The Bioscan 19(2): S.I (1), 506-510, 2024 www.thebioscan.com
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

Potential of Bacterial species as biocontrol agents of Fusarium wilt disease

Annadurai. R[1] and Kamaraj. M[2*]

[1]Research Scholar, Department of Botany, Jamal Mohamed College (A), Triuchirappalli-620 020, Tamil Nadu, India, nishanthiniac2014@gmail.com

[2]Associate professor, Department of Botany, Jamal Mohamed College (A), Triuchirappalli-620 020, Tamil Nadu, India, kamarajjmc@gmail.com

Affiliated to Bharathidasan University, Tiruchirrappalli-620 024, Tamil Nadu, India

*Corresponding Author

Dr. Kamaraj. M, Associate professor, Department of Botany, Jamal Mohamed College (A), Triuchirappalli, Tamil Nadu, India E-mail: kamarajjmc@gmail.com, Mobile: +91 9384503406.

DOI: https://doi.org/10.63001/tbs.2024.v19.i02.S.I(1).pp506-510

KEYWORDS
Efficiency, Fusarium oxysporum, Retention, Screening, Trichoderma.

Received on:

20-07-2024

Accepted on:

26-11-2024

Abstract

In this work aimed to evaluate the efficiency of biological products based on Bacterial species in the control of *Fusarium* species under in vitro conditions. Six rhizospheric bacterial isolates obtained from various field of cotton crop were screened *in vitro* by dual plate assay for antagonistic activity against Fusarium wilt pathogens. Their ability to B4 was found to be the most potential antagonist with a mycelial inhibition percentage of against FO3 and FO1 mycelia followed by FO4 and FO2 respectively. For the secondary metabolites screening in liquid media, 6 antagonistic bacteria against *Fusarium* sp. were selected with broad spectrum of PGPR. The result of the ethyl acetate extracted product of the bacterial isolates namely B3 and B4, against the two pathogen F3 and F1 are as given the NIST and WILEY library based on their retention time, molecular weight, molecular formula and one chemical structure of the compounds are illustrated by GCMS analysis.

INTRODUCTION

India's 4 million farmers cultivate the cotton crop over an area of 7.4 million hectares, making it a significant commercial crop. India holds the top spot in terms of acreage, accounting for about 25% of the world's cotton crop. In India, cotton has a prominent place among all cash crops. About 65% of the raw materials used by the Indian textile industry are cotton, making it a vital raw material. With approximately 1500 mills, 4 million handlooms, 1.7 million power looms, and hundreds of garment, hosiery, and processing facilities, the Indian textile sector plays a vital role in the nation's economy and employs over 35 million people directly or indirectly (Sankaranarayanan *et al.*, 2011).

Fusarium species is a soil-borne fungal disease that affects plants through their roots at all stages of growth, causing significant economic losses by generating necrosis and wilting symptoms in many agricultural plants and having a significant overall influence on production. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield. *Fusarium* sp. found in its many pathogenic forms, is the most damaging species of the genus where in plants are concerned. A number of new disease reports on Fusarium have been submitted to the literature pool on agricultural research Bokhari anajat and perveen kahkashan, (2012).

It is deemed safe to employ *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Paenibacillus polymyxa*, which are often found in soils, in the environment and with animals (Zhao *et al.*, 2015). They generate antagonistic actions directed against many bacterial and fungal diseases (Aarab *et al.*, 2015) Thus, the primary root colonisers are Pseudomonas and Bacillus strains, which are examples of PGPR (Manikandan *et al.*, 2010) can be applied to the control of illnesses caused by dirt. In this study, we tested selected *Trichoderma* species for their biocontrol ability against the Fusarium wilt of cotton under in vitro conditions.

MATERIALS AND METHODS

Isolation of pathogenic fungi

Evaluation of infected parts of the cotton plant resulted in isolation and identification of *Fusarium* species based on the examination under microscope. Parts of plants with symptoms of Fusarium wilt infection were surface sterilised by immersion in 0.3% sodium hypochlorite for 10 minutes, and then in 70% ethanol and later rinsed thoroughly with sterile distilled water. They were transferred to potato dextrose agar (PDA) medium in petriplates and incubated at 26 ± 2oC for seven days (Aneja, 2001). The characteristic growth of the fungus with morphological characters of micro-conidia and macro-conidia and chlamydospores were observed by (Agrios, 2005). Pure cultures were maintained on PDA slants and stored at 4oC in the refrigerator.

Isolation of antagonistic bacteria

Antagonistic bacteria were isolated from the rhizosphere soil sampled during the survey. One gramme of rhizosphere soil underwent repeated dilution treatment following the thorough

mixing of the soil with the root pieces (Aneja, 2001). Biochemical characterizations were performed to identify bacterial isolates using conventional methods. Cell and colony morphology and motility, Gram reaction and catalase and oxidase activities were determined by Allen *et al.*, (2002) and Ogutcu *et al.*, (2020). Tricalcium phosphate-containing Pikovskaya (PVK) medium was used to test for phosphate solubilization (Nautiyal, 1999). Schwyn and Neilands' (1987) description of the chromeazurol (CAS) agar assay medium was used to screen isolates for siderophore production. Using the dual culture method, the antagonistic activity of biocontrol drugs against *F. oxysporum* f. sp. *vasinfectum* was evaluated (Dennis and Webster, 1971). GC-MS was used to examine the chemical components found in nine isolates (B3) as (Verma *et al.*, 2015).

STATISTICAL ANALYSIS

The experimental design was Completely Randomized Design (CRD) with three replicates as described by Gomez and Gomez, (1984). Test of variance was calculated using Analysis of variance (ANOVA). Differences among treatment means for each measured parameter were further separated using fishers Least Significance Difference (LSD) to determine levels of significance according to Cochran and Cox, (1992).

RESULTS AND DISCUSSION

The colonies formed by the bacterial isolates on the Yeast Extract Mannitol Agar (YEMA) were found to be translucent as or opaque and the surface texture were smooth, circular in shape and having entire margin. The outcomes of the biochemical tests and gramme reaction that were carried out to identify the isolates of Bacterial species shown that the findings of the gramme staining for each isolate were identical and urease test was negative, indole, catalase test and utilization of citrate was positive. Two isolates such as B2 and B5 were appeared Oxidase as negative. The detail characteristics are given table 1. Gram-positive, rod-shaped, spore-producing Bacillus mycoides bacteria develop into certain rhizoids, which produce cells that are arranged in filamentous bundles and extend end to end (Yi, 2018). According to Public Health England (2018), Bacillus species are Gram-positive rods that are frequently paired or chain cells with round or square ends. They typically have a single endospore, which is typically oval or occasionally spherical and extremely resistant to harsh environments.

The Gram-positive bacteria retain the crystal violet stain and appear purple when observed under a microscope. Meanwhile, the Gram-positive cell wall appears as a broad, 20-80 nm thick dense wall consisting of multiple interconnected layers of 60 to 90% peptidoglycan (Kaiser 2021).

Table – 1. Biochemical characterization of Bacterial species

S.No	Biochemical Test	B1	B2	B3	B4	B5	B6
1.	Cell Shape	Rod	Cocci	Cocci	Cocci	Rod	Cocci
2.	Gram Stain	-	-	-	-	-	-
3.	Indole test	+	+	+	+	+	+
4.	Urease	-	-	-	-	-	-
5.	Catalase	+	+	+	+	+	+
6.	Oxidase	+	-	+	+	-	+
7.	Citrate	+	+	+	+	+	+
8.	Hydrogen pro duction	+	+	+	+	+	+

The six isolates of bacterial species B1, B2, B3, B4, B5 and B6 were showed potential in biocontrol activity such as antagonistic activity against *Fusarium* wilt pathogens as well as PGPR activity (Table 2). The chitinase activities and surface area of production was more in B2, followed by B4, B1 and B5 isolate, although, B3 and B6 don't appear in chitinase activity. All isolates of bacterial species produced yellow pigmentation in blue coloured succinate medium. Further, When tetrazolium salt was added to a siderophore sample, certain bacteria species observed an immediate appearance of yellow colour, whereas B1 isolates showed a delayed emergence of yellow colour, suggesting that the siderophore is hydroxamate type. Ghazy and El-Nahrawy, (2021) Using the CAS double-layer plate approach, two strains with strong siderophore production were tested. The strains may successfully prevent the hypha development of maize blight, as demonstrated by the results. Rouag *et al.*, (2019) conducted a six possible the capacity of *Bacillus* isolates to stimulate plant growth against Foc. The results be seen that all of the isolate generated IAA, four of them also produced chitinases and cellulases, and one isolate even solubilized phosphates. Aarab *et al.*, (2015).

Table – 2. Activities of rhizobacteria that promote plant development by the selected potential bacterial isolates.

S.No	Isolates	PGPR Attributes		
		Colour of siderophore pigments	**Surface area (mm^2)**	**Chitinase activity (U/ml)**
1	B1	Yellow	79.29	0.53
2	B2	Yellow	84.26	0.62
3	B3	Yellow	82.80	-
4	B4	Yellow	80.78	0.57
5	B5	Yellow	78.19	0.56
6	B6	Yellow	73.72	-

Values = mean ± SE, (n = 3)

The nitrogenase activities of all isolates were conformed to acetylene reduction assay and acetylene reduction activities as shown in Fig. 1. Isolates B4 and B1 able to converts maximum atmospheric nitrogen into ammonium under microaerophilic conditions at low nitrogen levels through the action of nitrogenase and able to grow as a pellicle when grow in Nfb semisolid medium were found to be 215.54 n moles of C_2H_4 per mg^{-1} of cell protein h^{-1} for B4 and 180.2 n moles of C_2H_4 per mg-1of cell protein $h^{-1}$for B1. In the same context, Isolates B2 able to converts minimum atmospheric nitrogen into ammonium under microaerophilic conditions through the action of nitrogenase activities. Around the world, isolates of the bacteria Penibacillus sp. have been found in a range of habitats, animals, plants, soils, and people. Numerous Penibacillus species are known to exhibit PGP effects, such as phosphate solubilization (Wang *et al.*, 2012) and nitrogen fixation (Liu *et al.*, 2019). Therefore, Rhizobiales, Sphingomonadales, Rhodospirillales, Nitrosomonadales, and Frankiales take role in the nitrogen cycle Sun *et al.*, (2014); Dun-Dun *et al.*, (2011).

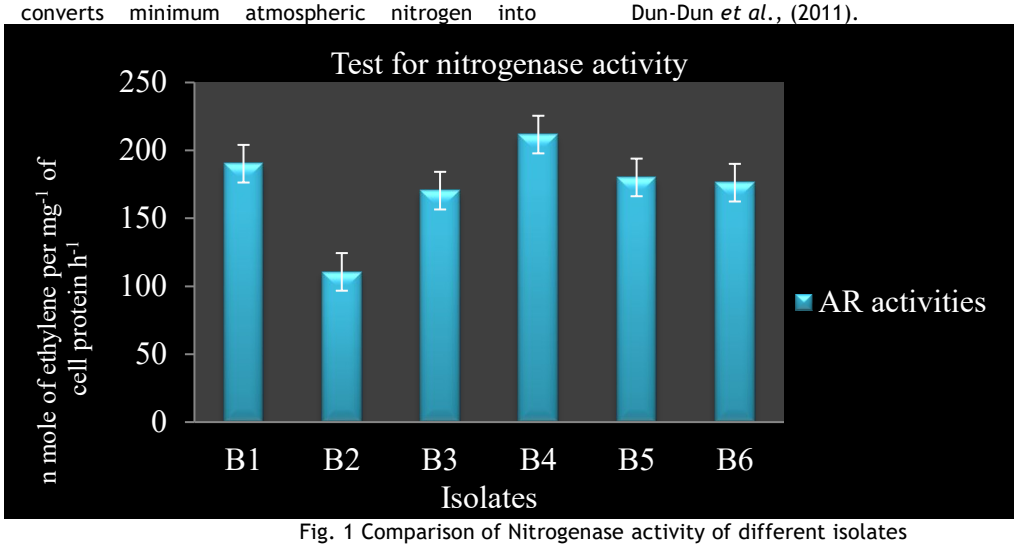


Fig. 1 Comparison of Nitrogenase activity of different isolates

Six rhizospheric bacterial isolates obtained from various field of cotton crop were screened in vitro by dual plate assay for antagonistic activity against *Fusarium* wilt pathogens for their ability to inhibit mycelial growth over control (Fig 2). Isolates, B4 and B3 was found to be the most potential antagonist with a mycelial inhibition percentage of 76.27 and 72.90 % against FO3 mycelia and 72.96 and 67.38 % against FO1 mycelia followed by FO4 (71.55 %) > FO2 (71.55 %) respectively. Among of all the bacterial isolates, isolate B2 was the least effective, reducing FO2 mycelial development by only 51.44 percent, the minimal inhibitory level. Therefore, the isolate B4 was most promising in controlling growth of all isolates of *F. oxysporum* f. sp. *vasinfectum* and B2 showed low ability to inhibit growth of the pathogen. *B. subtilis* is a somewhat effective biocontrol formulation against club root pathogens (Zhu *et al.*, 2019). However, reports on the antibacterial properties of Bacillus subtilis FA26 were assessed against *Clavibacter michiganesis* ssp. *Sepedonicus* (Cms), the causative agent of potato bacterial ring rot (Rajer *et al.*, 2017).

Burkholderia cenocepacia demonstrated 44.4% inhibition in vitro and 86.1% field reduction in the incidence of FocR4T in *Cavendish bananas* (Ho *et al.*, 2015). Conversely, Pseudomonas bacteria produce antibiotics, They have been identified as antagonistic agents against pathogens in suppressive soils, along with 2,4-diacetylphloroglucinol (2,4-DAPG), phenazine-1-carboxylic acid (PCA), pyrrolnitrin, rhizoxin, pyoluteorin, cyanide hydrogen (HCN) and 2-hexyl-5-propyl resorcinol (HPR) (Loper *et al.*, 2012).

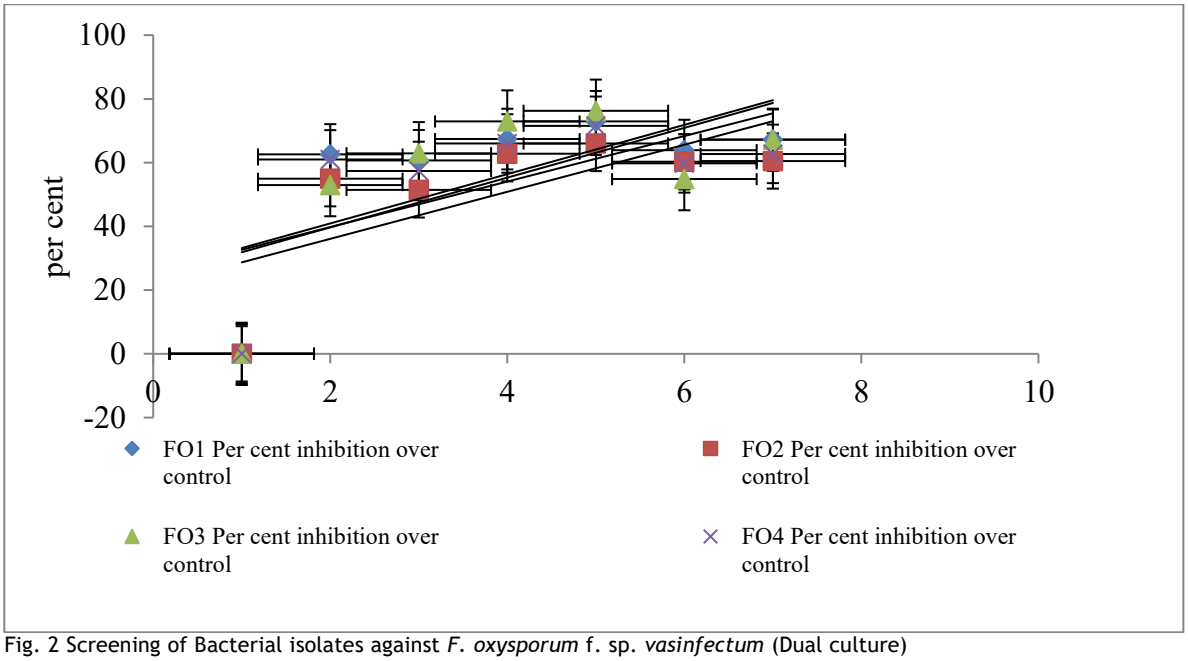


Fig. 2 Screening of Bacterial isolates against *F. oxysporum* f. sp. *vasinfectum* (Dual culture)

Two antagonistic bacteria against *Fusarium oxysporum* with a broad spectrum of PGPR were chosen for the secondary metabolites screening in liquid medium. An organic solvent (ethyl acetate) was used to extract the extracellular bioactive

compounds that the bacterial isolates in the culture filtrate generated. The outcome of the bacterial isolates B3 - *Bacillus subtilis* ethyl acetate extracted product against the two pathogens F3 and F1 are as given below (Table 3).

Thirty chemicals were found to be present in the ethyl acetate extract of *Bacillus subtilis* according to GC-MS analysis. By comparing the retention time, molecular formula, and molecular weight with the Wiley spectrum library search software, the identity of the chemical ingredients was verified. Fig. 2 display the active principles together with their retention time (RT), molecular weight, and molecular formula. Benzothiazole and 4-ditert-butylthiophenol were identified by Gao *et al.*, (2018) as the primary inhibitory volatile organic compounds generated by *B. subtilis* CF-3. The postharvest fungus Colle and *Monilinia fructicola* were significantly inhibited in their growth. Furthermore, Three-methyl-2-pentanone, 2-heptanone, 2-octanone, 2-decanone, 5-methyl-2-hexanone, 2-nonanone, 2-dodecanone, 2-undecanone, and 5-methyl-2-heptanone and 2-pentanone were among the eight Bacillus strains that produced ketones, which are abundant sources of bioactive volatile organic compounds and were found to be effective in preventing the growth of *Fusarium solani* (Li *et al.*, 2015) and Mycelial growth and spore germination were strongly inhibited by 2-methylenecyclohexanol, 2-nonanone, 2-nonanol, 2-(2-methylpropyl)-3-(1-methylethyl)pyrazine, and 2-dodecanone (Goswami and Kistler, 2004).

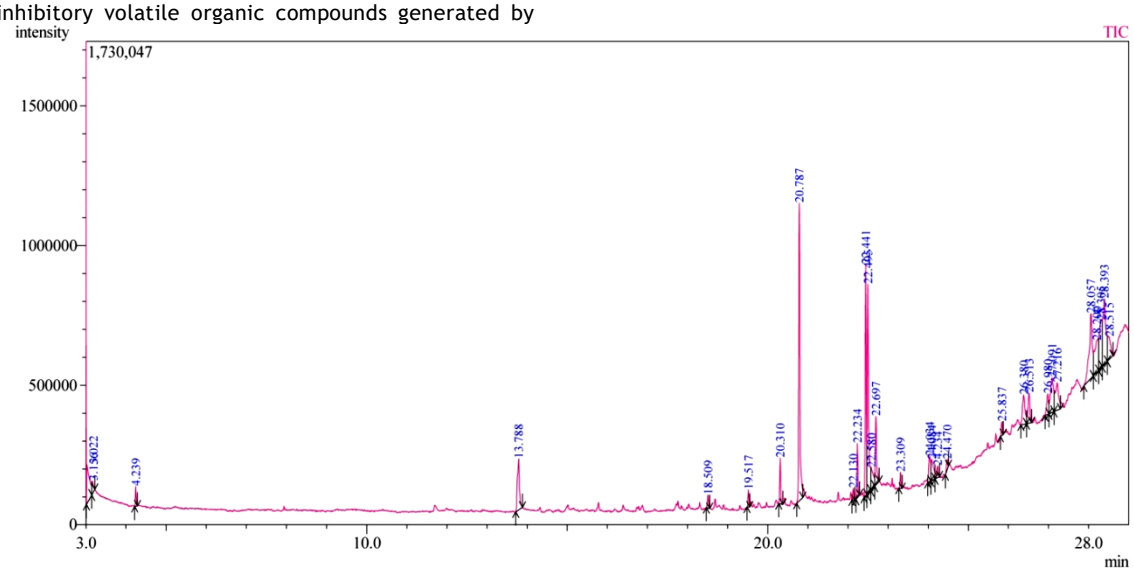
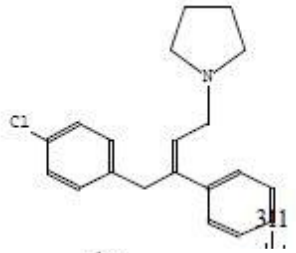
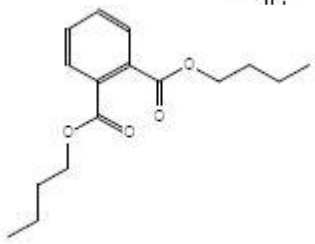


Fig. 2 GC-MS chromatogram of Bacillus subtilis

Table 3. Chemical compounds detected through GCMS

S. No.	R.Time	Area%	Height%	Name of the compound	Structure
1.	3.022	3.83	2.51	ethyl acetate	
2.	13.788	0.70	0.83	Pyrrolidine	
3.	18.509	0.58	1.25	Dibutyl ester	

CONCLUSION

The most promising antagonists were determined to be isolates B4 and B3, with a mycelial inhibition percentage of 76.27 and 72.90 % against FO3 mycelia and 72.96 and 67.38 % against FO1 mycelia followed by FO4 (71.55 %) > FO2 (71.55 %) respectively, and their antagonists were the most effective in reducing the occurrence and severity of *Fusarium oxysporum*, an incidental pathogen that causes cotton wilt. There is strong evidence that microbial inoculants based formulations may be highly effective in suppressing soil borne diseases and enhancing crop productivity.

REFERENCES

- Aarab S. Ollero F.J. Megias M. Laglaoui A. Bakkali M and Arakrak, A. 2015. Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An in vitro study. Int. J. Curr. Microbiol. App. Sci 4(1): 260-269.
- Agrios G. N. 2005. Plant Pathology. Elsevier Academic Press.
- Allen R, Andrewes O.N, Beger H, Bier O, Otto Borman E.K, Branham S.E and Breed, S.E. 2002. Bacterial

morphology and staining. Laboratory Exercises in Microbiology, McGraw-Hill Companies, 5th Edition, New York, 31-36.
- Aneja K.R. 2001. Experiments in Microbiology, Plant Pathology Tissue Culture and Mushroom Production Technology. New Age International, New Delhi.
- Aneja, K.R. 2001. Experiments in microbiology plant pathology tissue culture and Mushroom production technology, 3rd edn. New age international publishers 192 - 195.
- Bokhari anajat, perveen kahkashan. 2012. In vitro inhibition potential of *Phoenix dactylifera* extracts on the growth of pathogenic fungi. Journal of Medicine Plants Research 6(6)114-123.
- Cochran, G.W. Cox, G.M. Experimental Designs (2nd edn). John willey and Sons Inc, pp: 611. 1992.
- Dennis L and Webstar J. 1971. Antagonistic properties of species-groups of *Trichoderma*. The production of non volatile antibiotics. Trans. Bri. Mycol. Soc 57: 25-39.
- Dun-Dun J.I. Qiu H.D and Xie H. 2011. The improvement of N-ammonia degradation of photosynthetic bacteria by protoplast fusion, Journal of Dalian Ocean University. 26, 407–413.
- Gao H. Li P. Xu X. Zeng Q. Guan W. 2018. Research on volatile organic compounds from *Bacillus subtilis* CF-3: biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation. Front Microbiol 9:456.
- Ghazy, N and El-Nahrawy, S. 2021. Siderophore production by *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 and their efficacy in controlling *Cephalosporium maydis* in maize plant. Arch Microbiol 203:1195–1209.
- Gomez K.A. Gomez, A.A. 1984. Statistical procedures for Agricultural Research (2nd edn). John Wiley and sons. 680.
- Goswami R.S and Kistler H.C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops, Molecular Plant Pathology 5(6), 515–525.
- Ho Y.N. Chiang H.M. Chao C.P. Su C.C. Hsu H.F. Guo C.T. Hsieh J.L and Huang C.C. 2015. In planta biocontrol of soil borne Fusarium wilt of banana through a plant endophytic bacterium, *Burkholderia cenocepacea* 869T2. Plant Soil 387: 295-306.
- Kaiser G. 2021. The Gram-Positive Cell Wall. Catonsville (MD): The LibreTexts libraries; [accessed 2021 Feb 21].
- Li X.Y. Mao Z.C. Wu Y.X. Ho H.H. He Y.Q. 2015. Comprehensive volatile organic compounds profling of Bacillus species with biocontrol properties by head space solid phase micro extraction with gas chromatography-mass spectrometry. Biocontrol. Sci. Technol 25:132–143.
- Liu X. Li Q. Li Y. Guan G and Chen, S. 2019. *Paenibacillus strains* with nitrogen fixation and multiple beneficial properties for promoting plant growth. Peer J 7, e7445.
- Loper J.E. Hassan K.A. Mavrodi D.V. Davis E.W. Lim C.K and Shaffer, B.T. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: Insights into diversity and inheritance of traits involved in multitrophic interactions. PloS Genet 8 (7), 1–27.
- Manikandan R. Saravanakumar D. Rajendran L. Raguchander T and Samiyappan R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. Biol. Control 54: 83-89.
- Nautiyal C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett.170:265–270.
- Ogutcu H, Erbey Y, Güllüce M and Alaylar, B. 2020. Isolation and identification of *Bacillus pumilus* YHH-2, a potential pathogen to the alfalfa weevil (*Hypera postica* Gyllenhal). Turkish J. Agric. For. 44: 461-464.
- Public Health England. 2018. Identification of Bacillus species. UK Standards for Microbiology Investigations. London (UK): Standards Unit, Microbiology Services, PHE. 9(3.1):p. 9.
- Rajer F.U. Wu H. Xie Y. Xie S. Raza W. Tahir HAS. Gao X. 2017. Volatile organic compounds produced by a soil-isolate, *Bacillus subtilis* FA26 induce adverse ultra-structural changes to the cells of *Clavibacter michiganensis* ssp. *sepedonicus*, the causal agent of bacterial ring rot of potato. Microbiology 163:523–530.
- Rouag N. Abed H. Moutassam D. Mehmah S and Benabid S. 2019. Antifungal activity of isolated Bacillus species against chickpea Fusarium wilt. J Crop Prot 8(4):449–464.
- Sankaranarayanan K, Nalayini P, Sabesh M, Usha Rani S, Nachane R.P and Gopalakrishnan N, 2011. Low Cost Drip–Cost Effective and Precision Irrigation Tool in Bt Cotton. Technical Bulletin No.1/2011, Published by Central Institute for Cotton Research, Regional Station, Coimbatore 641 003.
- Schwyn, B and Neilands, J.B. 1987. Universal chemical assay for the detection & determination of siderophores. Analytical Biochemistry 160, 47-56.
- Sun J. Zhang Q. Zhou J and Wei Q. 2014. Pyrosequencing technology reveals the impact of different manure doses on the bacterial community in apple rhizosphere soil, Applied Soil Ecology. 78, 28–36,
- Verma B, Kumar P, Dhanasekaran D, Babalola O.O and Banakar S.P. 2015. Gas chromatography-mass spectrometry analysis and antibacterial activity of bluish-green pigment from *Pseudomonas* sp. JJTBVK (KF836502). Brazilian Archives of Biology and Technology 58(4), 628-635.
- Wang Y. Shi Y. Li B. Shan C. Ibrahim M and Jabeen A. 2012. Phosphate solubilization of *Paenibacillus polymyxa* and *Paenibacillus macerans* from mycorrhizal and non-mycorrhizal cucumber plants. Afr. J. Micro. Res 6, 4567–4573.
- Yi Y. 2018. *Bacillus mycoides*: novel tools for studying the mechanisms of its interaction with plants.
- Zhao L. Xu Y. Lai X.H. Shan C. Deng Z and Ji Y. 2015. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz. J. Microbiol 46, 977–989.
- Zhu M. He Y. Li Y. Ren T. Liu H. Huang J. Jiang D. Hsiang T and Zheng L. 2019. Two new biocontrol agents against club root caused by *Plasmodiophora brassicae*. Frontiers in Microbiology 10 - 19.