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Isolation and Identification of Zinc Dissolving Bacteria and Their Potential on Growth of *Zea mays*

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TWENTY-FIVE bacterial isolates were isolated from mature compost and evaluated for their solubilization potential of insoluble zinc oxide and zinc carbonate. An efficient zinc-solubilizing bacterial isolate (Zn14) were chosen. Thereafter, some morphological and biochemical characteristics as well as 16S rRNA sequence indicated that the isolate belonged to *Enterobacter cloacae*. The effect of various carbon and nitrogen sources on the efficiency of Zn solubilization were investigated under shaking and static conditions. The selected bacterial strain could solubilize Zn compounds under a wide range of nutrient types.

A pot experiment was conducted at Sakha Agricultural Research Station, Sakha, Kafrelsheikh, Egypt to study the efficiency of using *Enterobacter cloacae* as an *in vivo* Zn solubilizer either alone or with amended zinc compounds (ZnO and $ZnCO_3$) to the soil and its effect on growth parameters of *Zea mays* under sterile and non-sterile soil at the age of 30 and 60 days.

There was a highly significant response for increased Zn uptake in all treatments compared to the control. The treatment with ZnO and *E. cloacae* was the more effective in terms of total chlorophyll plant dry weight compared to the control.

To solve zinc nutrition problem using a biofertilizer, it is obvious that *E. cloacae* is capable of solubilizing insoluble zinc compounds by inoculation to increase crop growth and improve soil fertility.

Keywords: ZnO, $ZnCO_3$, *Enterobacter cloacae*, *Zea mays*

Zinc is one of the eight essential trace elements or micronutrients needed for the normal healthy growth and reproduction of crop plants, and it is vital to the crop nutrition as required in various enzymatic reactions, oxidation reduction reactions and metabolic processes, which requires in relatively small concentrations in plant tissues (5-100 mg kg^{-1}) (Parker *et al.*, 1992). Furthermore, Zn is also essential for many enzymes that are needed for nitrogen metabolism, protein synthesis and energy transfer. Insufficiency retards growth and yield of plants, but it also affects humans. More than 3 billion people worldwide suffer from Zn deficiencies (Hafeez *et al.*, 2013).

Normal soils inherit their micro elements which contain Zn, primarily from rocks through pedochemical and geochemical weathering processes. In addition, mineralogical composition of the parent material and the total amount of Zn in the soil during the process of soil formation are also dependent on the type, intensity of weathering, climate and numerous other factors (Saeed & Fox, 1977). Meanwhile, increase in pH and contents of $CaCO_3$, clay, phosphate and organic matter can fix Zn in the soil and cause the reduction of available Zn (Imtiaz, 1999). According to the Food and Agriculture Organization (Sillanpaa, 1990) about 30% of the cultivable soils of the world includes low levels of available Zn to the plant due to the low solubility of Zn in soils rather than a low total amount of Zn.

Zinc plays a role inside the cell, which affects the proper functioning of enzymes that perform a pivotal role in more than 300 enzymes (Vallee, 1991) and it found in all enzyme classes (transferases, oxidoreductases, lyases, hydrolases, ligases and isomerases). Some of these enzymes are involved in CO_2 regulation digestion of proteins, as well as found in DNA-binding proteins.

In the rhizosphere, it is important to study the interactions between plants, soil and microorganisms especially solubilization of insoluble Zn compounds and the benefits for crops. Many bacteria can solubilize Zn compounds, these include: *Microbacterium saperdae*, *Pseudomonas monteilli* (Whiting *et al.*, 2001), *Pseudomonas fluorescens* (Di Simine *et al.*, 1998) and *Pseudomonas aeruginosa* (Fasim *et al.*, 2002). The mechanisms of solubilization of Zn compounds are dependent on production of organic acids, especially 2-ketogluconic acid and H^+, as well as other metabolites, siderophores, and CO_2 from respiration. These processes are variable due to the organisms and the growth conditions (Nautiyal, *et al.*, 2000).

Maize (*Zea mays L.*) is one of the cereal crops produced more than other cereal grain worldwide (FAO, 2011). It is an important cereal crop in nutrition for humans, poultry, and livestock (Nuss & Tanumihardjo, 2010). Thus, it's important to increase Zn concentration in maize grain for people whose diet relies directly or indirectly on maize-derived food.

In last decades, Zinc deficiency in the soil-crop system has become more prevalent so that maize is consider the most susceptible cereal crop to Zn deficiency due to high yielding maize varieties which are selectively grown with chemical fertilizers to improve the cropping and quality (Fageria *et al.*, 2002).

Zinc applications are reported to increase in maize plant around world (Hossain *et al.*, 2008 and Potarzycki & Grzebisz, 2009). Biofertilization is an effective strategy, which has been well documented to increase Zn levels in wheat and rice (Cakmak, 2008 and Shivay *et al.*, 2008). Recent studies indicated that it's possible to increase Zn levels in maize by increasing soil Zn fertilizer or using biofertilizers to transfer insoluble Zn compounds form to soluble forms (Harris *et al.*, 2007).

Whiting *et al*. (2001) discuss the efficiency of Zn solubilizing bacterial strains isolated from the rhizosphere of a Zn hyper-accumulating plant Thlaspi caerulescens that has Zn concentration greater than 10,000 mg g^{-1} of plant tissue. These strains were inoculated into the rhizosphere of the germinating seeds of Thlaspi plants, the bacteria increased the water soluble Zn portion in the soil system and increasing Zn concentrations in roots and shoots 22–67% higher than the control. The maximum increases in Zn uptake and all plant growth parameters of *Vigna radiata* was seen when seedlings were inoculated with Zn solubilizing bacteria (Iqbal *et al*., 2010).

In this study, we report the isolation, characterization, and identification of a Zn solubilizing bacterial isolate, and its ability to enhance the growth of *Zea mays* plants.

Materials and Methods

Microorganism and growth conditions

Twenty five bacterial isolates from mature compost were screened for its ability to solubilize zinc compounds based on clear zone formation on the modified Bunt and Rovira medium described by Abdel Hafez (1966) which containing gl^{-1} (0.4 KH_2PO_4, 0.5 $(NH_4)_2SO_4$, 0.5 $MgSO_4 \cdot 7H_2O$, 0.1 $MgCl_2$, 0.1 $FeCl_3$, 0.1 $CaCl_2$, 1.0 peptone, 1.0 yeast extract, 5.0 glucose, 250.0 ml soil extracts, 20.0 g agar, 750.0 ml tap water, pH 7.0), as well as 0.1% insoluble ZnO and $ZnCO_3$ as described by Saravanan *et al.* (2003). The test organisms were inoculated by stab onto agar plats and incubated at 30ºC for 48 h. The diameters of clear zone around the colonies were measured and the best one was chosen. Pure isolate were maintained on nutrient agar slants at 4°C in a refrigerator for further use.

Characterization of the selected isolate

One selected isolate was characterized based on morphological, biochemical, and culturing characteristics according to Bergy & Holt (1984) and Somasegaran & Hoben (1985).

Molecular identification on the basis of 16S rRNA

The most effective isolates for zinc solubilizing were chosen to identify by polymerase chain reaction (PCR) at Sigma Scientific Services Co., Giza, Egypt. Genomic DNA of the test bacterial isolates grown on nutrient broth was extracted with GeneJet Bacterial Genomic DNA Extraction Kit (Fermentas). The 16S rRNA gene of the isolate was amplified by using universal primers Forward and Reverse (F, 5-AGA GTT TGA TCC TGG CTC AG-3 and R, 5- GGT TAC CTT GTT ACG ACT T-3) used to obtain a PCR product of ~ 1.5 kb. The sample was placed in a hybridthermal reactor thermocycler (Maxima Hot Start PCR Master Mix (Fermentas), initially denatured (enzyme activation) for 10 min at 95°C for one cycle and denaturated for 30s at 95°C, annealing for 1min at 65°C then extention for 1min at 72°C. This was followed by a final elongation step for

10 min at 72°C. The PCR products were analyzed on 1% (w/v) agarose gels and sent to GATC (Germany) for sequencing using ABI 3730xl DNA sequencer.

Sequence data were imported into the BioEdit version 5.0.9 sequence editor; base-calling was examined, and a contiguous sequence was obtained. The full sequence was aligned using the RDP Sequence Aligner program. Sequences used in the phylogenetic analysis were obtained from the RDP and GenBank databases. A dendrogram was constructed using the neighbour-joining method. Also, confidence in tree topology was determined.

Effect of various nutrient parameters on efficiency of Zn solubilization

Carbon sources

In this experiment modified Bunt and Rovira broth medium was used where glucose present originally in these medium was substituted with different carbon sources included glucose, sucrose, mannitol, fructose, starch, galactose, sodium citrate, sodium acetate, carboxy methyl cellulose and dextrose. Carbon sources stock solutions were sterilized by filtration and added to the broth medium.

Nitrogen sources

Different nitrogen sources included ammonium sulphate, ammonium chloride, urea, sodium nitrate, peptone, yeast extract, ammonium molybdate or ammonium oxalate were used.

With different carbon and nitrogen sources, the flasks contain the modified Bunt and Rovira medium and/or 0.1% insoluble ZnO and $ZnCO_3$. 0.5 ml suspension of the test culture strain with a cell load of 1×10^8 cells ml^{-1} were inoculated and incubated at 30°C and 150 rpm. Experiments were done under shaking and static conditions in triplicate. After 48h, the samples were withdrawn and centrifuged at 15°C and 6000 rpm to remove the cells and debris. Twenty ml of this solution was fed to Atomic Absorption Spectrophotometry (AAS) PERKIN ELIMER 3300 to determine the available Zn content.

Determination of pH

The pH of the Zn solubilizing bacterial culture filtrates samples were determined at 48h after inoculation. The pH was determined using pH meter HANNA HI 98127 and compared with the uninoculated control.

Pot experiment

A pot experiment was carried out in a greenhouse at Sakha Agricultural Research Station, Sakha, Kafr el-sheikh, Egypt. The greenhouse used for this study was basically an open net house. The mean temperature during the experimental period (June to July 2015) ranged from 30°C to 40°C with a 10–12-h daylight and 60–65% relative humidity. The experimental plan was based on six treatments. These were T1, control (no Zn source, no *Enterobacter cloacae*); T2, inoculation with *Enterobacter cloacae* (Zn solubilizer); T3, ZnO alone; T4, $ZnCO_3$ alone; T5, ZnO + *Enterobacter cloacae* (Zn solubilizer); T6,

$ZnCO_3$ + *Enterobacter cloacae* (Zn solubilizer). The experiment was conducted in a split plot design under sterile and non-sterile soil conditions with four replicates.

Soil used

The pot experiment was conducted in silty clay soil in texture having the following characteristics: pH, 8.11; EC, 0.186 dSm^{-1}; organic matter (%), 1.12; particle size distribution sand, silt and clay (%), 33.65, 24.17 and 42.18, respectively; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (meq L^{-1}), 0.86, 0.49, 0.50 and 0.12, respectively; soluble anions CO_3^{-}, HCO_3^{-}, Cl^{-} and SO_4^{-} (meq L^{-1}), 0.0, 1.0, 0.66 and 0.31, respectively; available N (mg kg^{-1}), 6.44; available P (mg kg^{-1}), 5.80; available K (mg kg^{-1}), 351.1; available Zn (mg kg^{-1}), 17.39. Also, total count of bacteria, 150 x 10^6 CFU g^{-1}; total count of fungi, 75 x 10^4 CFU g^{-1}; and total count of actinbacteria, 45 x 10^5 CFU g^{-1} (Allen, 1959). These soil was put in pots of 30 cm diameter and 35 cm depth at the rate of 6 kg soil pot^{-1}. The soil moisture maintained at 60% of water holding capacity. The mineral fertilizers were added as recommended by Egyptian Ministry of Agriculture for mineral fertilizers which comprised Urea (46.5% N) with the rate of 1.2 g pot^{-1}, calcium super-phosphate (15% P_2O_5) with the rate of 1.2 g pot^{-1}, potassium sulphate (48% K_2O) with the rate of 0.3 g pot^{-1} and Zn source fertile (ZnO or $ZnCO_3$) with the rate of 0.048 g pot^{-1}.

Preparation of Enterobacter cloacae inoculum

A loop full of nutrient agar slant *Enterobacter cloacae* KX034162 cells was cultivated in 250 ml nutrient broth medium in 500 Erlenmeyer flask at 30 °C on a rotary shaker for 24h at 150 rpm. All plants were inoculated with bacterial suspension 3 ml $plant^{-1}$ as shown from plate counting technique, this procedure yielded 10^7-10^8 CFU ml^{-1}.

Grains used

Grain of maize (*Zea mays* L. cv. Hybrid 10) was surface sterilized in a 3.5% (w/v) solution of calcium hypochlorite for 10 min then immersed in distilled water several times then one grain was sown in each pot. The grains were kindly supplied by Field Crops Research Institute, Department of Cereal Crops, Sakha Agricultural Research Station, ARC.

Estimation of available zinc content in soil

Available zinc content of the soil was estimated using the diethylene triamine penta acetic acid extract following the method of Lindsay and Norvell (1978).

Chemical component assay of plant materials

The shoots were harvested at 30 and 60 days after sowing, rinsed twice in distilled water, dried at 80°C for three days, and dry weight $plant^{-1}$ determined as well as their Zn content were determined by atomic absorption technique (PERKIN ELIMER 3300) according to Cottenie *et al*. (1982). Equipments were calibrated and uncertainties were calculated. Internal and external quality assurance systems were applied in the laboratory according to ISO/IEC 17025 requirements for laboratory accreditation. In all measurements, blanks, triplicate measurements of metals in extracts, and analysis of certified reference materials for each metal (Merck) were routinely included for quality control.

Total chlorophyll determination

Total chlorophyll was estimated by Minolta chlorophyll metter SPAD-502 in the green house after 30 and 60 days of sowing.

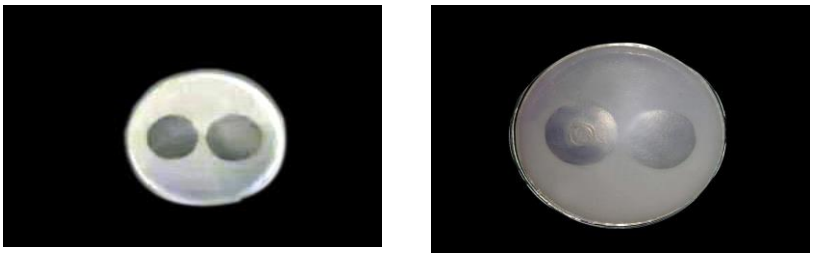
Statistical analysis

Data obtained were subjected to the analysis of variance and treatment means were compared using the L.S.D methods according to Steel & Torrie (1981).

Results and discussion

Isolation and screening of Zinc solubilizing bacteria in solid medium

One of 25 isolates was screened according to hydrolysis capacity (HC) using the diameter of clear zone and diameter of colony on the modified Bunt and Rovira solid medium containing insoluble ZnO and $ZnCO_3$ to select the most potent Zn solubilizing (Fig. 1 and Tables 1, 2).



A **B**

Fig. 1. Clear zone of Zn14 on the modified Bunt and Rovira media agar plates. A) ZnO B) $ZnCO_3$ each at 0.1%.

TABLE 1. Solubilization capacity of zinc oxide by isolates under study.

Code of bacterial isolate	Colony diameter (cm)	Clear zone Diameter (cm)	HC value	Code of bacterial isolate	Colony diameter (cm)	Clear zone diameter (cm)	HC value
Zn1	0.43±0.01	1.3±0.11	3.02	Zn14	0.48±0.01	2.6±0.06	5.42
Zn 2	0.13±0.00	0	0	Zn15	0.61±0.01	1.36±0.09	2.23
Zn3	0.39±0.001	1.54±0.03	3.95	Zn16	0.47±0.05	2.36±0.06	5.02
Zn4	0.22±0.002	0	0	Zn17	0.53±0.03	1.15±0.07	2.17
Zn5	0	0	0	Zn18	0.36±0.01	1.41±0.02	3.92
Zn6	0.44±0.005	2.1± 0.14	4.77	Zn19	0.60±0.02	1.99±0.15	3.32
Zn7	0.31±0.00	1.25± 0.17	4.03	Zn20	0	0	0
Zn8	0.16±0.006	0	0	Zn21	0.36±0.00	1.46±0.05	4.05
Zn9	0.52±0.01	0.66± 0.03	1.27	Zn22	0.65±0.02	1.81±0.02	2.78
Zn10	0.15±0.00	0	0	Zn23	0.12±0.00	0	0
Zn11	0.31±0.006	0.65±0.03	2.1	Zn24	0	0	0
Zn12	0.57±0.03	1.86±0.09	3.26	Zn25	0.53±0.02	1.11±0.00	2.09
Zn13	0.45±0.02	1.69±0.15	3.75				

Hydrolysis capacity (HC): diameter of clear zone / diameter of colony

TABLE 2. Solubilization capacity of zinc carbonate by isolates under study.

Code of bacterial isolate	Colony diameter (cm)	Clear zone diameter (cm)	HC value	Code of bacterial isolate	Colony diameter (cm)	Clear zone diameter (cm)	HC value
Zn1	0.39±0.01	1.10±0.12	2.82	Zn14	0.50±0.01	2.39±0.06	4.78
Zn 2	0.11±0.00	0	0	Zn15	0.67±0.01	1.40±0.09	2.08
Zn3	0.39±0.001	1.30±0.11	3.33	Zn16	0.57±0.08	2.28±0.04	4.0
Zn4	0.24±0.002	0	0	Zn17	0.50±0.03	1.11±0.06	2.22
Zn5	0	0	0	Zn18	0.32±0.01	1.30±0.02	4.06
Zn6	0.40±0.005	1.80± 0.11	4.5	Zn19	0.68±0.06	1.85±0.15	2.72
Zn7	0.30±0.00	1.11± 0.14	3.7	Zn20	0	0	0
Zn8	0.12±0.005	0	0	Zn21	0.39±0.00	1.49±0.06	3.82
Zn9	0.48±0.01	0.71± 0.03	1.47	Zn22	0.60±0.02	1.77±0.02	2.95
Zn10	0.17±0.00	0	0	Zn23	0.14±0.00	0	0
Zn11	0.31±0.006	0.72±0.03	2.32	Zn24	0	0	0
Zn12	0.51±0.02	1.65±0.07	3.23	Zn25	0.61±0.02	1.14±0.00	1.86
Zn13	0.41±0.02	1.79±0.17	4.36				

Hydrolysis capacity (HC): diameter of clear zone / diameter of colony

Characterization of rhizobacterial isolate Zn14

A rhizobacterial isolate designated as Zn14 was recovered from mature compost. The isolate was characterized by morphological and biochemical reactions and 16S rRNA gene sequencing methods (Table 3). The morphological characterization revealed that the colony morphology of the isolate was circular, with entire margin, smooth, peach colored, gram and endospore negative with a small rod shaped. Under *in vitro* liquid conditions, the isolate grew on MacConkey broth and was positive toward motility and Indole Acetic Acid production, Voges–Proskauer, citrate utilization, oxidase, catalase and urease. Negative results occurred for starch, gelatin and casein hydrolysis. Phylogenetic analysis based on 16S rRNA gene sequence (1.5 kb) indicated that isolate was found to be closely related to *Enterobacter cloacae* strain ATCC 13047 (99.1 %). Sequence data were submitted to GenBank and it provided a GenBank accession number KX034162 as shown in Fig. 2.

TABLE 3. Some morphological, biochemical characterizations and 16S rRNA gene sequencing of studied rhizobacterial isolate Zn14.

Characteristics	Response	Characteristics	Response
Colony configuration	Circular	Gram reaction	-
Colony margin	Entire	Cell shape	Rod
Colony surface	Smooth	Endospore	-
Colony pigmentation	Peach color	Motility	+
Indole production	+	Oxidase	+
Methyl red test	-	Catalase	+
Voges-Proskauer test	+	Urease	+
Growth on MacConkey broth	+	Starch hydrolysis	-
Citrate utilization	+	Gelatin hydrolysis	-
H_2S production	-	Casein hydrolysis	-

+: Positive, -: Negative

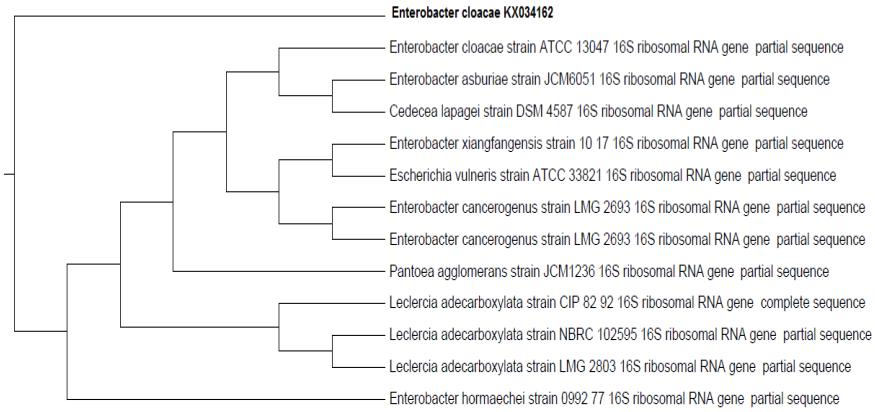


Fig. 2. Neighbor-joining phylogenetic tree reconstructed on the basis of 16S rRNA gene sequence (1.5 kb) showing the phylogenetic *Enterobacter cloacae* KX034162

Effect of various nutrient parameters on efficiency of zinc solubilization

The observations reported during the growth of liquid cultures of *E. cloacae* KX034162 under different carbon and nitrogen sources on zinc solubilizing are recorded in Table 4. In general, analysis of the supernatant, performed by atomic absorption technique, showed an increase in the concentration of soluble Zn with decrease in pH. Also, during the time course of the experiment, the increased Zn concentration caused by shaking as compared with static conditions.

The best carbon source was glucose followed by succrose and galactose, (530.00, 512.98 and 500.22 mg L^{-1} Zn solubilization, respectively) under shaking conditions for ZnO. Results of $ZnCO_3$ followed the same trend.

On the other hand, ammonium chloride was a nitrogen source giving the highest values of Zn solubilizing (mg L^{-1}), 830.88 and 751.98 for ZnO and 848.00 and 768.43 for $ZnCO_3$ under shaking and static conditions, respectively.

Colonies of the microorganism produced clear halos on solid medium incorporating zinc phosphate or Zn solubilization in liquid culture when glucose was provided as the carbon source (Di Simine *et al.*, 1998).

Morley *et al.* (1996) and Nautiyal *et al.* (2000) showed that production of organic acids and H^+ appear to be the most significant mechanisms for heterotrophic metal solubilization although some contribution may also arise from excretion of other metabolites, siderophores and CO_2 from respiration, the significance of all these processes being variable and dependent on the organisms and the growth conditions. The gluconic acid is subsequently taken up by transport systems of the cell and utilized by cellular metabolic pathways; the

external oxidation of glucose therefore usually produces only transient increases in the concentration of gluconic acid (Drosinos & Board, 1994).

TABLE 4. Effect of *E. cloacae* KX034162 solubilizing ZnO and $ZnCO_3$ under different carbon, nitrogen sources and final pH.

Carbon source	ZnO			$ZnCO_3$		
	Final pH	Zn concentration (mg L^{-1})		Final pH	Zn concentration (mg L^{-1})	
		Shaking	Static		Shaking	Static
Glucose	7.1	530.00	481.57	5.9	753.75	680.33
Succrose	7.3	512.98	466.74	7.1	513.88	436.71
Mannitol	7.4	496.11	433.95	7.5	494.23	410.65
Fructose	7.4	492.64	427.27	7.4	505.43	422.01
Starch	7.6	460.44	394.88	7.5	478.10	424.96
Galactose	7.3	500.22	456.32	6.5	708.45	640.50
Sod. citrate	7.9	238.55	181.74	8.7	113.12	60.77
Sod. acetate	7.8	246.99	182.70	8.3	138.43	82.87
CMC	7.9	229.01	166.98	8.0	145.55	89.35
Dextrose	7.5	482.66	412.89	7.7	251.00	184.98
Nitrogen source	**ZnO**			**$ZnCO_3$**		
	pH	Zn concentration (mg L^{-1})		pH	Zn concentration (mg L^{-1})	
		Shaking	Static		Shaking	Static
Amm. sulphate	4.3	822.61	750.50	4.0	853.74	779.86
Amm. chloride	4.1	830.88	751.98	4.0	848.00	768.43
Urea	5.1	796.32	719.04	4.8	817.00	740.23
Sod. nitrate	5.6	784.54	698.00	5.0	801.00	739.98
Peptone	5.0	804.29	736.98	4.8	815.35	738.93
Yeast extract	5.9	763.75	691.33	5.5	788.98	703.21
Amm. molybdate	5.3	792.00	711.34	5.0	807.00	743.90
Amm. oxlate	5.3	790.88	714.97	4.9	815.85	744.12

Pot experiment

Total chlorophyll content and dry weight (g plant^{-1})

Significant variations among the treatments were observed in total chlorophyll content and dry weight (g plant^{-1}) of *Zea mays* plants at two stages of crop growth under non-sterile and sterile soils conditions (Table 5). Zinc applications and *E. cloacae* inoculation had no significant effect on total chlorophyll content at 30 days under unsterile and sterile soils but, there were large differences between the treatments at 60 days. The treatments *E. cloacae* + ZnO and *E. cloacae* + $ZnCO_3$ were the superior treatments in this context which recorded values at 41.97 and 41.77 under unsterile soils and 45.93 and 43.87 under sterile soils, respectively, compared to the control at 60 days.

The differences mostly were significant for *E. cloacae* + ZnO followed by *E. cloacae* + $ZnCO_3$ treatments gave the highest dry weight under circumstances of non-sterile or sterile soils. It recorded 3.93 and 3.84 compared to control 2.67 g $plant^{-1}$ for unsterile soils and 4.91 and 4.15 compared to control 2.70 g $plant^{-1}$ for sterile soils at 30 days. Similarly trend as dry weight g $plant^{-1}$ are showed at 60 days. The increase in total chlorophyll content due to combined mineral fertilizer and inoculation of one or more organisms has been documented by several workers (Balamurgan & Gunasekharan, 1996 and Devananda, 2000). Similarly, Biswas *et al.* (2000) and Senthilkumar (2003) have documented the increase in total chlorophyll content when using mineral fertilizer and inoculation with *E. cloacae* in wheat, corn and radish.

TABLE 5. Effect of Zn applications and *E. cloacae* inoculation on chlorophyll content and dry weight (g $plant^{-1}$) in *Zea mays* plant under sterile and Non-sterile soils at 30 and 60 days.

Treatments		Chlorophyll		Dry weight	
Main	**Sub main**	**30 days**	**60 days**	**30 days**	**60 days**
Non-sterile soil	Control	29.90	40.40 ^{cd}	2.67 ^{g}	12.77 ^{h}
	E. cloacae	31.10	41.50 ^{bc}	3.11^{f}	13.17 ^{g}
	ZnO	31.17	40.90 ^{bc}	3.42 ^{ef}	13.49 ^{fg}
	$ZnCO_3$	30.33	40.40 ^{cd}	3.38 ^{ef}	13.93 ^{e}
	E. cloacae + ZnO	33.83	41.97 ^{bc}	3.93 ^{bc}	14.98 ^{bc}
	E. cloacae +$ZnCO_3$	32.17	41.77 ^{bc}	3.84 ^{bcd}	14.68 ^{cd}
Sterile soil	Control	28.53	38.03 ^{d}	2.70 ^{g}	13.65 ^{ef}
	E. cloacae	30.97	41.37 ^{bc}	3.34 ^{ef}	14.51 ^{d}
	ZnO	30.87	41.30 ^{bc}	3.60 ^{cde}	14.62 ^{d}
	$ZnCO_3$	30.43	42.23 ^{bc}	3.48 ^{def}	14.52 ^{d}
	E. cloacae + ZnO	36.03	45.93 ^{a}	4.91 ^{a}	15.83 ^{a}
	E. cloacae +$ZnCO_3$	32.43	43.87 ^{b}	4.15 ^{b}	15.22 ^{b}
F. test		n.s	**	**	**
L.S.D 5%		--	2.04	0.31	0.32
Significance due to:					
Main (soil)		n.s	n.s	n.s	**
L.S.D 5%		--	--	--	0.27
Sub main (treatment)		**	**	**	**
L.S.D 5%		1.28	1.44	0.22	0.23

The increase in dry weight of plant may be due to the effective specific Zn applications and *E. cloacae* inoculation used in the applied fertilizer and inoculum. Sarathambal *et al.* (2010) showed that the inoculation of *Gluconacetobacter diazotrophicus* in maize rhizosphere with ^{65}ZnO and $^{65}ZnCO_3$ increased the dry weight of plant compared to the control.

Ramesh *et al.* (2014) reported that inoculation significantly (with *E. cloacae*) enhanced shoot and seed dry weight up to 14 and 16 %, respectively, in soybean, and 39 and 49 % in wheat compared to un-inoculated control.

Mehnaz *et al.* (2010) and Montañez *et al.* (2012) trend a similar increase in growth parameters with inoculation by *Enterobacter*.

Zinc content (mg kg^{-1})

Data presented in Table 6 indicated that inoculation with *E. cloacae* alone or with different zinc applications variably increase zinc percentages of *Zea mays*. The highest content of Zn in plants were noted for inoculation with *E. cloacae* and ZnO (57.48 and 56.71 mg kg^{-1}, respectively) followed by inoculation with *E. cloacae* and $ZnCO_3$ (51.43 and 48.86 mg kg^{-1}, respectively) under non-sterile and sterile soils at 30 days. A similar trend of Zn content occurred at 60 days.

TABLE 6. Effect of Zn applications and *E. cloacae* inoculation on Zinc content (mg kg^{-1}) in *Zea mays* plant under sterile and Non-sterile soils at 30 and 60 days.

Treatments		Zn (mg kg^{-1})	
Main	**Sub main**	**30 days**	**60 days**
Non-sterile soil	Control	37.57 ^{d}	47.78 ^{f}
	E. cloacae	60.65 ^{a}	68.32 ^{ab}
	ZnO	50.55 ^{bc}	62.51 ^{d}
	$ZnCO_3$	39.33 ^{d}	52.31 ^{e}
	E. cloacae + ZnO	57.48 ^{a}	70.35 ^{a}
	E. cloacae +$ZnCO_3$	51.43 ^{bc}	65.46 ^{c}
Sterile soil	Control	34.15 ^{e}	44.77 ^{g}
	E. cloacae	53.14 ^{b}	67.85 ^{b}
	ZnO	47.90 ^{c}	60.76 ^{d}
	$ZnCO_3$	38.64 ^{d}	49.61 ^{f}
	E. cloacae + ZnO	56.71 ^{a}	70.55 ^{a}
	E. cloacae +$ZnCO_3$	48.86 ^{c}	67.47 ^{bc}
F. test		*	**
L.S.D 5%		3.41	2.18
Significance due to			
Main (soil)		**	n.s
L.S.D 5%		2.65	--
Sub main (treatment(		**	*
L.S.D 5%		2.41	1.54

The inoculation of *E. cloacae* with ZnO and $ZnCO_3$ increased the available zinc content in the soil. However, the available zinc content was greater in non-sterile than the sterile soils conditions. This clearly indicated that microorganism other than *E. cloacae* may also involve the solubilization of insoluble zinc sources. Zinc solubilizing potential of a few bacterial genera namely *Pseudomonas fluorescens*, *Thiobacillus ferroxidans*, *Bacillus* spp. and *T. thiooxidans* have been reported (Hutchins *et al.*, 1986 and Di Simine *et al.*, 1998).

Sarathambal *et al.*, (2010) Irrespective of soil types, the inoculation of *Gluconacetobacter diazotrophicus* showed higher plant zinc content. The total zinc content was more in unsterile zinc deficient soil. This may due to more zinc available due to solubilization of insoluble zinc compounds by soil microorganisms or *G. diazotrophicus* in non-sterile soil. On the other hand, Pascal (1962) reported the application of ZnO with *G. diazotrophicus* showed better uptake of the nutrient. ZnO is a sparingly soluble compound ($3–5 \times 10^4 g^{-1}$ 100^{-1} water at 25°C) compared to $ZnCO_3$ which might be the added advantage of ZnO.

Conclusions

The artificial application of zinc as fertilizer in crop production increases cost of cultivation. The available Zn in soil in different forms may be unavailable to plants, but it can be solubilized by inoculating with Zn solubilizing bacterial species.

Zinc solubilization depends on the metabolic activities of microorganisms. Many factors such as nitrogen and carbon sources may affect the efficiency of solubilization. In this research, *E. cloacae* was able to solubilize Zn compounds with a wide range of nutrients.

The over-all results of the present study suggest that ZSB inoculation with zinc oxide would be a better results for the present Zn-fertilization, which could also improve crop growth and improve soil fertility.

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عزل وتعريف البكتيريا المذيبة للزنك ومدى كفائتها على نمو نبات الذرة الشامية

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تم عزل ٢٥ عزلة بكتيرية من الكمبوست الناضج وذلك بغرض تقييم إمكانية إستخدامها في تيسير مركبات الزنك الغير ذائبة وهي أكسيد الزنك وكربونات الزنك. تم إختيار أكفأ العزلات البكتيرية وهي Zn14 . أجريت بعض الخصائص الظاهرية والبيوكيميائية بالإضافة إلى التعريف بإستخدام 16S rRNA والتى أسفرت نتائجها عن أن هذه العزلة هى *Enterobacter cloacae*.

تم دراسة تأثير مصادر الكربون والنيتروجين على كفاءة الذوبان لمركبات الزنك الغير ذائبة تحت ظروف الرج والثبات. وقد أظهرت النتائج قدرة السلالة على إذابة مركبات الزنك تحت مدى واسع من المصادر الغذائية.

أجريت تجربة أصص بمحطة البحوث الزراعية – سخا – كفرالشيخ – مصر وذلك بغرض دراسة كفاءة إستخدام بكتيريا *Enterobacter cloacae* كمذيب لمركبات الزنك المضافة للتربة ومدى تأثيرها على مقاييس النمو لنباتات الذرة الشامية تحت ظروف التربة المعقمة والغير معقمة خلال ٣٠ و ٦٠ يوم من عمر النبات.

أظهرت النتائج أن هناك إستجابة معنوية عالية في زيادة الزنك الممتص في كل المعاملات بالمقارنة بالكنترول. أيضا أثبتت المعاملة بالتلقيح ب*E . cloacae* + ZnO أنها الأكفأ خاصة في مقاييس النمو المختلفة ومنها الكلوروفيل، الوزن الجاف للنبات (جرام/ نبات) وذلك بالمقارنة بالكنترول.

لذا، نخلص من هذه الدراسة أن التلقيح ببكتيريا *Enterobacter cloacae* *يساهم في* تيسير مركبات الزنك الغير ذائبة للنبات مما يؤدى إلى زيادة نمو النبات وكذا المحافظة على خصوبة التربة.