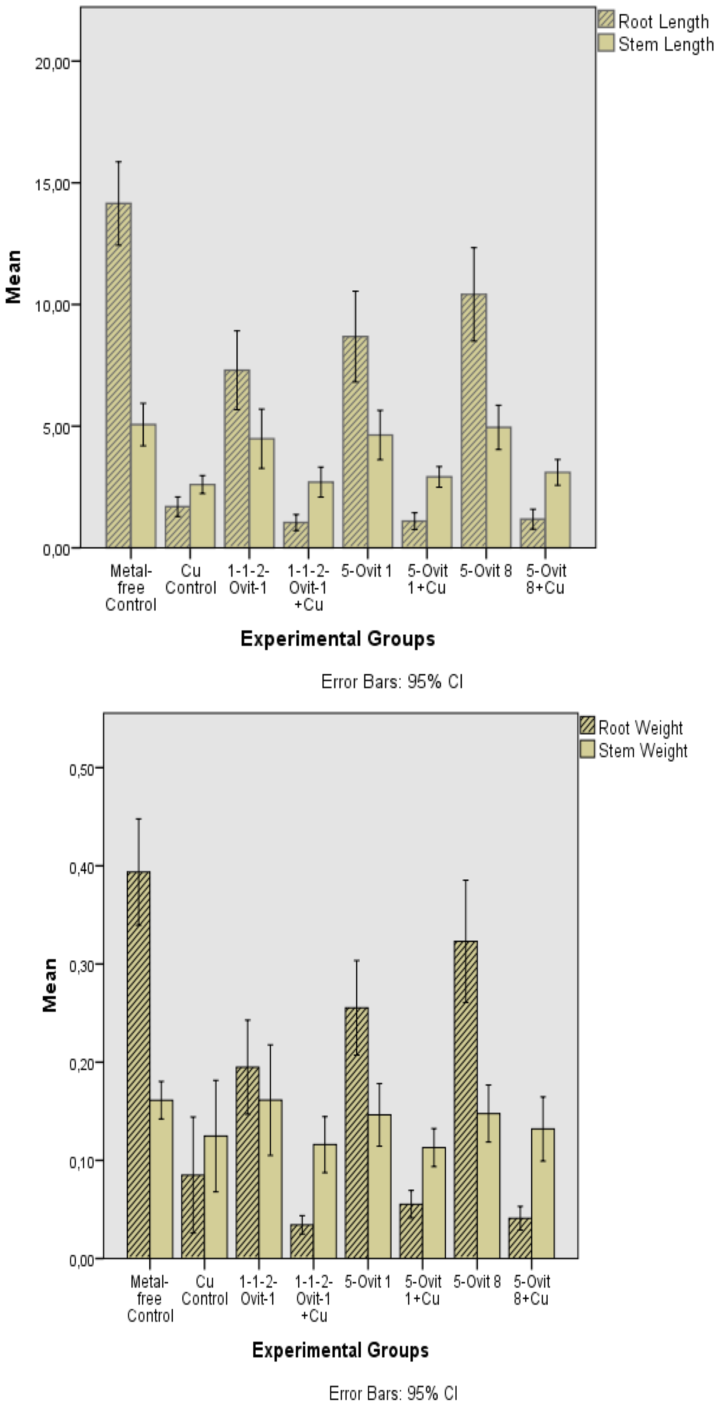

Figure 3- The effects of bacteria on root-stem length and root-stem weight in the presence and absence of Cu

It was determined that there is a significant difference ($P<0.01$) in the combination of both copper and bacteria with copper in the number of fringes among all parameters examined compared to the control group. It has been observed that the bacteria alone increase the fringing and there is a low change in the presence of copper (Figure 4). These results show that the bacteria are of rhizoid origin, stimulate plant growth and can perform metal bioremediation.

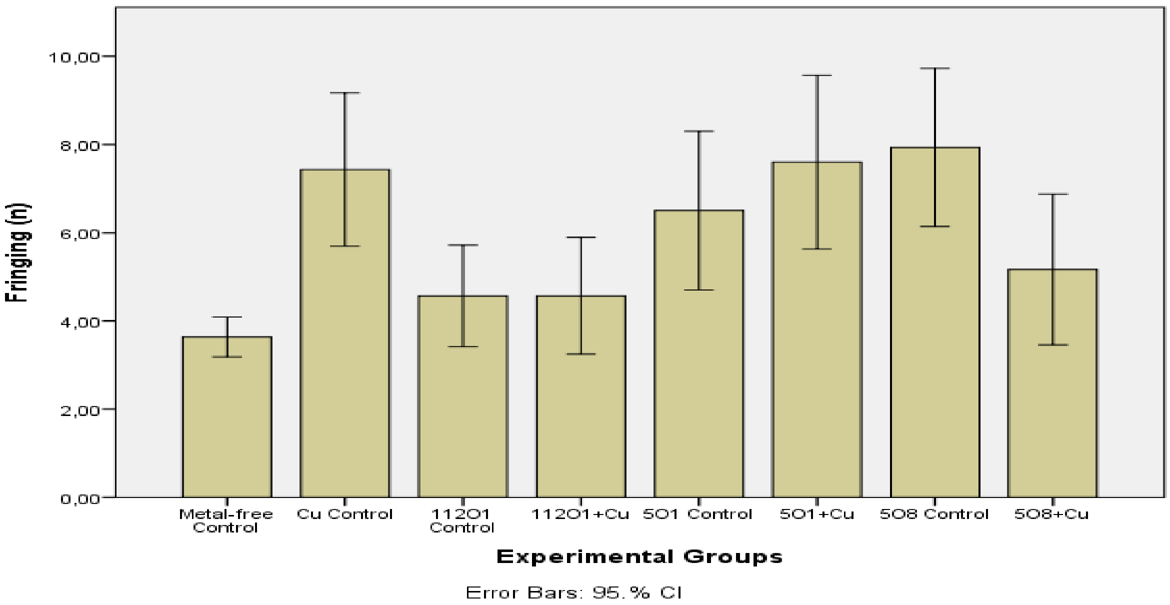

Figure 4- Effects of bacteria on fringing in the presence and absence of Cu

All seeds germinated at the end of 5th day in maize control groups with and without copper metal. Although germination was not affected in the presence of copper metal compared to the copper-free control medium, it was observed that copper metal decreased the root and shoot growth of maize, but increased fringe root formation. In general, when the germination success is examined, it is seen that germination decreases in the presence of bacteria compared to control, germination is better in the presence of copper only compared to control, and the germination success increases in copper and bacteria associations. According to these results, it was observed that the presence of 1.5 mM copper increased the germination rate, decreased the germination rate of bacteria alone, and the presence of bacteria+copper increased the germination rate of the seed.

Bacillus bacteria produce various compounds that can be used to combat many plant pests. Growth and development of biological agents in field conditions is a problem due to adverse environmental conditions. With the excellent stabilizer that can optimize its activity in field conditions, the formulation of the active product is an alternative and more effective strategy for the management of plant pests rather than using live bacteria directly. Bacterial formulated products can be harmful to live bacteria when used in conjunction with other synthetic chemical pesticides. It is important in the formulation that can extend the shelf life of the bacterial product during storage, transportation as well as field application. For a stable and effective formulation, the bacterial active compound must be recognized correctly (Shafi et al. 2017). Systemically derived resistance and plant growth promoting properties of *Bacillus* strains play a fundamental role in biological plant protection. A large number of *Bacillus* strains have been developed to effectively combat plant pests and diseases. In our study, it was concluded that the strains isolated and conventional / molecularly diagnosed are potential biocontrol agents with high metal and salinity tolerances and capable of reproducing in a wide range of temperature and pH.

4. Conclusions

Since the intensive accumulation of environmental pollutants, especially in agricultural areas and water resources, negatively affects life, the subject of researching potential new bioremidant microbial resources is always up to date. It is considered that the importance of increasing or improving productivity in agriculture will increase in the future. Based on this, the effect of strains on germination in the presence and absence of copper was tested in our study. While germination in the presence of 1.5 mM Cu did not change much due to the trace element properties of copper, the bacteria slowed the growth by taking the copper in this range. It suggests that high concentrations of metal that will affect plant growth will be absorbed by bacteria and contribute to the continuity of development. It is obvious that the evaluation of plant-bacteria relationships and selection of bacteria with plant growth promoting properties, will significantly contribute to development of new microbial preparations for agricultural purposes, bioremediation of the soil, obtaining healthier and higher quality agricultural products. In the present study, the bioremediation potential of the three strains was evaluated, and it is thought that different metal groups can be tested in future studies. In addition, it is anticipated that germination experiments can be tried on different plant species and that suitable plants will be directed to greenhouse and field studies.

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