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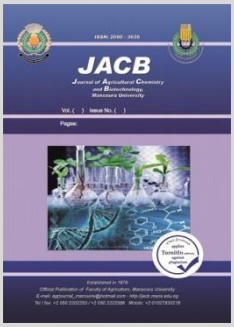
Biological control of two pathogenic fungi by silicate bacteria

Aida H. Afify[1*] and A. Z. A. Ashour[2]

[1] Department of Agricultural Microbiology, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
[2] Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza, Egypt



ABSTRACT

Silicate solubilizing bacteria (SSB) play a critical role in soil fertility by utilizing available forms of silicates minerals. Furthermore, SSB can also enhance plant defense mechanisms. Distribution of rhizobacteria in fields had been cultivated with flax and wheat which, are high silicate accumulators, and screened for available silicate on a selective medium, the bacterial counts varied markedly with a type of plantation in soil. Four bacterial strains were capable of solubilizing biotite (source of silicate mineral): *Pseudomonas fluorescens***,** *Bacillus subtilis*, *Proteus mirabilis*, and *Bacillus circulans*. By using dual culture antagonistic assays were carried out on these bacteria versus two pathogens fungi (*Fusarium oxysporum* and *Rhizoctonia solani*). The inhibition zone of these bacteria against two pathogenic fungi ranged 10 to 33 mm. The maximum inhibition zone by bacterial strains was shown by the strain *B. subtilis* followed by *Ps. fluorescens.* When detected the antagonistic metabolites, siderophores were produced by *Ps. fluorescens* and *B. subtilis* also these two strains with *B. criculans* produced chitinase. Only *Proteus mirabilis* produced hydrogen cyanide. The SSB strains showed different levels of efficiency in increasing the survival of flax seedlings however, two bacterial strains (*B. subtilis* & *Ps. fluorescens)* showed maximum efficiency under glasshouse conditions compared with the control. Finally, the strong action of silicate bacteria is very important in the development of bio fungicides against fungal pathogens.

***Keywords*:** Silicate bacteria; Biocontrol; Phytopathogenic fungi.

INTRODUCTION

Silicon (Si) promotes the growth and development of several crops (Afify, 1982 & 2022) and has been reported to reduce the incidence of many fungal diseases in different pathosystems by strengthening the cell walls, particularly the outer membrane of epidermal cells in leaves, thereby increasing resistance to the penetration of pathogenic fungi (Carver, *et al.*, 1987; Francois, *et al.*, 2005). Silicon remains in insoluble form unless solubilized by weathering action of minerals or biological activity of plant roots and microorganisms. Thus, silicate solubilizing bacteria (SSB) can play an important role by enhancing plant defense mechanisms (Vasanthi, *et al.*, 2012). Silicate bacteria are naturally occurring in soils (Zahra, 1969). Cultivation leads to an increase in their numbers, usually higher in the rhizosphere than in the soil outside this zone (Vintikova, 1964). Many microorganisms such as *Bacillus circulans* (Afify, 1982) *Proteus mirabilis, B. subtilis* (Afify and Bayoumy 2001), and *Rhizobium* sp. (Chandrakala, 2019) are capable of decomposing silicates. There are many ways by silicate bacteria to antagonize fungal pathogens. These include the production of many antagonistic metabolites as bioagents against fungal pathogens (Naureen, *et al.*, 2015; Ashour and Afify, 2016). Flax (*Linum usitatissimum* L.) is one of the most important sources of fiber and oil. Flax is susceptible to several fungal diseases such as seedling blight caused by *R. solani* Kuhn and *F.oxysporum* schlech. These fungi attack the plant at the seedling stage causing great economic losses under favorable conditions (Nyvall, 1981). In light of present-day constraints on plant disease control, biological control is increasingly capturing the attention of plant pathologists all over the world as a possible means of controlling soil-borne pathogens (Katz and Demain, 1977). Moreover, the antagonistic bacteria were repressed and decreased the disease incidence in plants (Afify and Ashour, 1995). Hence, this study aims to determine the distribution of bacteria in the rhizosphere of flax and wheat crops and evaluation of silicate bacteria for antagonistic activity against the two fungal pathogens *Fusarium oxysporum* and *Rhizoctonia solani* which cause flax seedling blight, also evaluation of these bacteria for increasing seedling survival under glasshouse conditions.

MATERIALS AND METHODS

Rhizobacterial count in soil samples and test of silicate solubilizing bacteria

Soil samples were collected from fields, which had been cultivated with flax (*Linum usitatissimum* L.) and wheat (*Triticam aestivum* L.) as these crops are high silicate accumulators. For rhizobacterial count, by the plate method as described by Naureen *et al.*, (2015). Plates containing agar medium supplemented with biotite as a source of silicate (Aleksandrov and Ternovs'ka, 1961). Plates were incubated at 30 ^{0}C for 24 - 48 hr then, morphologically distinct colonies were counted.

Source of silicate bacteria

Four bacterial isolates were isolated from the rhizosphere of flax and wheat identified at Agric. Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt as Pseudomonas fluorescens, Bacillus

* **Corresponding author.**
E-mail address: aidaafify@yahoo.com
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subtilis, Proteus mirabilis and Bacillus circulans (Afify, 2022). For studying their cultural characteristics, agar medium supplemented with biotite as a source of silicate was used according to Aleksandrov and Ternovs'ka (1961). These characteristics such as color, shape, and transparency were examined according to Prescott, et al., (1993).

Source of pathogenic fungi

Two pathogenic isolates of Fusarium oxysporum schlech and Rhizoctonia solani kuhn were used from the fungal collection of cotton pathol. Lab., Agricultural Research Center (ARC), Egypt. The isolates were originally isolated from roots blighted flax seedlings infected with damping-off.

Antagonistic assays

Antagonistic activity of selected bacterial isolates was tested against the two pathogenic fungi using dual culture assays as described by Naureen et al., (2009). The assay plates with PDA medium and incubated at 28 – 300 C for a week to record the inhibition zone (mm) of pathogenic fungal growth. There were three replicates (plates) for each bacterial strain.

Determination of bacterial antagonistic metabolites

These metabolites, include extracellular enzymes, siderophores, hydrogen cyanide (HCN), and ammonia (NH3). The ability of silicate bacteria to produce extracellular enzymes was detected according to the method of Nagarajkumer et al., (2004). Siderophore was determined on chrome-acurol-S-agar (CAS) according to Scher and Baker (1982). The method of HCN determination was described by Bakker and Schippers (1987) using tryptone soya agar supplemented with glycine (Dye, 1962).

Bacterial seed treatment

Each bacterial strain was grown for 48 h at 28ºC on a nutrient broth medium. For a single strain inoculation, 1.5 ml of a bacterial suspension and 5g of flax seeds (Sakha 1 cv.) were mixed in a small plastic bag, and sown in glasshouse potted soil (Mew and Rosales, 1986).

Preparation of fungal inoculum

The fungal inoculum was prepared by growing R.solani and F.oxysporum in 500 ml bottles containing barley grain medium (100 g of barley grains + 50 ml water), then incubated at 20ºC for 20 days. The inoculum was mixed throughout with soil at a rate of 0.1 g/kg of soil weight. A glasshouse study was conducted by using clay pots 20 cm in diameter with four replications. The soil used in the experiment was naturally clay soil (pH 7.5, E.C 1.4 mmhos/cm). 30 flax seeds treated with bacterial suspensions were planted in each pot one week after soil infestation. The percentage of surviving seedlings was recorded after 40 days from sowing.

Statistical analysis

Percentage data of glasshouse were transformed into arc sine angles before carrying out an analysis of variance (ANOVA) to produce an approximately constant variance. ANOVA was performed by the software MSTAT (A Micro-computer program for the Design, Management, and Analysis of Agronomic Research Experiments Michigan State Univ., USA).

RESULTS AND DISCUSSION

Counts of bacteria in soil

Soil samples were collected from fields that had been cultivating flax (F) and wheat (W) long time, which are rich in silicon and potassium minerals (Table 1). The bacterial counts (cfu/g dry soil) in the soil rhizosphere are given in Table (1). The counts appear to vary markedly with the type of plantation. The variation in densities of bacterial count revealed that the source of energy for microorganisms in virgin soil is a plant or animal residues in varying degrees of degradation. While in cultivated soils, the source of energy consists mainly of substances more easily available to microorganisms (Monib *et al.*, 1984).

Table 1. Effect of type plantation on the abundance of bacteria in soil

Soil sample No.	Standing crop	Location in soil	Counts $\times 10^5$ cfu / dry soil
1	Flax (F)	Rhizosphere	30
2	Wheat (W)	Rhizosphere	20

Silicate bacterial strains

The bacterial strains were checked for silicate solubilization on a selective agar medium (Aleksandrov and ternovs'ka, 1961) that was amended with the biotite as a source of silicate mineral. These four bacterial strains were observed for the cultural characteristics of silicate bacteria on a selective medium (Table 2).

Table 2. The cultural characterizations of silicate bacteria strains

Silicate bacteria isolates	Colony shape *
Pseudomonas fluorescens	Tear shape with raised
Bacillus subtilis	Tear shape and large white colour
Proteus mirabilis	Tear shape and mucous colonies
Bacillus circulans	Round, no colour mucous having the shape of a tear.

*** : colony shape on selective agar medium.**

It is also worth pointing out that Lauwers and Heinen (1974) have drawn attention to the role of such bacteria (*P. mirabilis*) to accumulate monomer silicate ions, which then deposited the silica again as a polymer in the stem and leaves. Urrutia and Beveridge (1995) stated that the bacteria *B. subtilis* produced a mineral phase in which a high proportion of silicate was found. Joseph *et al., (*2015) reported that the bacterial silicate can solubilize insoluble minerals such as silicates into soluble form by production of organic acids.

Antagonism

The selected four bacterial strains were tested for antagonistic activity against pathogenic fungi: *Fusarium oxysporum* and *Rhizoctonia solani*, and the inhibition zone was detected (Table 3). All the bacterial strains exhibited at least some antagonistic activities against the two pathogens. The inhibition zone of bacterial strains against the two pathogenic fungi ranged from 10 mm to 33 mm. Maximum antagonistic activity was detected in the case of strain *B. subtilis* isolated from the rhizosphere of flax, against two fungal pathogens while the lowest antagonistic activity was observed with *Proteus mirabilis.* These results agreed with Paulitz and Belanger (2001) & Kloepper *et al.*, (2004) who found that most microbes produce and secrete one or more compounds with antibiotic activity.

Table 3. Inhibition of phytopathogenic fungi by silicate solubilizing bacterial strains

Bacterial strains	Mean zone of inhibition after 7 days (mm)	
	Fusarium oxysporum	*Rhizoctonia solani*
Pseudomonas fluorescens	21	29
Bacillus subtilis	31	33
Proteus mirabilis	10	18
Bacillus circulans	17	18

Antagonistic activities

Microbial metabolic (enzymes and chemicals) production may be involved in the antagonistic activities of selected silicate bacterial strains are presented in Table 4. There are many ways by which these silicate solubilizing bacteria can antagonize fungal pathogens. These include the production of hydrolytic enzymes, siderophores, HCN, and antibiotics (Naureen *et al.,* 2015). Also, it can benefit the plants by the antagonistic activity against the pathogens (Raturi *et al.,* 2021).

Table 4. Detection of antagonistic metabolites from four selected silicate bacterial strains.

Metabolites production	Reaction against bacterial strains			
	Psudomonas fluorescens	*Bacillus subtilis*	*Proteus mirabilis*	*Bacillus circulans*
Enzymes				
Chitinase	+	+	-	+
Lipase	+	-	-	-
Amylase	-	+	-	+
Cellulase	-	+	+	+
Pectinase	-	+	+	+
Protease	-	-	+	-
Siderophores	+	+	-	-
Hydrogen cyanide	-	-	+	-
Ammonia	+	-	-	+

(+) : positive reaction, (-) : negative reaction

Glasshouse experiment

Data in Table (5) showed that all bacterial strains were effective in increasing the surviving seedlings whether they were applied under glasshouse conditions however, their efficiency was always much higher in the case of two strains (*B. subtilis* and *Ps. fluorescens*). Bacterial strains increased the percentage of surviving seedlings compared to the untreated control. These results are in agreement with previously reported results by Ashour and Afify, 1999.

Table 5. Effect of silicate bacterial strains on seedling survival of flax under glasshouse conditions.

Treatments	Fungi involved in flax seedling disease						Mean	
	R.solani		*F. oxysporum*		Non infested soil[c]			
Ps. fluorescens	65.2[a]	(53.85)[b]	78.0	(62.05)	78.0	(62.05)	69.6	(56.56)
B. subtilis	68.2	(55.56)	74.5	(59.68)	74.5	(59.68)	69.9	(56.77)
Proteus mirabilis	58.6	(49.97)	72.2	(58.19)	72.2	(58.19)	64.3	(53.28)
B. circulans	54.3	(47.48)	66.5	(54.63)	66.5	(54.63)	58.0	(49.59)
Nutrient broth	27.7	(31.76)	52.9	(46.67)	52.9	(46.67)	37.7	(37.87)
Control	24.9	(29.96)	52.2	(46.27)	52.2	(46.27)	33.8	(35.55)
Mean	49.8	(44.76)	66.0	(54.58)	66.0	(54.58)	55.5	(48.27)

[a] Percentage of surviving seedlings, [b] Arc sine- transformed data, [c] Soil non infested either *R. solani* or *F.oxysporum*, L.S.D. for bacterial agents = 3.71 (p = 0.05) and for fungal treatments = 1.86 (p = 0.05) .

CONCLUSION

Based on the obtained results, four silicate solubilizing bacterial strains; *Pseudomonas fluorescens*, *Bacillus subtilis*, *Proteus mirabilis*, and *Bacillus circulans* showed high antagonistic activity against two plant pathogens fungi (*Fusarium oxysporum* and *Rhizoctonia solani*) *in vitro* using the dual cultural technique. As well as the antagonistic metabolites were determined. In brief, siderophores were produced by *Ps. fluorescens* and *B. subtilis* also these two strains with *B. criculans* produced chitinase. Only *Proteus mirabilis* produced hydrogen cyanide. In addition, *B. subtilis* and *Ps. fluorescens* showed maximum efficiency in increasing the survival of flax seedlings under glasshouse conditions. From the current study, it could be recommended the possibility of using silicate solubilizing bacteria (SSB) for the development of bio-fungicides against fungal pathogens.

REFERENCES

Afify, Aida H. (2022). Silicate bacteria as biofertilizer. J . Agric. Chem. and Biotechn., Mansoura Univ., Vol. 13(9): 75 - 83

Afify, Aida H. (1982). Studies on silicate bacteria. M. Sc. Thesis, Fac. Agric., Mansoura Univ., Mansoura, Egypt, pp. 182.

Afify, Aida H. and A.Z.A. Ashour (1995). Biological and chemical control of *Rhizoctonia solani* of cotton. J. Agric. Sci., Mansoura Univ. 20: 1441-1452.

Afify, Aida H. and Samia , M.M. Bayoumy (2001). Effect of certain silicate bacteria on primary silicate minerals. J. Agric. Sci. Mansoura Univ., 26 (5) : 3111 – 3125.

Aleksandrov, V.G. and M.L. Ternovs 'Ka (1961). Methodical textbook on preparation and application of silicate bacteria liquid preparation. Odessa, U.S .S .R.

Ashour, A.Z.A. and Aida H. Afify (1999). Use of *Bacillus* strains mixture to improve biological control of cotton seedling disease. The second Internat. Conf. on Fungi. The regional center for Mycol. and Biotechnol. The African J. of Mycol. and Biotechnol. 7(3): 109-120.

Ashour, A.Z.A. and Aida, H. Afify (2016). Evaluation of antagonistic properties of rhizobacteria *in vitro.* J. Agric. Chem. and Biotechn., Mansoura Univ., Vol. 7(3) : 89-94.

Bakker, A.W. and B. Schippers (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol. Biochem. 19 : 45107.

Carver, T. L. W. ; R. J. Zeyen and G. G. Ahlstrand (1987). The relationship between insoluble silicon and success or failure of attempted primary penetration by powdery mildew *Erysiphe graminis* germlings on barley. Physliolgical and Molecular Plant Pathol., 31 , 133- 148.

Chandrakala, C. ; S.R. Voleti ; S. Bandeppa ; N. Sunil Kumar and P.C. Latha (2019). Silicate solubilization and plant growth promoting potential of *Rhizobium* sp. isolated from rice rhizosphere.Silicon,11:2895 –2906.

Dye, D.W. (1962). The inedequancy of the usual determinative tests for identification of *xanthomonas* spp. NZT Sci. 35 : 393 – 416.

Francois, F.; R.B. Wilfried; G. M. James and R. B. Richard (2005). Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiol. Letters, 249, 1 – 6.

Joseph, M.H.; T.S. Dhargave; C.P. Deshpande and A.K. Srivastava (2015). Microbial solubilization of phosphate: Pseudomonas versus Trichoderma. Annals of Plant and Soil Res., 17: 227-232.

Katz, E. and A.L. Demain (1977). The peptide antibiotics of Bacillus : chemistry biogenesis and possible functions. Bacteriol. Rev. 41, 449- 474.

Kloepper, J. W. ; C. M. Ryu and S. Zhang (2004). Induce systemic resistance and of plant growth by *Bacillus* spp. Phytopathol . 94 : 1259 - 1266.

Lauwers, A.M. and W. Heinen (1974). Bio-degradation and utilization of silica and quartz. Arch. Microbiol., 95: 67-78.

Mew, T.W. and A.M. Rosales (1986). Bacterization of rice plants for control of sheat blight caused by *Rhizoctonia solani.* Phytopathol., 76(11): 1260-1264.

Monib, M. ; M.K. Zahra; S.I. Abdel El-Al and A. Heggo (1984). Role of silicate bacteria in releasing K and Si from biotite and orthoclase. Soil Biology and Conservation of the Biosphere., 2: 733-743.

Nagarajkumar, M.; R. Bhaskavan and R. Vaiezhahan (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbial. Res.; 159 : 73-8.

Naureen, Z.; M. Aqeel; M. N. Hassan; S.A. Gilani; N. Bouqellah; F. Mabood; I. Hussain and F.Y. Hafeez (2015). Isolation and screening of silicate bacteria from various habitats for biological control of phytopathogenic fungi. Amer. J. of Plant Sci. 6 : 2850 – 2859.

Naureen, Z.; F.Y. Hafeez; J. Hussain; A. Al Harrasi; N. Bouqellah and M.R. Roberts (2015). Supperssion of incidence of *Rhizoctonia solani* in Rice by siderophore producing rhizobacterial strains based on competition for iron. European Scientific J., 11, 186-207.

Naureen, Z.; A.H. Price; M.J. Wilson; F.Y. Hafeez and M.R. Roberts (2009). Supperssion of rice blast disease by siderophore producing bioantagonistic bacterial isolates isolated from rhizosphere of rice grown in Pakistan. Crop Protect., 28, 1052-1060.

Nyvall, R.F. (1981). "Field Crop Diseases Handbook". Avi Publishing Company, INC Connecticut.pp:436.

Paulitz, T.C. and R.R. Belanger (2001). Biological control in greenhouse systems. Annu. Rev. phytopathol. 39 : 103 - 133.

Prescott, L. M.; J.P. Harley and D. A. Kelin (1993). Microbiology, second edition. Wm . C. Brown Publishers. Oxford, England, pp. 912.

Raturi, G.; Y. Sharma; V.Rana; V.Thakrat; B. Myaka; P.Salvi; M.Singh; H. Dhar and R. Deshmukh (2021). Exploration of silicate solubilizing bacteria for sustainable agriculture and silicon biogeochemical cycle. Plant Physiol. and Biochem. 166 : 827-838.

Scher, F. M. and R. Baker (1982). Effect of *Pseudomonas putida* and asynthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathol.; 72: 1567-73.

Urrutia, M.M. and T.J. Beveridge (1995). Formation of short-range orderd aluminosilicates in the presence of a bacterial surface (*Bacillus subtilis*) and organic ligands. Geoderma, 65(1-2): 149-165.

Vasanthi, N.; L.M. Saleena and S.A. Raj (2012). Silicon on day tody life. World Appl. Sci. J., 17: 1425 – 1440.

Vinticova, H. (1964). A contribution to the study of the efficiency of silicate bacteria. Rostl. Vyroba 37: 1219-1228. Soils and Fert. 28: 1738.

Zahra, M. K. S. (1969). Studies on silicate bacteria. M.Sc. Thesis, Fac. Agric., Cairo Univ., Giza, Egypt.

المقاومة الحيوية لفطرين من الفطريات الممرضة بواسطة بكتيريا السليكات

عايدة حافظ عفيفى [1] و عبد الودود زكى عبد الله عاشور[2]

[1]قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنصورة - المنصورة – مصر
[2]معهد بحوث أمراض النباتات – مركز البحوث الزراعية - الجيزة - مصر

الملخص

تؤثر البكتيريا المذيبة للسليكات فى التربة عن طريق إذابة وتيسير صور العناصر الغير ميسره فى مركبات السليكات وعلاوه على ذلك أن هذه البكتيريا لها دور فى تحسين وسائل مقاومة النباتات للفطريات الممرضه0 أمكن الحصول فى اطباق عد وتوزيع البكتيريا من ريزوسفير نباتات القمح و الكتان أن انتشار البكتيريا يتأثر بنوع المحصول المزروع وباستخدام أربعة عزلات بكتيريه لها القدرة على النمو واذابة السليكات فى البيئة المتخصصه وأن هذه العزلات هى : *Bacillus subtilis* , *Pseudomonos fluorescens* , *Bacillus circulans* و بطريقة الأطباق تم اختبار تضاد هذه العزلات البكتيرية مع إثنين من فطريات التربة الممرضه لنبات الكتان هما *Rhizoctonia solani* , *Fusarium oxysporum* أظهرت النتائج تثبيط نمو هذه الفطريات بقياس منطقة تثبيط بين هذه البكتيريا و الفطريات الممرضه والتى تراوحت من 10- 33مل و كان أعلى مستوى من التضاد بواسطة بكتيريا *B. subtilis* يليها بكتيريا *Ps. fluorescens* . وعند تقدير الموادالمنتجه لتثبيط نمو الفطريات بواسطة العزلات البكتيرية نتيجة الأيض الميكروبى وجد أن عزلتى البكتيريا السابقتين سجلت نتيجة موجبة فى انتاجها للسايدروفورز بالاضافة إلى انتاج الكيتتيز من ميكروب *Proteus mirabilis* , وعند تقييم العزلات البكتيرية بزراعة الكتان تحت ظروف البيوت الزجاجية أظهرت مستويات مختلفة من الكفاءة فى زيادة النسبة المئوية للبادرات السليمة للكتان حيث أظهرت العزلتان *B. subtilis* *Ps. fluorescens* أفضل تأثير تحت ظروف البيوت الزجاجية بالمقارنة بالكنترول و بناء على نتائج هذه الدراسة إستنتاج ان عوامل المقاومة الناتجه بواسطة هذه البكتيريا تساهم فى تطوير المقاومه الحيوية ضد الفطريات الممرضة.