MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Endophytes isolated from wheat and phragmites and their effect on maize grain priming

Ashraf Elsaye [1], Marwa Magdy Fawzy[1], Ahmed Shawkey Gebreil[1] and Amr Mohammed Mowafy[1]*

[1]*Botany Department, Faculty of Science, Mansoura University, 35516 Mansoura City, Egypt*

**** Correspondent author**: marwamagdi911993@gmail.com 01022931186*

Received:11/5/2019
Accepted: 1/6/2019

Abstract: The use of plant growth promoting bacteria (PGPB) is considered as a main part of today's sustainable agriculture practices. The intended target of this study was to use PGPB to stimulate maize priming. The endophytes inhabiting both Wheat and Phragmites were the source of the desired isolates. These plants were collected and then surface sterilized and the endophytes were isolated from their different parts. The obtained isolates were tested as priming agents to stimulate maize growth. Out of the twenty-one obtained isolates, the three isolates E1S2, MK2R2 and B2L2 stimulated the growth of maize grains to 98%, 95% and 95% compared to 85% for water and 80% for media priming. These isolates have been molecularly identified as Enterobacter cloacae, Klebsiella michiganensis and Bacillus subtilis respectively. Additionally, other germination vigor parameters such as length of plant root and hypocotyl in addition to number of lateral roots increased for various degrees in response to these biogenic treatments. The obtained isolates Enterobacter E1S2, Klebsiella MK2R2 and Bacillus B2L2 were found to be the best maize growth promoting bacteria according to the results obtained from the preliminary laboratory experiments shown in this study. These results pave the way for studying the plant growth promoting criteria of these isolates in order to use them to improve maize growth and metabolism, a study that is being carried out in the field conditions.

keywords: **:** *Bacillus*, Endophytes, *Enterobacter*, maize, germination percentage, *Klebsiella*.

1.Introduction

The increase in agricultural productivity is one of the most priorities for all governments to face the growing population that is expected to reach 9.1 billion in 2050 [1]. The achievement of this goal is becoming so difficult due to the reduced arable land through urban sprawl, climate change and poor land management practices, a situation that led researchers to explore non-traditional farming practices [2].

The purposeful use of plant growth promoting bacteria (PGPB) as biofertilizers in agriculture is a promising technology to get more effective and environmentally friendly solutions with the potentiality to ensure food security [3].

Plant growth promoting bacteria are classified into three categories: those that colonize the root surface (rhizosphere bacteria), others being in symbiotic relationships with host plants (symbiotic bacteria), in addition to those can enter into the root interior and colonize the plant (entophytic bacteria) [4, 5].

Generally, bacterial endophytes are neither organ nor host specific [6]. A lot of endophytes have been isolated from different tissue parts of many plant species [7]. Endophytic bacteria have been associated with the growth enhancement of many crops like potato, lettuce, maize, tomato, cucumber, cotton and can thus preserve or enhance crop yield [8-10].

Their effects on plant growth include the increase in plant height, root and shoot biomass, potato and tuber production, root leaf-hair formation, and lignification of xylem vessels [11, 12]. Evidence for the presence of endophytic bacteria in maize leaves has so far been provided by several authors [13-15].

Maize is an important economic cereal as a food source for both man and animal. For the increasing needs, several techniques have been developed to increase the productivity of maize in an environmentally friendly way and the use of plant growth promoting bacteria is one of these strategies. *Rhizobium, Bacillus and Azotobacter* have shown a significant effect on the growth and yield of maize plants [16]. Therefore, the objective of the current study is to isolate, identify and evaluate the endophytes obtained from wheat and *Phragmites*, for their belonging to the same family of maize "Poaceae", as a probiotic agent to promote priming of maize grains

2.M aterials and methods

1- Isolation of Endophytic bacteria

For the endophytic bacterial isolation, healthy leaves, stems, and roots of cultivated wheat and *Phragmites* were collected from different agricultural farmlands around Mansoura and Talkha cities, Dakahlia governorate, Egypt in summer season during May 2018. The samples were placed in sterilized plastic bags, brought to the laboratory and used for further experimental purposes.

Surface sterilization of the plant parts was the initial step for isolation of endophytic bacteria in order to eliminate all the surface undesired microbes. The collected plant parts were first sterilized by running tap water for 15 minutes, and then immersed in 70% ethanol for 3 min [17] followed by 5% NaOHCl washing [18]. After all, the samples were washed 3 times by sterilized distilled water. Validation of the surface sterilization procedures was attained by culturing aliquots of water from the last rinsing onto nutrient media [19].

After drying under aseptic conditions, each sample was divided into small pieces (1-3 cm) and plated on LB agar media [20]. The incubation period of these plates at 28°C was around 3 days [25]. The obtained bacterial colonies were further purified on LB media. The obtained pure isolates were maintained in 50% glycerol stocks at -20°C for further experiments [21].

2- Molecular identification of the endophytic bacteria

The isolates that gave the best results in maize seedling growth stimulation, as will be shown later, were molecularly identified. The genomic DNA was extracted for each sample by using GeneJET Genomic DNA Purification Kit (Sigma, 168 Third Avenue Waltham, MA, USA) according to the protocol supplied by the manufacturer. PCR amplification was done by using the genomic DNA as a template in 20 µl reaction mixture containing Maxima Hot Start PCR Master Mix (Thermo K1051) using the universal primers; 27 f (5′-AGAGTTTGATCC TGGCTCAG-3′) and p1492r (5′-TACGGCTACCTTGTTACGACT-3′).
Thermal cycling was carried as described previously [22].

The sequencing reactions were performed in a total volume of 20 µl (7 µl of the purified PCR product and 13 µl of the sequencing module) by adjusting the thermal cycler conditions as described previously [22]. The excess dye terminators and primers were removed from the cycle sequencing reaction using Dye Ex^{TM} 2.0 Spin Kit (Qiagen PN 63204). The sequences obtained were analyzed by Finch TV (version 1.4.0) software. The isolate sequences were submitted to the GenBank.

3- Priming of maize grains by the isolated endophytes

A- Inoculum preparation

The obtained 21 isolates were cultured in 250 mL LB broth containing spectinomycin as antifungal [100 µg/ml] at 28^{o}C for 48 h in an orbital shaking incubator (compact, model: JSR-100C/Korea) with 180 rpm. By using a spectrophotometer (Jenway, 7315), the optical density of these cultures were measured at 600 nm and adjusted to 0.5 to get a uniform population of endophytic bacteria [10^8 – 10^9 colony forming units (CFU)] for the inoculation step [23].

B- Maize grain treatment

Surface sterilization of maize grains (white single hybrid; Pioneer 30K8) was done as described previously [21, 22]. The grains were further primed by the obtained 21 isolates, prepared as described previously, for 30 minutes at 28^{o}C. Twenty surface-sterilized grains were transferred into sterilized plastic boxes containing sterile moisten filter papers

and incubated at 28°C for 96 h. The percent of germinated grains, length of plant root and hypocotyl in addition to the number of lateral roots were measured. Two controls have been used for this experiment the water control and the media control "grains pretreated with LB media without bacteria"[23].

Statistical analysis

The obtained data were analyzed using two-way ANOVA followed by Duncan's test at probability level 0.05 using COSTAT software program

3. Results and Discussion

1.Screening of endophytic bacteria for maize priming

Twenty-one bacterial isolates were obtained from wheat and *Phragmites* tissues (Table 1). The isolates have been coded as indicated in Tables 2 for wheat isolates and in Table 3 for *Phragmites* isolates.

Table (1) : Number of endophytic bacterial isolates from wheat and *Phragmites* parts.

Plant parts	Wheat endophytes	*Phragmites* endophytes
Root	5	3
Stem	4	3
Leaf	4	2

For priming by wheat isolates, the maximum significant increase in maize grain germination percentage (98.00%), length of plant roots (8.10) and hypocotyl (4.00) in addition to number of lateral roots (4.70) was observed by priming with the wheat isolate B2L2 followed by MK2R2 then 2R1w wheat isolates (Table 2). However, a significant decrease was recorded in response to 2L3w in all parameters compared with the control.

For priming by *Phragmites* isolates, the isolates E1S2 and 1R2p treatments led to a significant increase in all measured parameters (Table 3). Also, 2S1p and 1S1p isolates showed a significant increase in length of plant roots and hypocotyl in addition to the number of lateral roots. The 1L1p isolate led to a significant decrease in root length (3.45), number of lateral roots (1.86).

Upon these priming experiments, it has been found that the isolates 2R1w, MK2R2 and B2L2 from wheat and isolates E1S2, 1R2p and 2S1p from *Phragmites* were the best probiotic agents. In order to confirm these results, these isolates were tested again at the same time in one experiment. The obtained results were as previously obtained (Table 4) and the most affected parameter by priming was root length. However, the isolates E1S2, MK2R2 and B2L2 were the most potentials (Figure 1) and upon that they were selected for molecular identification

Table (2) : Effect of endophytic bacterial strains isolated from wheat (coded w) roots (coded R), leaves (coded L) and stem (coded S) on maize grains under laboratory conditions.

Treatment	Germination %	Root Length (Cm)	No. of Lateral Roots	Hypocotyl Length (Cm)
Control	$85^{cd} \pm 0.03$	$4.35^{d} \pm 0.28$	$3.36^{cd} \pm 0.03$	$1.23^{def} \pm 0.03$
Media	$85^{cd} \pm 0.03$	$4^{def} \pm 0.14$	$2.2^{fg} \pm 0.45$	$1.45^{d} \pm 0.06$
1R1w	$80^{d} \pm 0.03$	$4^{def} \pm 0.27$	$4.1^{defg} \pm 0.2$	$1.22^{def} \pm 0.07$
1R2w	$80^{d} \pm 0.03$	$3.5^{fg} \pm 0.06$	$3.2^{cd} \pm 0.13$	$1.05^{ef} \pm 0.11$
1R3w	$80^{d} \pm 0.03$	$3.4^{g} \pm 0.02$	$2.1^{gh} \pm 0.12$	$0.89^{f} \pm 0.07$
2R1w	$95^{ab} \pm 0.03$	$6.7^{c} \pm 0.07$	$4.25^{ab} \pm 0.06$	$3.6^{b} \pm 0.16$
MK2R2	$97^{ab} \pm 0.02$	$7.3^{b} \pm 0.09$	$4.35^{ab} \pm 0.06$	$3.8^{ab} \pm 0.13$
1L1w	$85^{cd} \pm 0.03$	$3.67^{efg} \pm 0.03$	$2.48^{efg} \pm 0.06$	$1.13^{def} \pm 0.05$
2L1w	$85^{cd} \pm 0.02$	$4.1^{de} \pm 0.38$	$3.7^{bc} \pm 0.37$	$1.83^{c} \pm 0.08$
B 2L2	$98^{a} \pm 0.02$	$8.1^{a} \pm 0.2$	$4.7^{a} \pm 0.07$	$4^{a} \pm 0.06$
2L3w	$55^{e} \pm 0.03$	$2.75^{h} \pm 0.12$	$1.53^{h} \pm 0.08$	$1.13^{def} \pm 0.28$
1S1w	$86^{cd} \pm 0.02$	$3.4^{g} \pm 0.06$	$2.8^{def} \pm 0.38$	$1.47^{d} \pm 0.17$
1S2w	$80^{d} \pm 0.03$	$3.8^{defg} \pm 0.03$	$2.4^{fg} \pm 0.04$	$1.4^{de} \pm 0.01$
2S1w	$90^{bc} \pm 0.03$	$4.2^{de} \pm 0.11$	$3.15^{cde} \pm 0.29$	$2.1^{c} \pm 0.09$
2S2w	$85^{cd} \pm 0.03$	$4.13^{de} \pm 0.14$	$3.12^{cde} \pm 0.01$	$1.13^{def} \pm 0.01$
LSD	7.56	0.48	0.61	0.33

The mean values followed by the same letter in each column are not significantly different from each other as detected at $\alpha = (p \leq 0.05)$, LSD= Least Significant Difference.

Table (3) : Effect of the endophytic bacterial strains isolated from *Phragmites* (coded p) roots (coded R), leaves (coded L) and stem (coded S) on maize grains under laboratory conditions.

Treatment	Germination%	Root Length (Cm)	No. of Lateral Roots	Hypocotyl Length (Cm)
Control	$85^{b} \pm 0.03$	$4.5^{ef} \pm 0.64$	$2.6^{e} \pm 0.2$	$2.4^{d} \pm 0.22$
Media	$68^{c} \pm 0.33$	$2.7^{h} \pm 0.14$	$1.6^{g} \pm 0.28$	$1.4^{e} \pm 0.08$
1L1p	$65^{c} \pm 0.03$	$3.45^{gh} \pm 0.08$	$1.86^{fg} \pm 0.12$	$1.6^{e} \pm 0.11$
2L1p	$85^{b} \pm 0.03$	$3.65^{fg} \pm 0.14$	$2.35^{ef} \pm 0.23$	$2.1^{d} \pm 0.12$
1S1p	$90^{ab} \pm 0.03$	$5.5^{cd} \pm 0.38$	$3.3^{cd} \pm 0.07$	$3^{bc} \pm 0.15$
E1S2	$98^{a} \pm 0.02$	$8.8^{a} \pm 0.016$	$4.9^{a} \pm 0.12$	$4.4^{a} \pm 0.11$
2S1p	$93^{ab} \pm 0.02$	$7.3^{b} \pm 0.4$	$4.1^{b} \pm 0.25$	$4^{a} \pm 0.17$
1R1p	$85^{b} \pm 0.03$	$5^{de} \pm 0.35$	$2.7^{de} \pm 0.15$	$2.1^{d} \pm 0.04$
1R2p	$95^{a} \pm 0.03$	$6.2^{c} \pm 0.1$	$3.43^{c} \pm 0.31$	$3.3^{b} \pm 0.21$
2R1p	$85^{b} \pm 0.03$	$5.1^{de} \pm 0.27$	$3.3^{cd} \pm 0.1$	$3^{c} \pm 0.18$
LSD	7.8	0.9	0.55	0.42

The mean values followed by the same letter in each column are not significantly different from each other as detected at $\alpha = (p \leq 0.05)$, LSD= Least Significant Difference.

Table (4) : Effect of candidate endophytic bacteria isolated from wheat and *Phragmites* on maize grains under laboratory conditions.

Treatment	Germination %	Root Length (Cm)	No. of Lateral Roots	Hypocotyl Length (Cm)
Control	$85^{bc} \pm 0.03$	$3.6^{cd} \pm 0.13$	$1.7^{de} \pm 0.12$	$1.6^{cd} \pm 0.07$
Media	$80^{c} \pm 0.03$	$3.3^{d} \pm 0.08$	$1.45^{e} \pm 0.06$	$1.35^{d} \pm 0.13$
2R1w	$90^{ab} \pm 0.03$	$3.87^{c} \pm 0.06$	$2.45^{c} \pm 0.01$	$1.5^{cd} \pm 0.06$
MK2R2	$95^{a} \pm 0.03$	$5.86^{a} \pm 0.4$	$2.9^{b} \pm 0.2$	$1.8^{abc} \pm 0.11$
B 2L2	$95^{a} \pm 0.03$	$6.3^{a} \pm 0.18$	$3.1^{b} \pm 0.16$	$2^{a} \pm 0.08$
E1S2	$98^{a} \pm 0.02$	$6^{a} \pm 0.25$	$3.6^{a} \pm 0.17$	$2^{a} \pm 0.05$
1R2p	$85^{bc} \pm 0.03$	$4.1^{bc} \pm 0.06$	$2.7^{bc} \pm 0.04$	$1.6^{bcd} \pm 0.01$
2S1p	$85^{bc} \pm 0.03$	$4.6^{b} \pm 0.04$	$1.85^{d} \pm 0.02$	$1.4^{d} \pm 0.03$
LSD	7.9	0.56	0.35	0.22

The mean values followed by the same letter in each column are not significantly different from each other as detected at $\alpha = (p \leq 0.05)$, LSD= Least Significant Difference.

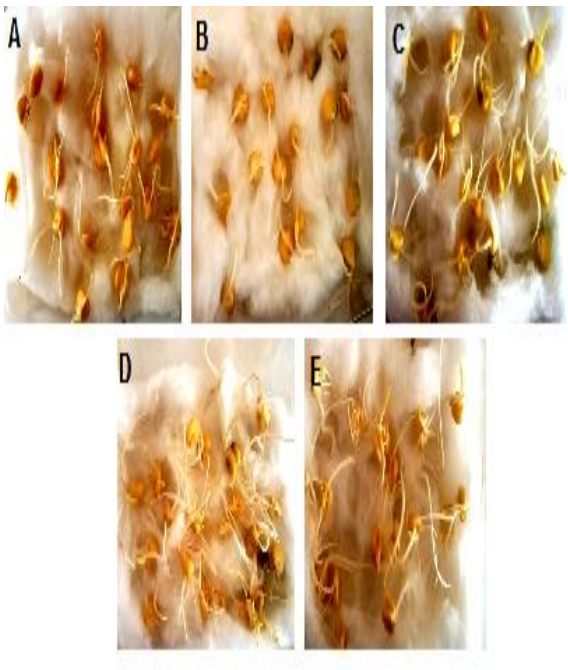


Fig. (1) : Germination of maize grains treated with the candidate endophytic bacteria A: control; B: media; C: MK2R2; D: B2L2 and E: E1S2 isolates. The germination percent and parameters were found to be at the maximum level in E followed by D and C compared with the control ones A and B.

From morphology of the three selected isolates, E1S2 and MK2R2, have been regarded as Gram negative rod shape bacteria and the other one B2L2 has been regarded as Gram positive rod shaped one (Table 5 and Figure 2 .(

Table (5) : Characteristic features of the three selected endophytic bacterial isolates.

Characteristic (S)	E1S2	MK2R2	B2L2
Cell Shape	Small Rod	Rod	Irregular Rod
Gram Reaction	Gram-ve	Gram-ve	Gram+ve
Pigmentation	Creamy	White Translucent	White
Margin	Smooth (Entire)	Undulate	Undulate (Wavy)
Texture	Shiny	Mucoid	Dry/Rough
Temp	28-30	28-30	28-30
Elevation	Convex	Umbonate	Umbonate (Raised)
Motility	Motile	Non- Motile	Motile

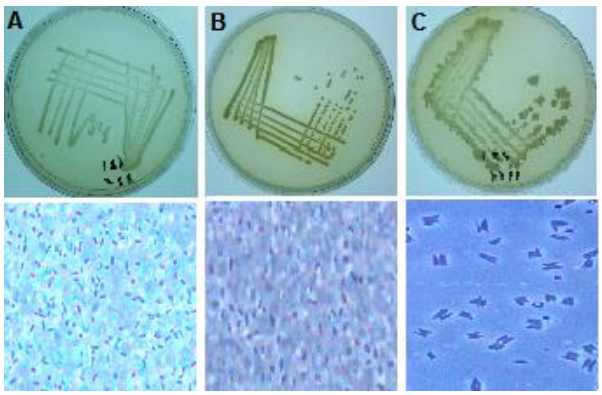
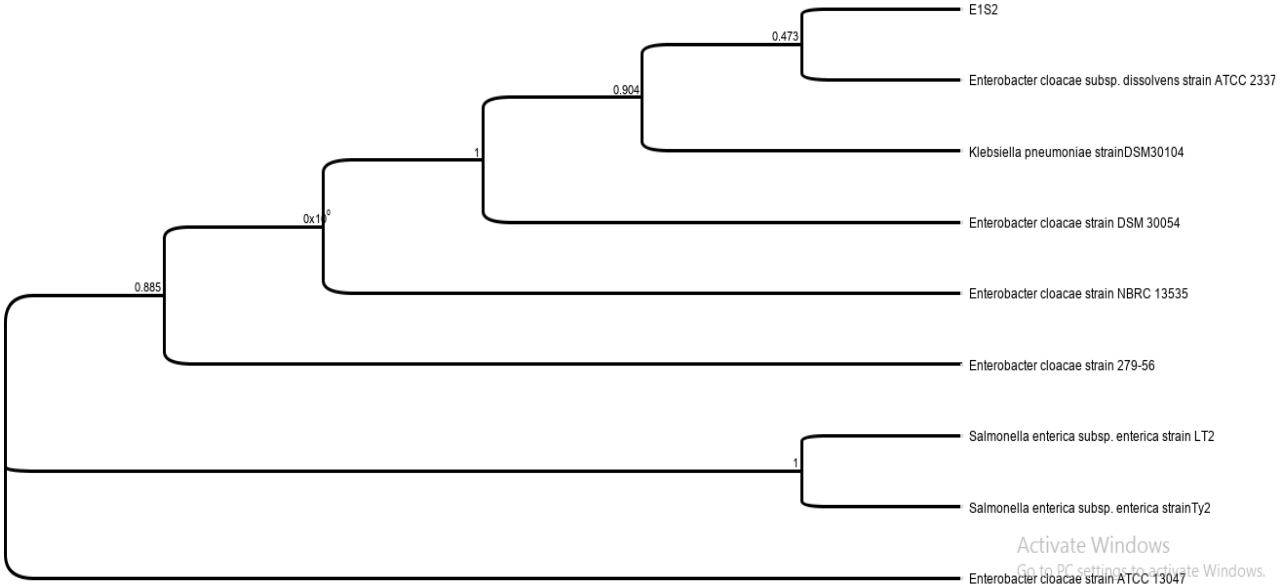


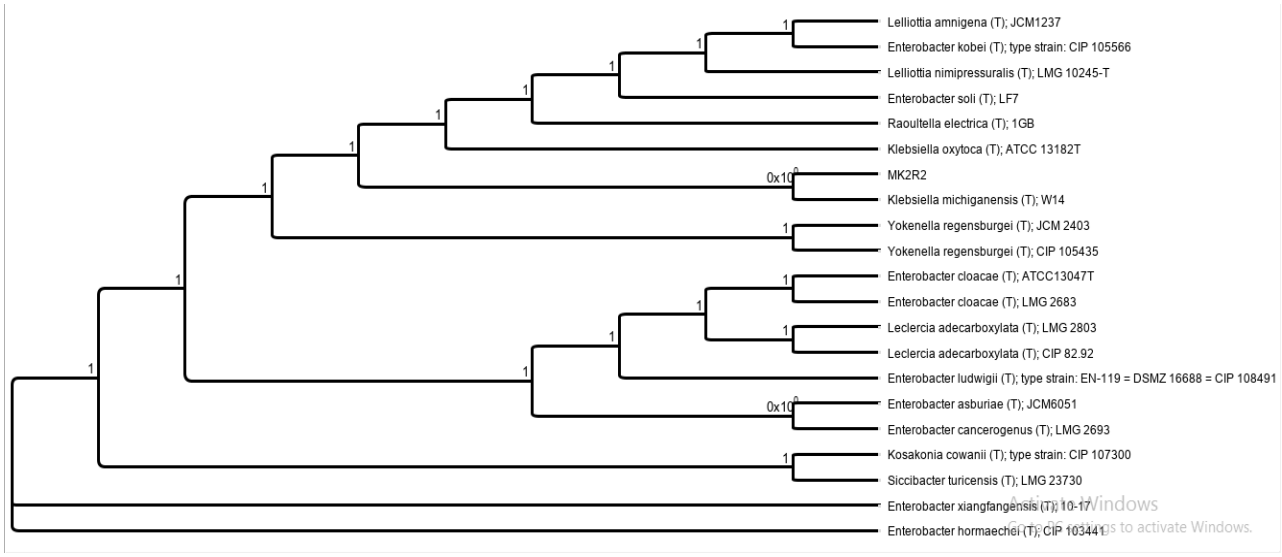
Figure 2: Cultural morphology of the three selected endophytic bacterial isolates (A: E1S2; B: MK2R2 and C: B2L2 isolates) on LB agar media and microscopic examination of unstained living cells using phase contrast microscope at 1000X. The isolate E1S2 had a creamy pigmentation with convex elevation, while isolates MK2R2 and B2L2 had a white pigmentation with elevation. All of them are shown to be unicellular rod-shaped but B2L2 isolate showed unusual bicellular aggregations.

Molecular identification of isolated bacterial endophytes

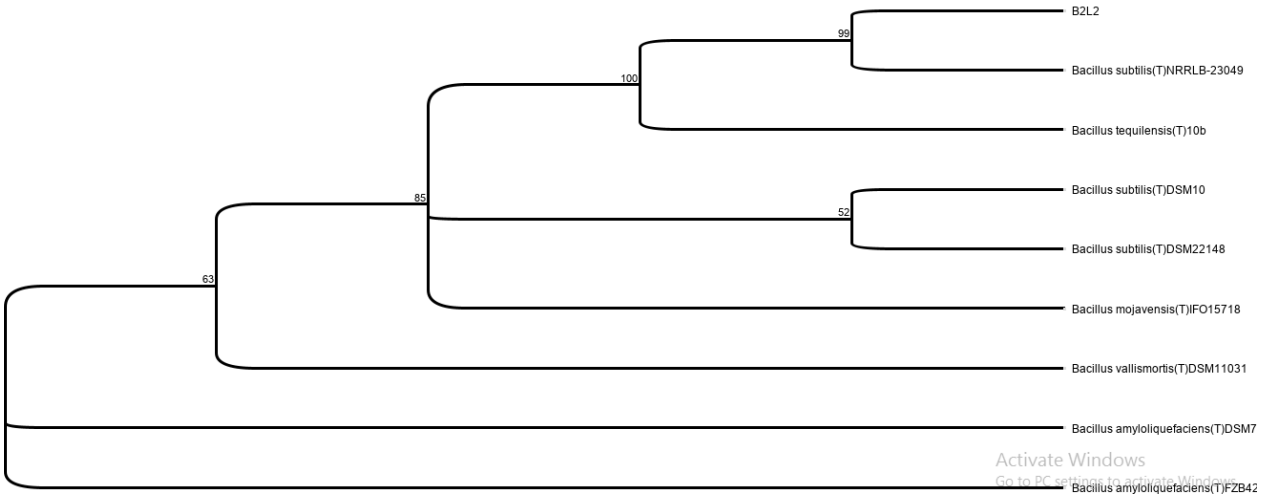
The obtained sequence for each of the three isolates was compared to type strains in the GenBank (NCBI). The 16S rRNA sequence of isolates E1S2, MK2R2 and B2L2 showed high levels of sequence similarity to Enterobacter cloacae, MK2R2 to Klebsiella michiganensis and B2L2 to Bacillus subtilis respectively. The obtained sequences were submitted to GenBank with accession numbers MK574871, MK464251 and MK574870 respectively. Phylogenetics is important for describing taxonomic classification of an organism based on their evolutionary history (Figure 3)



A



B



C

Figure 3: Neighbor-joining phylogenetic analysis resulting from the multiple alignment of 16S rRNA gene sequence of endophytic bacterial isolates **A**: E1S2; **B**: MK2R2; **C**: B2L2)

Discussion

The use of PGPB is currently gaining worldwide interest as a promising alternative to the chemical fertilizers and pesticides. PGPB may employ different mechanisms to promote plant growth [24, 25]. In general, a bottom-up approach is employed to select strains, which are promising for field application. Initially, laboratory based tests of various activities potentially involved in plant growth promotion are used, followed by field application [26 - 28]. Many studies have been performed in different species of agricultural interest, like maize [29, 30], wheat [31], potato [32], and rice [33.]

In this study, twenty-one bacterial isolates obtained from wheat and Phragmites were tested as probiotic factor to enhance maize seedling growth. Among them, three endophyte strains showed high plant growth-promoting activity. The molecular identification of these isolates showed the sequence identity of E1S2 to Enterobacter cloacae, MK2R2 to Klebsiella michiganensis and B2L2 to Bacillus subtilis.

In this study, the identified strains might be able to produce some growth regulating hormones such as auxins based on the significant increase of germination percentage, length of plant roots and hypocotyls in addition to the increase in number of lateral roots by 20-50% compared to the control.

Enterobacter has been recognized as endophytic bacteria in several plants such as sweet orange soybean and other crop plants [34, 35], as well as maize [36]. Species from this strain have been identified as plant-growth promoters as they have multiple growth-enhancing activities [37 - 40.]

Phytohormones such as auxins, and cytokinins41 can be produced by Enterobacter spp. and enhance plant root growth [42]. Enterobacter also promotes growth by siderophores synthesis [43] in addition to its ability to fix nitrogen [44]

Several species of Bacillus have been reported as maize kernel endophytes [36] and they have been isolated from sweet corn and cotton [29]. Among various reported plant growth-promoting and biocontrol bacterial species, Bacillus showed the highest potentiality at in vitro and in vivo assays [45]. It has been reported that B. subtilis produces various phytohormones such as indole-3-acetic acid (IAA), cytokinins, zeatin, gibberellic acid and abscisic acid which transported into the shoot through the xylem, delay senescence and thus boost production of lettuce, tomato, cucumber and pepper [46, 47]. Some rhizobacterial Bacillus strains have been found to promote plant growth by releasing volatiles [48]. Also they have been reported to enhance growth in crops such as corn due to the production of growth stimulating hormones [49] and phosphate solubilization [50].

Some Klebsiella species are found to be naturally associated with plants as beneficial organisms [51] for their ability to fix atmospheric nitrogen [52, 53]. Klebsiella

michiganensis SBP-8 has been regarded as a potential PGPB for its 1-aminocyclopropane-1-carboxylatedeaminase activity beside its ability to protect wheat from salt stress [17.[

The obtained isolates Enterobacter E1S2, Klebsiella MK2R2 and Bacillus B2L2 showed the ability to promote maize grains germination indicating their significance as PGPB. Their characteristic plant growth promoting activities as well as their effect on maize plant under field condition are being studied.

Conclusion

Summering up, the obtained data from this study showed that not all the endophytes obtained from wheat and Phragmites were compatible with maize as probiotic agent for growth. The isolates E1S2, MK2R2 and B2L2 those are closely relevant to Enterobacter cloacae, Klebsiella michiganensis and Bacillus subtilis respectively were selected as the best maize growth promoting bacteria according to the results obtained from the preliminary laboratory experiments shown in this study. These results pave the way for using these isolates to improve maize growth, a study that is being carried out under field conditions.

4. References

1. Alexandratos, N. and Bruinsma, (2012). World agriculture towards 2030/2050: the 2012revision, ESA Working paper FAO, Rome.Vol.**12** (**3**). Available from: http: //www.fao.org/3/a-ap106e.pdf.
2. Smith, P., J. I. House, M. Bustamante, J. Sobocká, R. Harper, G. Pan, P. Westand K.Paustian*et* al., (2016)."Global change pressures on soils from land use and management." Global Change Biology **22**(**3**): 1008-1028. Doi:10.1111/gcb.13068
3. Glick, B. R.,(2014). "Bacteria with ACC deaminase can promote plant growth and help to feed the world." Microbiological research**169**(1):3039.Doi:10.1016/j.micres .2013.09.009.
4. Stone, J. K., C. W. Bacon and Jr. J. F. White,(2000). An overview of endophytic microbes: endophytism defined. Microbial endophytes, CRC Press**:** 17-44.Doi: **10**. 1201/ 9781482277302.
5. Bacon, C. W. andD. M. Hinton, (2007). Bacterial endophytes: the endophytic niche, its occupants, and its utility. Plant-associated bacteria, Springer**:** 155-194. Doi:**10**.1007/978-1-4020-4538-7_5.
6. Rosenblueth, M. and E. Martínez-Romero,(2006) . Bacterial endophytes and their interactions with hosts. Molecular plant-microbe interactions **19**(**8**): 827-837. Doi: **10**.1094/MPMI-19-0827.
7. Palumbo, J. andD. Kobayashi,(2000). Bacterial endophytes and their effects on plants and uses in agriculture. Microbial Endophytes, CRC Press**:** 213-250. Doi: Https://doi.org/10.1201/9781482277302.
8. Hinton, D. M. and C. W. Bacon,(1995). Enterobacter cloacae is an endophytic symbiont of corn. Mycopathologia **129**(**2**): 117125.Doi:https://doi.org/10.1007/BF01 103471.
9. Zinniel, D. K., P. Lambrecht, N. B. Harris, Z. Feng, D. Kuczmarski and P. Higley*et al*., (2002). "Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants." Appl. Environ. Microbiol. **68**(**5**): 2198-2208. Doi**:** 10.1128/AEM.68.5.2198-2208.2002.
10. Asis, Jr. C. and K.Adachi,(2004). Isolation of endophytic diazotroph Pantoea agglomerans and nondiazotroph Enterobacter asburiae from sweetpotato stem in Japan. Letters in Applied Microbiology**38**(**1**):1923.Doi:10.1046/j.14 72-765X.2003.01434.x.
11. Pillay, V. and J. Nowak,(1997). "Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (Lycopersicon esculentum L.) seedlings inoculated with a pseudomonad bacterium." Canadian *Journal of Microbiology***43**(**4**):354361.Doi:10.1139/ m97-049.
12. Sturz, A. V., B. R. Christie, B. G. Matheson and J. Nowak,(1997). Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biology and Fertility of Soils **25**(1): 13-19. Doi: 10.1007/s003740050273.
13. McInroy, J. and J. Kloepper,(1991). "Analysis of population densities and identification of endophyte bacteria of maize and cotton in the field." Bulletin OILB SROP (France). ID=FR9202854.

14. Fisher, P. J., O. Petrini and H. L. Scott, (1992). The distribution of some fungal and bacterial endophytes in maize (Zea mays L.). New Phytologist **122**(**2**): 299-305. Doi: 10.1111/j.1469-8137.1992.tb04234.x.
15. Döbereiner, J., V. L. Baldani and V. M. Reis, (1995). Endophytic occurrence of diazotrophic bacteria in non-leguminous crops. Azospirillum VI and related microorganisms, Springer**:** 3-14. Doi: 10.1007/978-3-642-79906-8_1.
16. Singh, G., D. R. Biswas and T. S. Marwaha, (2010). Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (Zea mays) and wheat (Triticum aestivum L.): a hydroponics study under phytotron growth chamber. *Journal of plant nutrition* **33**(**8**): 1236-1251. Doi : 10.1080/01904161003765760.
17. Z in, N. M., N. I. Sarmin, N. Ghadin, D. F. Basri,N. M. Sidik, W. M. Hess and G. A. Strobel, (2007). "Bioactive endophytic streptomycetes from the Malay Peninsula. FEMS microbiology letters **274**(1): 83-88. Doi:10.1111/j.1574-6968.2007.00819.x.
18. Gardner, J. M., A. W. Feldmanand R. M. Zablotowicz, (1982). Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. Appl. Environ. Microbiol. **43**(6): 1335-1342. Https://www.ncbi.nlm.nih.gov/ pmc/ articles/ PMC244237/.
19. McInroy, J. and J. Kloepper, (1994). "Novel bacterial taxa inhabiting internal tissues of sweet corn and cotton." improving plant productivity with Rhizosphere bacteria: 190.Doi:10.1139/m97-131.
20. Ramalashmi, K., K. Prasanna Vengatesh, K. Magesh, R. Sanjana, S. Siril Joe, andK. Ravibalan, (2018). "A potential surface sterilization technique and culture media for the isolation of endophytic bacteria from Acalypha indica and its antibacterial activity." *J. Med. Plants Studies* **6**(**1**): 181-184. Http://www. Plantsjournal.com/ archives /2018/ vol6issue1/PartC/6-1-17-361.pdf.
21. Anjum, N. and R. Chandra, (2015). Endophytic bacteria: optimization of isolation procedure from various medicinal plants and their preliminary characterization. Asian *Journal of Pharmaceutical and Clinical Research* **8**: 233-238. Https:// innovareacademics.in /journals/index.php/ajpcr/article/view/6586
22. Gagne-Bourque, F., (2012). The Isolation, Identification and Characterization of Endophytes of Switchgrass (Panicum virgatum L.), a Bioenergy Crop, McGill University (Canada). Doi: 10.1111/jam.12088
23. Naveed, M., (2013). Maize endophytes-diversity, functionality and application potential.Dataset_id=9826&property_id=107
24. Dobbelaere, S., J.Vander Leyden and Y.Okon, (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical reviews in plant sciences **22**(2): 107-149.Doi: 10.1080/713610853
25. Mitter, B., G. Brader, M. Afzal, S. Compant, M. Naveed, F. Trognitz and A. Sessitsch, (2013). Advances in elucidating beneficial interactions between plants, soil, and bacteria. Advances in agronomy, Elsevier. **121:** 381445.Doi:10.1016/B978-0-12-407685-3.00007-4
26. Khalid, A., M. Arshad and Z. A. Zahir, (2004). "Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat." *Journal of Applied Microbiology* **96**(**3**): 473-480. Doi: 10.1046/j.1365-2672.2003.02161.x
27. Smyth, E. M., J. McCarthy, R. Nevin, M. R. Khan, J. M. Dow, F. O'gara and F. M. Doohan, (2011). "In vitro analyses are not reliable predictors of the plant growth promotion capability of bacteria; a Pseudomonas fluorescens strain that promotes the growth and yield of wheat." *Journal of applied microbiology* **111**(**3**): 683-692. Doi: 10.1111/j.1365-2672.2011.05079.x
28. Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. van Themaat and P. Schulze-Lefert, (2013). "Structure and functions of the bacterial microbiota of plants." Annual review of plant biology **64:** 807-838. Doi: 10.1146/annurev-arplant-050312-120106

29. McInroy, J. A. and J. W. Kloepper, (1995). "Survey of indigenous bacterial endophytes from cotton and sweet corn." Plant and soil **173**(2): 337-342. Doi: 10.1007/BF00011472
30. Gutiérrez-Zamora, M. and E. Martınez-Romero,(2001). "Natural endophytic association between Rhizobium etli and maize (Zea mays L.)." *Journal of biotechnology* **91**(**2-3**): 117-126.Doi:10.1016/S0168-1656(01)00332-7
31. Germida, J. and S. Siciliano,(2001). "Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars." Biology and Fertility of Soils **33**(**5**): 410-415.Doi: 10.1007/s003740100343
32. Sessitsch, A., J. G. Howieson, X. Perret, H. Antoun and E. Martinez-Romero, (20020. "Advances in Rhizobium research." Critical Reviews inPlant Sciences **21**(4): 323-378.Doi:10.1080/0735-260291044278
33. Sun, L., F. Qiu, X. Zhang, X. Dai, X. W. and Dongand Song, (2008). Endophytic bacterial diversity in rice (Oryza sativa L.) roots estimated by 16S rDNA sequence analysis.Microbial ecology **55**(3): 415-424. Doi: 10.1007/s00248-007-9287-1
34. Araújo, W. L., J. Marcon, W. Maccheroni, J. D. van Elsas, J. W. van Vuurde and J. L.Azevedo, (2002). "Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants." Appl. Environ. Microbiol. **68**(**10**):49064914.Doi**:** 10.1128/AEM.68.10.4906-4914.2002
35. Kuklinsky- Sobral, J., W. L. Araújo, R. Mendes, I. O. Geraldi, A. A. Pizzirani-Kleiner and J. L.Azevedo, (2004). "Isolation and characterization of soybean- associated bacteria and their potential for plant growth promotion." Environmental microbiology **6**(**12**): 1244-1251. Doi: 10.1111/j.1462-2920.2004.00658.x
36. Rijavec, T., A. Lapanje, M. Dermastia and M. Rupnik, (2007). Isolation of bacterial endophytes from germinated maize kernels. Canadian *journal of microbiology* **53**(**6**): 802-808.Doi: 10.1139/W07-048
37. Kämpfer, P., S. Ruppel and R. Remus, (2005). "Enterobacter radicincitans sp. nov., a plant growth promoting species of the family Enterobacteriaceae." Systematic and appliedmicrobiology **28**(**3**): 213-221. Doi: 10.1016/ j.syapm.2004.12. 007
38. Deepa, C. K., S. G. Dastager and A. Pandey, (2010). Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (Vigna unguiculata (L.) Walp.) seedling growth." World *Journal of Microbiology and Biotechnology* **26**(**7**): 1233-1240. Doi: 10.1007/ s11274-009- 0293-y
39. Montañez, A., A. R. Blanco, C. Barlocco, M. Beracochea and M. Sicardi, (2012). Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (Zea mays L.) and their inoculation effects in vitro.Applied Soil Ecology **58:** 21-28. Doi: 10.1016/ j.apsoil. 2012. 02. 009
40. Ramesh, A., S. K. Sharma, M. P. Sharma, N. Yadav and O. P.Joshi, (2014). Plant growth-promoting traits in Enterobacter cloacae subsp. dissolvens MDSR9 isolated from soybean rhizosphere and its impact on growth and nutrition of soybean and wheat upon inoculation. Agricultural Research **3**(1): 53-66. Doi: 10.1007/s40003-014-0100-3
41. Lodewyckx, C., J. Vangronsveld, F. Porteous, E. R. Moore, S. Taghavi, M. Mezgeay and D. V. der Lelie, (2002). Endophytic bacteria and their potential applications. Critical reviews in plant sciences **21**(**6**): 583-606. Doi:10.1080/0735-260291044377
42. Patten, C. L. and B. R. Glick, 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68**(**8**): 3795-3801. Doi: 10.1128/AEM.68.8.3795-3801.2002
43. Giongo, A., A. Beneduzi, A. Ambrosini, L. K. Vargas, M. R. Stroschein, F. L. Eltz, M. H. Bodanese-Zanettini and L. M. Passaglia, P.(2010). "Isolation and characterization of two plant growth-promoting bacteria from the rhizoplane of

a legume (Lupinus albescens) in sandy soil." Revista Brasileira de Ciência do Solo **34**(**2**): 361-369.Doi:10.1590/S0100-06832010000200009

44. James, E., (2000). Nitrogen fixation in endophytic and associative symbiosis. Field crops research **65**(2-3): 197-209. Doi:10.1016/S0378-4290(99)00087-8
45. Islam, M. T., M. Rahman, P. Pandey, C. K. Jha and A. Aeron, (2016). Bacilli and agrobiotechnology, Springer. Doi: 10.1007/978-3-319-44409-3
46. Vessey, J. K., (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and soil **255**(**2**): 571-586.Doi: 10.1023/A:1026037216893
47. Yobo, K. S., M. D. Laing and C. H. Hunter, (2004). Effect of commercially available rhizobacteria strains on growth and production of lettuce, tomato and pepper. South African *Journal of Plantand Soil* **21**(**4**): 230-235. Doi :10.1080/ 02571862.2004.10635054
48. Ryu, C. M., M. A. Farag, C. H. Hu, M. S. Reddy, H. X. Wei, P. W.Paré and J. W. Kloepper, (2003). Bacterial volatiles promote growth in Arabidopsis. Proceedings of the National Academy of Sciences **100**(**8**): 4927-4932. Doi: 10.1073/pnas.0730845100
49. Araujo, F. F. d., (2008). Seed inoculation with Bacillus subtilis, formulated with oyster meal and growth of corn, soybean and cotton. Ciência e Agrotecnologia **32**(**2**): 456-462.Doi: 10.1590/S1413-70542008000200017
50. Khan, A. A., G. Jilani, M. S. Akhtar, S. M. Naqvi and M. Rasheed, (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *J agric biol sci* **1**(1): 48-58. Https:// www.researchgate.net /profile/ SM_Saqlan_Naqvi/publication/307108426.
51. Martínez, J., L. Martínez, M. Rosenblueth, J. Silva and E. Martínez-Romero, (2004). How are gene sequences analyses modifying bacterial taxonomy? The case of Klebsiella." International Microbiology **7**(**4**): 261-268.DOI:10.2436/im.v7i4.9481
52. Triplett, E. W., (1996). Diazotrophic endophytes: progress and prospects for nitrogen fixation in monocots. Plant and Soil **186**(**1**): 29-38. Doi: 10.1007/BF 00035052
53. Iniguez, A. L., Y. Dong and E. W.Triplett, (2004). "Nitrogen fixation in wheat provided by Klebsiella pneumoniae 342." Molecular Plant-Microbe Interactions ,**17**(**10**): 1078-1085. Doi: 10.1094/MPMI.2004.17.10.1078