Agricultural Communication

Biosci. Biotech. Res. Comm. 9(3): 530-538 (2016)

BBRC Bioscience Biotechnology Research Communications

Screening of salt tolerant sugarcane endophytic bacteria with potassium and zinc for their solubilizing and antifungal activity

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ABSTRACT

Nowadays high consumption of fertilizers and fungicides in agriculture can increase the problem of soil salinity. Some endobacteria survive in saline condition and induce plant resistance in harm environment. We examined the diversity of halotolerant endophytic bacteria in the internal tissues of sugarcane roots, stems and leaves, with zinc and potassium solubilizing ability and also their antifungal activity. Nutrient Agar medium was used to isolate endophytic bacteria, and then they were screened in view of salinity tolerance on nutrient agar medium containing different concentrations (100, 200, 400, 600 mM) of NaCl, $CaCl_2$ and $MgCl_2$ at ratio of 3: 2: 1. Zinc and potassium solubilizing ability of isolates was respectively assessed using PVK medium containing 0.1% insoluble zinc compound (zinc oxide) and Alexandrov agar medium containing vermiculite. The effect of superior isolates inoculation to supply potassium for wheat was examined in greenhouse condition. As well as antifungal activity of isolates against *Fusarium* sp. was determined using a dual culture technique. DNA of superior isolates was extracted and the 16S rRNA gene was partially sequenced and used for molecular identification. From 55 endophytic bacteria, 5 halotolerant isolates which solubilize potassium and zinc selected to assay antifungal activity. The isolates were divided into three genus were composed of *Enterobacter cloacae, Bacillus pumilus*, *Pseodomonas* sp. Inoculating *Enterobacter cloacae* (R-1) with higher potassium solubilizing index into pot caused to increase uptake of K by wheat. Antifungal activity of *Pseodomonas* sp (S-49) and *Enterobacter cloacae* (R-10) was higher than other isolates. These results showed that some of isolates are integral part of sugarcane as endophytic bacteria survive in saline environment and have antifungal activity.

KEY WORDS: BIOFERTILIZER, INHIBITION, SALINITY, DISSOLUTION

ARTICLE INFORMATION:

**Corresponding Author: n.enayatzamir@scu.ac.ir*
Received 10th Sep, 2016
Accepted after revision 30th Sep, 2016
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007
Thomson Reuters ISI ESC and Crossref Indexed Journal
NAAS Journal Score 2015: 3.48 Cosmos IF : 4.006

Online Contents Available at: http//www.bbrc.in/

INTRODUCTION

Bacteria that exist within plant tissues during at least one period of their life-cycles without any adverse influences on plant growth called endophyte. These microorganisms set up a mutualistic relationship with the plants, because of ecological advantages to them. Endophytic bacteria provide more benefits than rhizospheric bacteria because: endophytic bacteria living inside of organs and tissues of the plant that keeps safe them from unfavorable environmental conditions than in the rhizosphere; they cannot be washed away by rainfall, runoff or irrigation such as rhizospheric bacteria; they are less exposed to UV radiation and there isn't a lot of competition among them as in the rhizosphere, (Coêlho et al. 2011, Gaiero et al. 2013, Hidayati et al. 2014, Jhala et al. 2015 and Yuan et al. 2015).

Endophytic bacteria have been distributed in many plant species and isolated from different plant organs such as roots, stems, leaves, fruit, flowers and seeds. This microbial community may play an important role in agriculture by contributing plant development through producing phytohormones, siderophores increasing resistance to pathogens, promoting biological nitrogen fixation and antibiotic production, (O'Sullivan and Gara 1992, Pal et al. 2001, Han et al. 2005 Strobel and Daisy 2003 Karthikeyan et al. 2005 and Feng et al. 2006 and El-Deeb et al. 2013).

Sugarcane (*Saccharum officinarum* L.) is a major crop in Iran, where it is grown for production of sugar, bioethanol, and its waste such as bagasse and vinasse can be used to conserve soil against erosion. Consequently, sustaining and enhancing the growth and yield of sugarcane have become a major focus of research. The growth and performance of sugarcane in the field are adversely affected by a number of abiotic and biotic factors, including soil salinity and a wide range of fungal and bacterial diseases. On the other hand to get sustainable and organic agriculture it is necessary to use soil potential and reduce utilization of chemical fertilizers and pesticides. Potassium is one of the major nutrients, essential for plant growth. Potassium is associated with movement of water, nutrients, and carbohydrates in plant tissue. If potassium is deficient or not supplied in adequate amounts, growth is stunted and yields are reduced, (Ashley et al. 2006).

Most of the potassium in soil exists in various insoluble minerals, (Goldstein 1994). Microorganisms play an important role to release potassium from minerals and supply soluble K for plant. These bacteria are usually known as potassium solubilizing bacteria or biological potassium biofertilizers. Among the micronutrients, zinc deficiency often happens in crops due to low solubility of zinc in soil, (Iqbal et al. 2010). The solubility of Zn depends upon soil pH, cationic competition and soil moisture, (Vasanthi et al. 2012). The majority of soils under sugarcane cultivation in the Khuzestan province have more than 40% of lime and their pH is greater than 7, hence are often zinc deficient. Some microorganisms are able to dissolve the zinc-containing compounds and release Zn, (Han and Lee 2006, Sharma et al. 2012 and Diep and Hieu 2013).

Excessive using of pesticides causes environmental problems and adversely affects the health of living organisms and this has prompted researchers to look for new environment friendly solutions for controlling plant pathogens. Recently, many studies have revealed the potential of endophytic bacteria for biological control of fungal diseases, (Munif et al. 2012), (Szilagyi-Zecchin et al. 2014), (Xu et al. 2007). The antifungal properties of endophytic bacteria are attributed to their ability to produce antibiotics, (Wang et al. 2013) or/and hydrolytic enzymes (Bacon and Hinton 2011).

In sugarcane, most of the research on endophytic bacteria has focused on diazotrophs (Muangthong et al 2015), (Boddey et al. 2003), (Ramos et al. 2011), and to our knowledge there is not any report on investigation of potassium and zinc solubilizing ability of endophytic bacteria from sugarcane. So, the present study aimed to isolate salt tolerance endophytic bacteria from sugarcane with potassium and zinc solubilizing ability and antifungal activity. Understanding the diversity of beneficial endophytic bacteria and their role in plant production has important implication in agriculture to encourage using them as eco-friendly approaches to manage crop production and sustain agro-ecosystem, (Schenk et al., 2012).

MATERIAL AND METHODS

ISOLATION OF ENDOPHYTIC BACTERIA

Bacteria were isolated from the tissues of sugar cane grown on the Debal-Khazaei agro-industrial unit located in Ahvaz-Abadan road in the province of Khozestan, Iran (latitude 31°05'; longitude 48°30'). To isolate endophytic bacteria, samples of roots, leaves and stems weighing 10.0 g were washed in tap water and surface sterilized according to the method of Marcon *et al.* (2002). The inner section of the stem was removed using a sterilized hole punch. The organs were placed in separate sterile mortar and were well crushed into 90 ml sterile physiologic serum until to obtain a homogeneous suspension and serially diluted with sterile physiologic serum. About 50µl of 10^3-10^8 dilutions was inoculated onto nutrient agar medium and incubated at 28°C for 72 h. All bacterial colonies were purified according to their morphology on nutrient agar.

SCREENING OF ENDOPHYTIC BACTERIA FOR CHARACTERISTICS OF SALT TOLERANCE

To determine salt tolerance of isolates, from the overnight culture of each isolates were dropped in triplicate on nutrient agar plate containing different concentrations (100, 200, 400, 600 mM) of NaCl, $CaCl_2$ and $MgCl_2$ mixture at a ratio of 3: 2: 1 and incubated at 30°C for three days. Growth colony diameter was recorded every day. A control without any salt addition was kept to compare colony growth. Percentage reduction of growth in salt amended media was calculated by using the formula (100 x A-B /A), where A is colony diameter growth in control plate in 'mm' of the isolate and B is colony diameter growth in salt amended plate.

POTASSIUM SOLUBILIZATION ASSAY

Alexandrov culture medium was used to assay the isolates potassium solubilizing ability with following ingredients: 0.5% glucose, 0.2% $Ca_3(PO_4)_2$, 0.05% $MgSO_4.7H_2O$, 0.01% $CaCO_3$, 0.0006% $FeCl_2$, 0.15% K_2HPO_4 or vermiculite and Agar with pH7.0 (Aleksandrov et al. 1967). 7µl from the overnight culture of isolates were dropped on Alexandrov agar medium and were incubated at 30°C for 3 days. Colony diameter was measured at third day. Solubility index was calculated by using the clear zone diameter / colony diameter formula (Shanware et al. 2014).

EFFECT OF SELECTED ISOLATE ON WHEAT PLANT GROWTH

Based on solubilizing ability, one isolate were selected to test its effect on potassium uptake by wheat. For this, greenhouse experiment consisted of a 2×3 factorial in complete randomized design whit four replications was arranged. The factors included two levels of inoculation (with and without inoculant) and three levels of potassium. The nitrogen fertilizer used 140 kg/ha urea, 90 mg/kg P as single super phosphate before seed sowing to prevent possible effects of nutrient deficiency. Potassium from source of potassium sulfate applied at three levels of 120 mg/kg (K3), 60 mg/kg (K2) and without potassium application (K1). The soil was completely mixed and irrigated by distilled water to field capacity (70%). Seeds were surface sterilized in 10% sodium hypochlorite solution for 10 min, then rinsed with sterilized distilled water and air dried (Ahmad and Haddad 2011).The seeds were planted in pots containing 4kg of steam sterilized clay loam soil. Overnight culture of isolate was diluted to $10^6 CFUmL^{-1}$ and then applied under seeds. Potassium concentration of leaves three month after growing was analyzed after dry digestion of organ using flame photometer (Gupta 2004). The data were analyzed statistically via SAS version 9.1. Mean comparisons was done using Duncan test at 5%.

ZINC SOLUBILIZATION ASSAY

The isolates were examined for zinc solubilization ability by using modified PVK medium (Pikovskaya 1948). The medium including: 10.0 g glucose, 1.0 g ammonium sulphate, 0.2 g potassium chloride, 0.2 g dipotassium hydrogen phosphate, 0.1 g magnesium sulphate, 0.2g Yeas, 0.1% insoluble zinc from source of ZnO in1000 ml distilled water with pH 7.0. From the overnight culture of isolates 7µl were dropped on plates containing the mentioned medium and incubated at 30°C for 72 h. Colony and halo zones diameter was recorded. Solubility index was calculated by using the clear zone diameter / colony diameter formula, (Ramesh et al. 2014).

ANTIFUNGAL ACTIVITY ASSAY

The isolates with salinity tolerance and potassium and zinc solubilization were screened for in vitro antagonism against *Fuzarium*. sp on PDA plates using a dual culture technique. The controls were prepared using pure cultures of fungi. The plates were incubated at 30°C until fungal mycelia covered the agar surface of the control plates. Radial growth of *Fuzarium*. sp was measured on the 5th days after inoculation. The inhibition percent in the mycelial development of the pathogen fungus was calculated by the formula: R1=(C-T)/C x 100; where RI is the inhibition percentage of the radial mycelial growth, C is the radial growth of the pathogen in the control (mm), and T is the radial growth of the pathogen in dual culture, (Ohike et al. 2013).

IDENTIFICATION OF ENDOPHYTIC BACTERIA

The superior isolates from point of characteristics related to plant growth promotion were identified by morphological,physiological and biochemical characteristics with reference to Bergeys Manual of Systematic Bacteriology and by sequencing the 16S rRNA, (Weisburg et al., 1991).

RESULTS AND DISCUSSION

In total, 55 endophytic bacteria from the root (10 isolates), stem (21 isolates) and leaves (24 isolates) of the sugarcane were isolated. The results of isolates response to different level of salt concentration have presented in table 1. Salt tolerance of microorganisms depends on the range of external salinity over which it is able to sustain these conditions in the cytoplasm [(Yeo 1998). The salinity tolerance of isolates was classified as very resistant (0-25% growth inhibition), resistant (25-50% growth inhibition),

Table 1: Mean inhibition percentage of isolates growth under different level of salinity

		Salinity level (mM)				Situation			Salinity level (mM)				Situation
Isolate code	Source	100	200	400	600		Isolate code	Source	100	200	400	600	
1N- R-1	Root	60.0	50.0	30.0	40.0	Resistant	29N- S-29	Stem	30.0	30.0	20.0	34.5	Resistant
2N- R-2	Root	63.6	63.6	36.4	45.0	Resistant	30N- S-30	Stem	33.3	22.2	0.0	5.0	Very resistant
3N- R-3	Root	38.5	34.6	26.9	15.0	Very resistant	33N- S-33	Stem	42.9	28.6	14.3	5.0	Very resistant
4N- R-4	Root	50.0	53.6	28.6	22.5	Very resistant	34N- B-34	Leaf	14.3	0.0	-7.1	-5.0	Very resistant
5N- R-5	Root	57.4	57.9	36.8	40.0	Resistant	35N- B-35	Leaf	50.0	40.0	30.0	35.0	Resistant
6N- R-6	Root	42.9	42.9	32.1	29.0	Resistant	36N- S-36	Stem	68.8	75.0	68.8	65.0	
7N- R-7	Root	63.6	56.8	45.5	52.5	Moderate	37N- B-37	Leaf	50.0	50.0	25.0	-15.0	Very resistant
8N- R-8	Root	60.0	55.0	50.0	45.0	Resistant	38N- S-38	Stem	56.3	46.9	37.5	55.0	Moderate
9N- R-9	Root	61.4	47.7	39.5	47.5	Resistant	39N- B-39	Leaf	100	55.6	38.9	27.5	Resistant
10N- R-10	Root	56.7	47.2	50.0	35.0	Resistant	40N- S-40	Stem	100	37.5	40.6	35.0	Resistant
11N- B-11	Leaf	81.7	76.7	73.3	63.5	Moderate	41N- S-41	Stem	100	50.0	25.0	22.5	Very resistant
12N- B-12	Leaf	43.3	33.3	22.2	40.0	Resistant	42N- S-42	Stem	100	68.8	70.3	69.0	Moderate
13N- B-13	Leaf	50.0	50.0	40.0	45.0	Resistant	43N- S-43	Stem	73.4	68.8	65.6	59.5	Moderate
14N- S-14	Stem	30.0	25.0	39.5	45.0	Resistant	45N- S-45	Stem	100	66.7	58.3	57.5	Moderate
15N- B-15	Leaf	35.3	26.5	28.8	20.0	Very resistant	46N- S-46	Stem	70.3	62.5	62.5	58.0	Moderate
16N- B-16	Leaf	50.0	50.0	25.0	20.0	Very resistant	47N- B-47	Leaf	81.7	76.7	73.3	70.0	Moderate
17N- B-17	Leaf	68.1	65.6	62.5	60.0	Moderate	48N- S-48	Stem	47.4	36.8	26.3	25.0	Resistant
18N- S-18	Stem	62.5	62.5	59.4	60.0	Moderate	49N- S-49	Stem	65.0	52.5	40.0	32.5	Resistant
19N- B-19	Leaf	75.0	65.0	67.0	65.0	Moderate	50N- S-50	Stem	61.5	61.5	38.5	30.0	Resistant
20N- B-20	Leaf	100	100	73.7	69.0	Moderate	51N- B-51	Leaf	83.0	82.1	74.1	75.0	Moderate
21N- B-21	Leaf	68.8	65.6	62.5	61.0	Moderate	52N- B-52	Leaf	100	75.0	55.6	55.0	Moderate
22N- B-22	Leaf	66.7	60.0	60.0	60.0	Moderate	53N- B-53	Leaf	28.1	25.0	25.0	25.0	Resistant
23N- B-23	Leaf	45.0	45.0	49.0	35.0	Resistant	54N- S-54	Stem	100	60.0	57.5	57.5	
24N- B-24	Leaf	44.4	11.1	0.0	-10.0	Very resistant	55N- B-55	Leaf	56.8	54.5	50.0	60.0	Moderate
25N- S-25	Stem	66.7	53.3	51.7	51.0	Moderate	56N- B-56	Leaf	60.0	53.3	50.0	50.0	Resistant
26N- B-26	Leaf	62.5	54.7	56.3	55.0	Resistant	57N- B-57	Leaf	100	100	37.5	30.0	Resistant
27N- S-27	Stem	64.1	53.1	50.0	49.0	Resistant	59N- S-59	Stem	100	25.0	21.9	22.5	Resistant
28N- S-28	Stem	62.5	59.4	56.3	52.0	Moderate	29N- S-29	Stem	44.4	44.4	33.3	15.0	Very resistant

moderate resistant (50-75% growth inhibition), sensitive (75-100% growth inhibition) and very sensitive (100% growth inhibition). Isolates growth decreased by increasing salt concentration in the medium. The results revealed that increasing salt concentration induced some isolates growth and seams they are halophile. Most of isolates classified in moderate group. Microorganisms possess multiple strategies to overcome salinity. One of these strategies is the osmoprotectants accumulation in the cytoplasm, (Mishra and Sharma 2012). This important feature demonstrates the potential of endophytic bacteria to alleviate salt stress of host plant by exopolysaccharides production which restricts sodium adsorption by plant (Milosevic et al. 2012) or indirectly by auxin production, (Yaish et al. 2015).

POTASSIUM SOLUBILIZATION ASSAY

The dissolution of insoluble potassium base on halo zone around the colonies of the isolates has shown in table 2. Highest solubility index recorded in R-1 isolate (*Enterobacter*) in presence of both vermiculite and K_2HPO_4 followed by S-49 isolate *(Pseudomonas*).

Most of the potassium solubilizing bacteria obtained from the plant rhizosphere [(Murali et al 2005), (Zhang and Kong 2014)] and identified as *Bacillus* sp., *Pseudomonas* sp., *Bacillus mucilaginosus* [(Liu 2001), (Murali et al. 2005), (Zhou et al. 2006), (Sugumaran and Janarthanam 2007)]. However, there are relatively few studies on potassium solubilizing bacteria in the sugarcane rhizosphere. Ghevariya and Deasi (2014) identified *Pseudomonas* sp. capable solubilize potassium from mica. The ability of potassium solubilizing by *Enterobacter* spp. has been already reported (Zhang and Kong 2014). Yuan et al. (2015) isolated *Enterobacter* spp. as endophytic bacteria from rhizome, root, leaves and stem of Moso Bamboo with the ability of dissolving potassium.

PLANT GROWTH AND POTASSIUM UPTAKE BY WHEAT

Inoculation influence on plant growth and potassium concentration has been shown in figure1.The results showed that potassium concentration of leaves of wheat significantly ($P \leq 0.05$) increased by inoculation and increased level of potassium application. The least potassium concentration was recorded in un-inoculated plants without potassium application, and the highest observed in inoculated plant and with potassium application (K3) followed by inoculated plant with second level of potassium application (K2) without significant difference between them. From this obtained results, it is clear that bio-fertilizer application were more effective to increase potassium concentration.

Increasing the bioavailability of P and K in soils with inoculation of PGPR such as *Bacillus mucilaginosus*, by producing organic acids and other chemical which may lead to increased K and P uptake and plant growth, was reported by many researchers, (Sheng et al. 2002; Lin et al. 2002). Nutrient uptake enhance by inoculated plants may attribute to the production of plant growth regulators by the bacteria at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil, (Abbasi et al. 2011).

Table 2: Colony and halo Diameter of isolates in Alexandrov medium

Isolate code	sample	Diameter / Genus name	Vermiculite			K_2HPO_4		
			Colony diameter (mm)	Halo diameter (mm)	HD/CD (mm)	Colony diameter (mm)	Halo diameter (mm)	HD/CD (mm)
1N- R-1	Root	*Enterobacter*	5	10	2	4	5	1.25
5N- R-5	Root	*Streptococcus*	4	1	0.25	4	1	0.25
7N- R-7	Root	*Enterobacter*	3.5	0.5	0.14	-	-	-
8N- R-8	Root	*Streptococcus*	4	1	0.25	-	-	-
9N- R-9	Root	*Enterobacter*	4	2	0.5	5	5	1
10N- R-10	Root	*Enterobacter*	4	1	0.25	-	-	-
41N- S-41	Stem	*Arthrobacter*	8	2	0.25	8	1	0.125
49N- S-49	Stem	*Pseudomonas*	6	10	1.6	6	4	0.66
51N- B-51	Leaf	*Arthrobacter*	5	3	0.6	8	2.5	0.31
52N- B-52	Leaf	*Enterobacter*	5	4	0.8	10	2	0.2
56N- B-56	Leaf	*Bacillus*	-	-	-	-	-	-

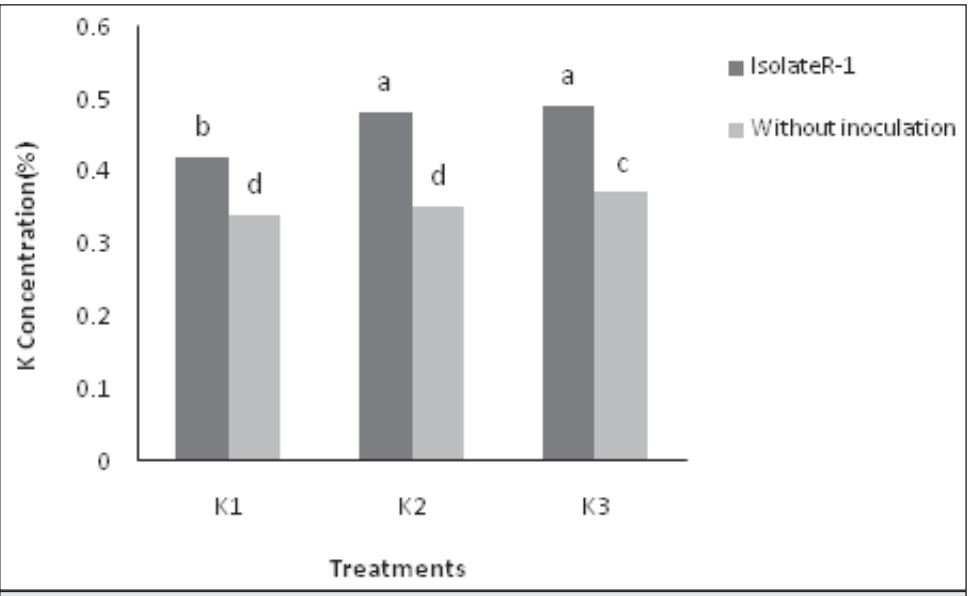

FIGURE 1. Mean comparison of treatments effect Potassium level (K) and bacterium on the dry matter of wheat and K concentration in plant Means followed by the same letters are not significantly difference based on duncan at α=5%.

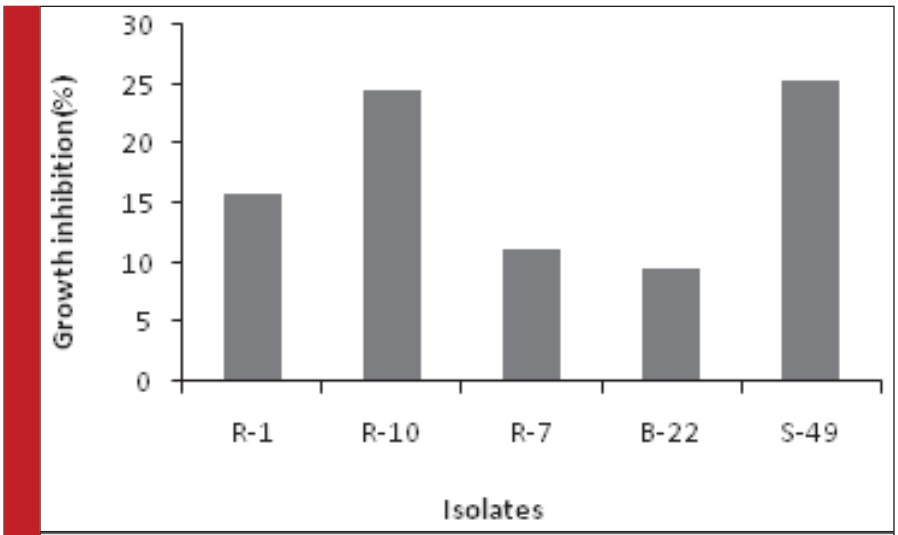

FIGURE 2. Growth inhibition of *Fusarium* sp. by endophytic bacteria

ZN SOLUBILIZATION ASSAY

The bacteria were able to dissolve zinc in plates produced clear halo around them (Table 3). Five isolates had the ability of zinc oxide dissolution. Isolates R-7 and R-10 had the highest rate of zinc dissolution among the five zinc solubilizing bacteria. Both of the bacteria were isolated from the roots of sugarcane and are belonging to the genus *Enterobacter*. Zinc solubilizing bacteria have been isolated from different plant rhizhosphere. *Enterobacter aerogenes* and *Pseudomonas aerugi-*

Table 3: Colony and halo Diameter of isolates in medium containing ZnO

Isolate code	Colony diameter (mm)	Halo zone diameter (mm)	HD/CD (mm)
1N- R-1	7	7	1
7N- R-7	5.5	6.5	1.18
10N- R-10	5	7	1.4
22N- B-22	3	3	1
49N- S-49	4	4	1

nosa from agricultural fields (Sunithakumari et al. 2016), *Bacillus* sp. from sugarcane (Vasanthi et al. 2012) and *Pseudomonas sp*. and *Bacillus sp*. from soybean rhizosphere (Sharma et al. 2012) have been reported to solubilize insoluble zinc. Of the basic mechanisms for the dissolution of zinc-containing compounds is the secretion of organic acids which reduce the pH and thereby increase the availability of zinc (Sunithakumari et al. 2016).

The biochemical information and molecular identification of isolates showed R-1, R-7, R-10 isolates are belonging to *Enterobacter cloacae* under accession number of KX262849, KX262850 and KX262851 respectively and B-22 and S-49 isolates were *Bacillus pumilus* (KX262852) and *Pseudomonas sp*. (KX262853) respectively. *Enterobacter* isolate has the ability to withstand different abiotic stresses such as salinity (Tantawy et al. 2009) and increased the resistance, growth and nitrogen fixing of inoculated plant under salt stress (Tantawy et al. 2009).

IN VITRO ANTAGONISTIC ACTIVITY OF ISOLATES

Results showed that each isolate could prevent *Fusarium* growth but there was difference among them. Highest growth inhibition was recorded by applying S-49 isolate which followed by R-10 isolate (Figure 1). Three other isolates ability to inhibit *Fusarium* growth in dual culture was less than mentioned two isolates which proves their efficacy in management of crop diseases. However, it is necessary to do complementary experiment to confirm their antagonistic ability and also their mechanisms to inhibit pathogens growth.It has been reported antagonistic activity of *Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida* isolated from the stalks of sugarcane against *Colletotrichum falcatum* (Viswanathan et al. 2003), *Bacillus amyloliquefaciens*, *B. subtilis*, and *B. thuringiensis* from banana against *Fusarium oxysporum* f. sp *cubense* and *Colletotrichum guaranicola*, (Souza et al. 2014).

Most bacterial species with biological control potential isolated from the soil rhizosphere, but their use is limited because they can hardly colonize plant roots, perhaps endophytic bacteria can be good choice for this purpose (Chang-Qing, Zhao et al. 2008). The biocontrol potential of enophytic bacteria against *Verticilium dahlia* has been reported by Ferrara et al. (2012). Plant growth promoting endobacteria may induce the plant's defense system against pathogens or enhance plant resistance through production of antimicrobial compounds (Heydari and Pessarakli, 2010). The growth inhibition of pathogen may be related to antibiotic and toxin secretion (Wang et al. 2013), compete with pathogens for space and nutrients impoverishment and pH alteration in the medium (Backman and Sikora 2008) or cell wall degrading enzymes secretion such as chitinase and β-1, 3-glucanase (Roberts and Selitrennikoff 1988).

CONCLUSION

Salinity is one of the most widespread constraints to soil fertility. During the latest years, a great attention has been paid to saline soils due to the reducing arable land, and of the increasing demand for agricultural production of areas influenced by secondary salinisation processes. Our study revealed a high plasticity of bacterial phyla that evidently possess genera and species adaptable to salinity conditions with plant growth promoting properties.There is scope for use of zinc and potassium solublizing bacteria as potential biofertilizers for reclamation saline soils of local area because isolates belongs to the same soil. From 59 isolates, five isolate having plant growth promotion (potassium and zinc solubilizing ability) identified. We also showed that *Pseudomonas sp*. and *Enterobacter cloacae* have the ability to inhibit the growth of *Fusarium*. It is interesting to investigate which mechanisms would be related to fungal inhibition activity of strains in our study. Our experiment demonstrated the advantage of isolate R-1 (*Enterobacter cloacae*) inoculation on potassium uptake by wheat. High cost of chemical fertilizers and harmful environmental effects of them caused to recommend using of biological fertilizer to increase soil fertility. Future studies are promising to test the biotechnological potential of these strains under field conditions in the hope that they will contribute as an alternative source of biological fertilizer and biological control.

ACKNOWLEDGMENTS

We thank Shahid Chamran University of Ahvaz for academic support and Iran National Science Foundation for financial support under grant number of 93045179.

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