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# Evaluation of Water Stress Resilient Endophytic Bacteria for Seed Vigor Index and Antagonistic activity in Maize (*Zea mays* L.)

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**ABSTRACT: For maize productivity water deficit stress is the major abiotic limitation and disease is one of the major biotic stresses that declines crop yield and furthermore deteriorate the quality of product that ultimately affects the market cost. Plants are more contingent on microorganisms which are capable to enhance their metabolic activity to combat stress. Thus, present study was aimed to screen maize endophytic bacteria for their osmotolerance and antagonistic activity in *in vitro* conditions. The endophytes with less per cent of decreased growth at all treated water potential (-0.05, -0.65, - 1.57, -2.17 and -2.70 MPa) in nutrient agar supplemented with PEG 6000 are found to be *Kosakonia radicincitans* (NL3E3), *Priestia aryabhattai* (PL3E2) and *Bacillus licheniformis* (VaR3E1). The maximum antagonistic activity against pathogenic fungi *Rhizoctonia solani* was shown by the bacterial isolates *K. radicincitans* (96%) and *P. aeruginosa* (96%), against *Fusarium oxysporium* 92% of mycelia inhibition was shown by *P. aeruginosa* and *B. licheniformis* inhibited 89% of mycelia growth of *Exserohilum turcicum.* Maximum germination per cent was shown by *Bacillus licheniformis,* while the seed vigor index is high for *Pantoea dispersa* (KS3E1). Further, influence of screened bacteria should be studied under water stress conditions in fields and their effect on plant growth and disease management to develop a microbial consortium for agricultural crops.**

**Keywords:** Endophytic bacteria, Osmotolerance, Antagonistic activity, Seed vigor.

## INTRODUCTION

Due to adverse environmental conditions plants are subjected to variety of biotic and abiotic stresses. World's agriculture production is getting affected in food crops due to abiotic and biotic stresses. In this regard, various techniques including genetic engineering and other technologies have been used to overcome abiotic and biotic stress decreasing crop growth. Among these, moisture stress has major impact on crop growth and productivity throughout the world. By 2050 more than 50% of arable lands are expected to have negative impact on crop growth because of drought (Vinocur and Altman, 2005). Drought stress has been reported to cause yield reductions of up to 21% in wheat and 40% in maize around the world (Daryanto *et al.*, 2016). On the other hand direct yield losses caused biotic stress including pathogens, animals and weeds will cause yield losses in the range of 20 to 40 % of worldwide agricultural productivity (Savary *et al.*, 2012). However, the interaction of plants with endophytic bacteria has emerged as an intriguing era of knowledge that can be used for new agricultural practices to mitigate stress situations.

A protection system is possessed by plants naturally that can tackle adverse stress conditions, even so, plants also interact with a many microorganisms that can alleviate the stress and protects the plant (Marulanda *et al.*, 2006). Plants are more contigent on microorganisms which are capable to enhance their metabolic activity to combat stress (Kavamura *et al.*, 2013). Upon exposure of plants to hostile conditions microbes can pre sensitize the plant cell metabolism, and so microbial treated plants will respond more quickly than untreated plants (Compant *et al.*, 2005). Thus, use of beneficial microbial inoculants as a stress protecting agent for

plants may reassure for sustainable and chemical free eco-friendly agriculture.

Plants harbour several microbes, which reside within the cells in intercellular spaces or in vascular system, without affecting the plants are known as endophytes (Sandhya *et al.,* 2017). Bacterial endophytes are beneficial over rhizospheric bacteria as they reside within plant tissue with more intimate contact and they have no competition with rhizosphere microorganisms (Naveed *et al.,* 2014). Their ability for plant growth and to alleviate the biotic and abiotic stresses is well studied (Chandran *et al.,* 2020; Ullah *et al.,* 2019; Sandhya *et al.,* 2017). The interaction of plant with endophytic bacteria alleviates the tolerance towards drought stress (Paul and Lade 2014). On the other hand endophytic bacteria have been reported possess variety of defense mechanisms to control plant pathogen by de novo synthesis of structural compounds, producing antibiotics and antimicrobial products, competing for niche and plant immunity development or induced systemic resistance (Pandey *et al.,* 2019). Four endophytic bacteria isolated from maize plant niche characterized successfully and found have the potential to be developed as biopesticides to control maize disease, especially *R. solani* and *Pantoea* sp. (Prihatiningsih and Soesanto, 2020). A few isolated strains of the genera *Bacillus* and *Pseudomonas* were characterized by high activity against fungal phytopathogens (Esikova *et al.,* 2021). Around 80% of bacterial endophytes isolated from roots of soybean have shown PGP traits, 20% showed antagonistic activity against pathogenic fungi *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Alternaria alternate* while only three of them showed drought tolerance up to −0.3 MPa of water potential (Dubey *et al.,* 2021).

Thus, present investigation was aimed to screen the plant growth promoting endophytic bacteria isolated from maize plant tissues of which efficient isolates were identified by 16S rRNA gene sequencing (Moturu *et al.,* 2021) for their osmotolerance ability and significant water stress resilient isolates were further characterized for their biochemical traits, seed vigor index, antagonistic activity and antibiotic sensitivity.

## MATERIALS AND METHODS

**Assaying endophtic bacteria for osmotolerance:** Bacterial isolates were inoculated in 50 ml nutrient broth medium supplemented with polyethylene glycol (PEG) 6000 at various concentrations (0, 10, 20, 30, 40%) to generate water potentials of -0.05, -0.65, -1.57, -2.17 and -2.70 MPa, respectively (Busse and Bottomley, 1989). Incubated the flasks in an orbital shaker with 100 rpm at 30°C. After 3 days of incubation bacterial growth was measured by taking optical density (OD) at 600 nm using spectrophotometer and per cent reduction in growth was calculated comparison to that obtained under control conditions (without PEG 6000). Bacterial isolates with lesser reduction in growth in presence of PEG 6000 were considered as osmotolerant and screened for further studies.

**Biochemical Characterization:** The effective plant growth promoting and drought tolerant endophytic bacterial isolates were characterized by various biochemical tests like starch hydrolysis, production of hdrogen sulphide, indole production, catalase & oxidase production, gelatin liquification, citrate utilization, nitrate reduction, methy red & voges prausker's test and carbohydrate utilization as per the standard methods (Cappuccino and Sherman, 1992).

**Seedling vigor assay:** For germination test paper towel method was used in which seeds primed with endophytic bacteria and distilled water are taken as treatments and control respectively. Bacterized and untreated seeds (50 each) were placed on wet germination paper towel, rolled and intermittently water was added to prevent from drying. The towels were unrolled after 15 days, and the data like root length, hypocotyl length and no of seeds germinated were measured on same day. Then germination percentage and seedling vigor index were analyzed. The vigor index (VI) was calculated using the formula VI = (mean root length + mean hypocotyl length) × % germination (Karthik *et al.,* 2017).

***In-vitro* Antagonistic Activity:** Biocontrol activity of the isolated endophytic bacteria was assayed against maize pathogens, *Exserohilum turcicum* (Turcicum leaf blight), *Rhizoctonia solani* (root and stalk rot) and *Fusarium oxysporium* following the dual culture technique (Dennis and Webster 1971; Ali *et al.,* 2014). On a Petri dish containing potato dextrose medium bacterial isolate was streaked on one side and perpendicular to it on the opposite side a mycelial disc of 8 mm diameter taken from a 7-day-old culture of the fungal pathogen was placed. Incubated the plates at 28 ± 2°C for seven days. Isolates with antagonistic activity inhibited the growth of the fungus when it grew towards the bacterial colony on PDA. The level of antagonism is calculated using the formula (Mugiastuti *et al.,* 2020).

$$I = \frac{C-T}{C} \times 100\%$$

where: I : The level of inhibition of antagonist (%)

C: The radius of pathogen colony opposite to antagonist

T: The radius of the colony of pathogen towards antagonist

**Intrinsic antibiotic sensitivity:** Antibiotic resistance profile of the isolates was screened by using antibiotics such as ampicillin, chloramphenicol, penicillin G, streptomycin, suphatriad and tetracycline on solid medium using antibiotic discs of different concentrations (Himedia, India) (Sandhya *et al.,* 2017).

## RESULTS AND DISCUSSION

**Osmotolerance of endophytic bacteria:** Osmotolaerance of bacterial isolates was measured by growing in nutrient broth supplemented with PEG 6000 to get varied water potential. Per cent decrease in the bacterial growth in treatments compared to control (No PEG) was measured. Isolates with less per cent decrease in their growth even at higher water potential were considered as osmotolerant or water stress resilient. The Table 1 showing the optical density of bacterial growth at varied water potential and the per cent decrease in their growth with respect to control.

**Table 1: Evaluation of endophytic bacterial growth (Optical Density) at varied water potential.**

| Isolate no. | | OD at varied water potential | | | | | Per cent decrease in OD | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Isolate code | -0.05 MPa (Control) | -0.65 MPa | -1.57 MPa | -2.17 MPa | -2.70 MPa | -0.65 MPa | -1.57 MPa | -2.17 MPa | -2.70 MPa |
| 1 | AR3E2 | 1.182 | 0.576 | 0.392 | 0.201 | 0.128 | 51.269 | 66.84 | 82.99 | 89.17 |
| 2 | AlR2E5 | 0.908 | 0.711 | 0.671 | 0.417 | 0.309 | 21.696 | 26.10 | 54.07 | 65.97 |
| 3 | GC3E2 | 1.388 | 0.407 | 0.381 | 0.207 | 0.086 | 70.677 | 72.55 | 85.09 | 93.80 |
| 4 | BS2E1 | 1.468 | 1.092 | 0.864 | 0.52 | 0.371 | 25.613 | 41.14 | 64.58 | 74.73 |
| 5 | VS1E1 | 1.463 | 0.826 | 1.123 | 0.781 | 0.595 | 43.541 | 23.24 | 46.62 | 59.33 |
| 6 | VR1E1 | 0.964 | 0.937 | 0.594 | 0.227 | 0.084 | 2.801 | 38.38 | 76.45 | 91.29 |
| 7 | NL3E2 | 1.388 | 0.407 | 0.381 | 0.207 | 0.086 | 70.677 | 72.55 | 85.09 | 93.80 |
| 8 | NL3E3 | 0.865 | 0.675 | 0.564 | 0.472 | 0.442 | 21.965 | 34.80 | 45.43 | 48.90 |
| 9 | NL3E4 | 1.532 | 0.921 | 0.473 | 0.195 | 0.2 | 39.883 | 69.13 | 87.27 | 86.95 |
| 10 | NL3E5 | 0.572 | 0.971 | 0.337 | 0.246 | 0.011 | -69.755 | 41.08 | 56.99 | 98.08 |
| 11 | NC3E1 | 1.36 | 0.978 | 0.256 | 0.265 | 0.224 | 28.088 | 81.18 | 80.51 | 83.53 |
| 12 | NC3E2 | 1.308 | 1.444 | 0.49 | 0.301 | 0.187 | -10.398 | 62.54 | 76.99 | 85.70 |
| 13 | NR3E1 | 1.503 | 1.439 | 0.583 | 0.299 | 0.239 | 4.258 | 61.21 | 80.11 | 84.10 |
| 14 | NR3E3 | 1.503 | 1.133 | 0.708 | 0.123 | 0.239 | 24.617 | 52.89 | 91.82 | 84.10 |
| 15 | PdC3E2 | 1.182 | 0.576 | 0.392 | 0.201 | 0.128 | 51.269 | 66.84 | 82.99 | 89.17 |
| 16 | PdC3E3 | 1.39 | 1.442 | 0.526 | 0.197 | 0.100 | -3.741 | 62.16 | 85.83 | 92.81 |
| 17 | PdC3E4 | 1.503 | 1.133 | 0.708 | 0.123 | 0.239 | 24.617 | 52.89 | 91.82 | 84.10 |
| 18 | PdS3E1 | 1.392 | 1.031 | 0.602 | 0.346 | 0.206 | 25.934 | 56.75 | 75.14 | 85.20 |
| 19 | PdS3E2 | 1.709 | 1.440 | 0.728 | 0.537 | 0.432 | 15.740 | 57.40 | 68.58 | 74.72 |
| 20 | RgL3E4 | 1.503 | 1.133 | 0.708 | 0.123 | 0.239 | 24.617 | 52.89 | 91.82 | 84.10 |
| 21 | RgC3E2 | 1.177 | 1.087 | 0.383 | 0.215 | 0.251 | 7.647 | 67.46 | 81.73 | 78.67 |
| 22 | JC3E1 | 0.908 | 0.711 | 0.671 | 0.417 | 0.309 | 21.696 | 26.10 | 54.07 | 65.97 |
| 23 | JC3E2 | 1.276 | 1.288 | 0.738 | 0.34 | 0.559 | -0.940 | 42.16 | 73.35 | 56.19 |
| 24 | PL3E2 | 1.331 | 1.092 | 0.852 | 0.703 | 0.446 | 17.956 | 35.99 | 47.18 | 66.49 |
| 25 | CC3E3 | 1.554 | 0.734 | 0.876 | 0.259 | 0.164 | 52.767 | 43.63 | 83.33 | 89.45 |
| 26 | VaR3E1 | 0.284 | 0.234 | 0.220 | 0.216 | 0.123 | 17.606 | 24.12 | 23.94 | 56.69 |
| 27 | VaL3E1 | 0.62 | 0.324 | 0.336 | 0.311 | 0.118 | 47.742 | 45.81 | 49.84 | 80.97 |
| 28 | VaS3E1 | 1.859 | 1.254 | 0.381 | 0.242 | 0.1 | 32.544 | 79.51 | 86.98 | 94.62 |
| 29 | KL3E1 | 1.103 | 1.635 | 0.633 | 0.373 | 0.228 | -48.232 | 42.61 | 66.18 | 79.33 |
| 30 | KL3E2 | 1.554 | 0.734 | 0.876 | 0.259 | 0.164 | 52.767 | 43.63 | 83.33 | 89.45 |
| 31 | KS3E1 | 1.177 | 1.087 | 0.383 | 0.215 | 0.251 | 7.647 | 67.46 | 81.73 | 78.67 |
| 32 | KS3E2 | 1.345 | 1.528 | 0.336 | 0.195 | 0.144 | -13.606 | 75.02 | 85.50 | 89.29 |
| 33 | LS3E1 | 1.133 | 1.575 | 0.852 | 0.439 | 0.216 | -39.011 | 24.80 | 61.25 | 80.94 |
| 34 | LS3E2 | 1.499 | 1.205 | 0.612 | 0.351 | 0.025 | 19.613 | 59.17 | 76.58 | 98.33 |
| 35 | LS3E3 | 1.388 | 0.845 | 0.689 | 0.207 | 0.086 | 39.121 | 50.36 | 85.09 | 93.80 |
| 36 | LL3E1 | 0.908 | 0.711 | 0.671 | 0.417 | 0.309 | 21.696 | 26.10 | 54.07 | 65.97 |

In current study isolates with less than 25% decrease in growth under given water potential are considered as efficient osmotolerant endophytic bacteria. Out of 36 isolates 21, 3 and 1 isolates/isolate were found to be effective osmotolerants at water potential of -0.65MPa, -1.57MPa and -2.17MPa respectively (Table 2). The endophytes with less per cent of decreased growth at all treated water potential are found to be *Kosakonia radicincitans*, *Priestia aryabhattai* and *Bacillus licheniformis*. Many endophytic bacteria similar to present ivestigation were identified as osmotolerants in previous studies that protects plant from drought stress including *Bacillus* sp. (Grover *et al.,* 2013; Kushwaha *et al.,* 2020; Vardharajula *et al.,* 2011) *Enterobacter cloacae* (Sandhya *et al.,* 2017) *Klebsiella* sp., *Pantoea alhagi* (Lei *et al.,* 2017; Chen *et al.,* 2017) *Pseudomonas* sp and *Bacillus cereus* (Dubey *et al.,* 2021). Many maize seed endophytic bacteria exhibited tolerance to salinity (10%) and osmotic stress (40% PEG 6000) (Bodhankar *et al.,* 2017).

**Table 2: Per cent decrease in bacterial growth at varied water potential.**

| Water Potential | Per cent decrease in bacterial growth | | | |
|---|---|---|---|---|
| | <25% | 25-50% | 50-75% | >75% |
| -0.65MPa | 21 | 8 | 7 | 0 |
| -1.57MPa | 3 | 15 | 16 | 2 |
| -2.17MPa | 1 | 4 | 10 | 21 |
| -2.70MPa | 0 | 1 | 10 | 25 |

Values in each column represents number of bacterial isolates

**Biochemical characterization:** According to the Bergey's manual of determinative bacteriology, the physiology and biochemical characteristics of the selected isolates was determined. All the isolates are positive for catalase production except for 3 isolates (PdC3E2, PdC3E3 and VaL3E1). Isolate VaL3E1 is negative for citrate utilization while remaining strains have shown positive result. 20 isolates were found to be $H_2S$ producers, 8 isolates are positive for indole production, 4 isolates are positive for MR test and 25 isolates are positive for VP test. Isolates NL3E5, PdC3E3 and PdS3E1 are found to be negative for gelatin liqification while remaining 33 isolates have shown positive result (Table 3).

**Table 3: Biochemical characterization of bacterial endophytes.**

| Sr.No. | Isolate Code | Catalase | Citrate Utilization | H2S Test | Indole Production | MR | VP | Gel Liquification | Ammonia Production |
|---|---|---|---|---|---|---|---|---|---|
| 1. | AR3E2 | + | + | - | + | + | + | + | + |
| 2. | AlR2E5 | + | + | + | - | - | - | + | - |
| 3. | GC3E2 | + | + | + | - | - | + | + | + |
| 4. | BS2E1 | + | + | + | - | - | + | + | - |
| 5. | VS1E1 | + | + | + | - | - | - | + | + |
| 6. | VR1E1 | + | + | + | - | - | + | + | + |
| 7. | NL3E2 | + | + | + | - | - | + | + | + |
| 8. | NL3E3 | + | + | + | - | - | + | + | + |
| 9. | NL3E4 | + | + | + | - | - | - | + | - |
| 10. | NL3E5 | + | + | - | + | + | - | - | + |
| 11. | NC3E1 | + | + | + | - | - | + | + | - |
| 12. | NC3E2 | + | + | + | - | - | + | + | + |
| 13. | NR3E1 | + | + | + | - | - | + | + | + |
| 14. | NR3E3 | + | + | + | - | - | - | + | + |
| 15. | PdC3E2 | - | + | - | - | - | - | + | + |
| 16. | PdC3E3 | - | + | - | - | - | - | - | - |
| 17. | PdC3E4 | + | + | - | - | - | + | + | + |
| 18. | PdS3E1 | + | + | - | - | + | + | - | + |
| 19. | PdS3E2 | + | + | - | - | - | + | + | + |
| 20. | RgL3E4 | + | + | + | - | - | + | + | + |
| 21. | RgC3E2 | + | + | - | + | - | + | + | - |
| 22. | JC3E1 | + | + | - | + | - | - | + | + |
| 23. | JC3E2 | + | + | - | + | - | + | + | + |
| 24. | PL3E2 | + | + | + | - | - | - | + | + |
| 25. | CC3E3 | + | + | + | - | - | - | + | + |
| 26. | VaR3E1 | + | + | + | - | - | + | + | + |
| 27. | VaL3E1 | - | - | - | + | + | - | + | + |
| 28. | VaS3E1 | + | + | + | - | - | + | + | - |
| 29. | KL3E1 | + | + | + | - | - | + | + | + |
| 30. | KL3E2 | + | + | - | - | - | + | + | - |
| 31. | KS3E1 | + | + | + | - | - | + | + | + |
| 32. | KS3E2 | + | + | + | - | - | + | + | - |
| 33. | LS3E1 | + | + | + | - | - | + | + | + |
| 34. | LS3E2 | + | + | - | + | - | + | + | + |
| 35. | LS3E3 | + | + | - | - | - | + | + | + |
| 36. | LL3E1 | + | + | - | + | - | + | + | + |

Ammonia production is an important trait that indirectly affect the plant growth. Out of all 27 isolates were found to be positive for ammonia production by changing color after addition of Nessler's reagent, indicating that these isolates may accumulate nitrogen in plants and promotes root and shoot elongation which indirectly influencing seed vigor index. Comparable results were reported by Marques *et al.,* (2010) that ammonia producing bacteria accumulate and provide nitrogen to host plant, elongation of plant root and shoot, consequently increasing plant biomass. Similarly Fouda *et al.,* (2021) showed the potency of bacterial endophytes for ammonia production ranging between low to high based on color change of inoculated growth media after adding Nesseler's reagent.

The carbohydrate utilization of endophytic bacterial isolates was evaluated for dextrose, sucrose, maltose and lactose. Dextrose was found to be mostly used carbon source while lactose was leastly used (Table 4). Our results were supported by similar observations reported by Shahab and Ahmed (2008) by testing 10 rhizospheric bacteria for their carbon sources utilization including Glucose, Fructose, Sucrose and Lactose. Out of all glucose was found to be most favorable carbon source for P solubilization while lactose is the least favorable carbon source.

**Table 4: Screening of bacterial endophytes based on carbon utilization.**

| Sr. No. | Isolate no | Dextrose | Sucrose | Maltose | Lactose |
|---|---|---|---|---|---|
| 1. | AR3E2 | + | - | + | - |
| 2. | AlR2E5 | + | - | + | - |
| 3. | GC3E2 | + | + | + | - |
| 4. | BS2E1 | + | + | + | + |
| 5. | VS1E1 | + | + | + | - |
| 6. | VR1E1 | + | + | + | - |
| 7. | NL3E2 | + | + | + | - |
| 8. | NL3E3 | + | + | + | - |
| 9. | NL3E4 | + | + | + | - |
| 10. | NL3E5 | + | + | + | - |
| 11. | NC3E1 | + | + | + | + |
| 12. | NC3E2 | + | + | + | + |
| 13. | NR3E1 | + | + | + | + |
| 14. | NR3E3 | + | + | + | - |
| 15. | PdC3E2 | + | + | + | + |
| 16. | PdC3E3 | + | + | + | + |
| 17. | PdC3E4 | + | - | - | - |
| 18. | PdS3E1 | + | + | + | + |
| 19. | PdS3E2 | + | + | + | + |
| 20. | RgL3E4 | + | + | + | + |
| 21. | RgC3E2 | + | + | + | + |
| 22. | JC3E1 | + | + | + | + |
| 23. | JC3E2 | + | + | + | + |
| 24. | PL3E2 | + | + | + | - |
| 25. | CC3E3 | + | + | + | + |
| 26. | VaR3E1 | + | + | + | - |
| 27. | VaL3E1 | + | + | + | - |
| 28. | VaS3E1 | + | + | + | - |
| 29. | KL3E1 | + | + | + | - |
| 30. | KL3E2 | + | + | + | + |
| 31. | KS3E1 | + | + | + | - |
| 32. | KS3E2 | + | + | + | - |
| 33. | LS3E1 | + | + | + | + |
| 34. | LS3E2 | + | + | + | - |
| 35. | LS3E3 | + | + | + | + |
| 36. | LL3E1 | + | + | + | - |

***In-vitro* Antagonistic Activity:** The bacterial isolates were screened based on their osmotolerance and biochemical characteristics. The prominent isolates were further tested for their antagonistic activity towards pathogenic fungi of maize i.e., *Rhizoctonia solani*, *Fusarium oxysporium* and *Exserohilum turcicum*. Bacterial isolates *Bacillus licheniformis* and *Pseudomonas aeruginosa* have shown antagonism effect for the three tested pathogenic fungi (Fig. 1).

Isolates *Kosakonia radicincitans* and *Kosakonia cowanii* were antagonistic to *Rhizoctonia solani* and *Fusarium oxysporium* while the strains of *Klebsiella pneumoniae* were antagonistic to *Rhizoctonia solani* and *Exserohilum turcicum*. The bacterial isolates *Priestia megaterium*, *Priestia aryabhattai* and *Methylorubrum populi* were found antagonistic to only *Rhizoctonia solani* (Table 6).

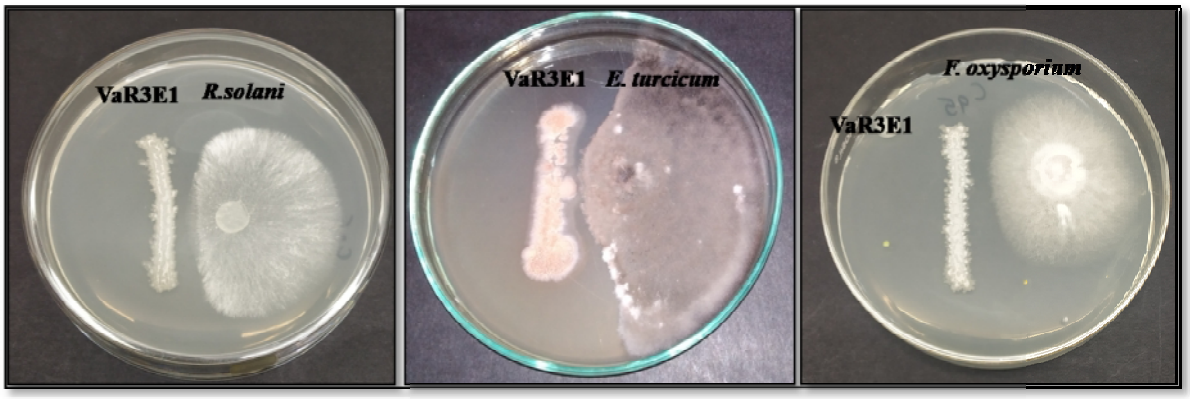


**Fig. 1.** In vitro Antagonistic activity by endophytic bacterial isolates.

**Table 5: Effect of maize seed bacterization by different endophytes on germination and seed vigor.**

| Isolate code | Isolate Name | Root length | Shoot lenght | % Germination | SVI |
|---|---|---|---|---|---|
| AR3E2 | *Gordonia hongkongensis* | 30.167$^{ef}$ | 16.333$^{ef}$ | 90$^{abcd}$ | 4184.8$^{fgh}$ |
| VR1E1 | *Microbacterium hydrothermale* | 29.933$^{f}$ | 19.3$^{abc}$ | 88.67$^{bcd}$ | 4365.1$^{defg}$ |
| NL3E3 | *Kosakonia radicincitans* | 32.3$^{d}$ | 17.167$^{de}$ | 86$^{d}$ | 4255.9$^{efgh}$ |
| NC3E2 | *Kosakonia cowanii* | 31.3$^{def}$ | 18.367$^{abcd}$ | 88$^{cd}$ | 4370.1$^{defg}$ |
| PdS3E1 | *Priestia megaterium* | 38.267$^{a}$ | 18.067$^{cd}$ | 94.67$^{ab}$ | 5334.9$^{ab}$ |
| PdS3E2 | *Priestia aryabhattai* | 30.233$^{ef}$ | 18.067$^{cd}$ | 92.67$^{abc}$ | 4473.3$^{def}$ |
| RgL3E4 | *Klebsiella pneumoniae* | 27.5$^{g}$ | 14.233$^{gh}$ | 94.67$^{ab}$ | 3949.3$^{gh}$ |
| JC3E2 | *Priestia megaterium* | 31.967$^{de}$ | 17.933$^{cd}$ | 94$^{abc}$ | 4688.2$^{cde}$ |
| PL3E2 | *Priestia aryabhattai* | 27.767$^{g}$ | 14.967$^{fg}$ | 90$^{abcd}$ | 3846.5$^{h}$ |
| CC3E3 | *Cellulosimicrobium funkei* | 34.167$^{c}$ | 12.833$^{h}$ | 93.33$^{abc}$ | 4388.9$^{defg}$ |
| VaR3E1 | *Bacillus licheniformis* | 36.067$^{b}$ | 19.167$^{abc}$ | 95.33$^{a}$ | 5265.2$^{ab}$ |
| KL3E1 | *Kosakonia cowanii* | 34.467$^{bc}$ | 18.167$^{bcd}$ | 94$^{abc}$ | 4946.8$^{bc}$ |
| KS3E1 | *Pantoea dispersa* | 38.333$^{a}$ | 19.833$^{a}$ | 92.67$^{abc}$ | 5390.8$^{a}$ |
| LS3E1 | *Pseudomonas aeruginosa* | 32.233$^{d}$ | 19.733$^{ab}$ | 76.67$^{e}$ | 3992.1$^{gh}$ |
| LS3E3 | *Klebsiella pneumoniae* | 31.633$^{def}$ | 19.233$^{abc}$ | 94$^{abc}$ | 4783.9$^{cd}$ |
| LL3E1 | *Methylorubrum populi* | 34.633$^{bc}$ | 19.4$^{abc}$ | 93.33$^{abc}$ | 5043.6$^{abc}$ |
| CONTROL | No inoculum | 30.467$^{def}$ | 17.3$^{de}$ | 88$^{cd}$ | 4204.1$^{fgh}$ |
| **CD** | | 1.747 | 1.436 | 5.600 | 393.055 |
| **CV** | | 3.232 | 4.880 | 3.695 | 5.175 |

Values in each column are means of three replications compared using Duncans Multiple Range Test (DMRT). Same letters in the column are not significantly different between treatments at P < 0.05

In present study *Bacillus licheniformis* and *Pseudomonas aeruginosa* were found to have antagonistic activity towards all the three tested pathogenic fungi with maximum per cent of mycelium inhibition in the range of 84-95%. The results are similar with the findings of Esikova *et al.,* (2021) as the genera *Bacillus* and *Pseudomonas* were characterized by high activity against fungal phytopathogens. In current invetigation the efficient antagonistic bacteria were isolated from root and stem of maize and the results were comparable with Mugiastuti *et al.,* (2020) that the endophytic *Bacillus* sp, isolated from maize root (BK.A1; BK.A3; PP.A5) and stem (PPD.B2) can suppress the growth of *R. solani* by more than 50% and considered to be prominant. Similarly *Bacillus* strains isolated from pearl millet niche showed antagonistic activity by inhibiting mycelium growth of *Sclerotium rolfsii* (45 – 78%) and *Rhizoctonia solani* (47 – 80 %) (Kushwaha *et al.,* 2019) and strong antagonistic activity was exhibited by maize endophytic *Bacillus* spp. against the pathogen *Fusarium moniliforme* (Gond *et al.,* 2015).

The efficient antagonistic bacteria may induce the systemic resistance against pathogenic fungi in host plants and can be applied as biopesticides. The results were in positive correlation with the findings of Pandey *et al.* 2012 that *Pseudomonas aeruginosa* PW09 isolated from wheat found to trigger an induced systemic resistance in cucumber plants infected with fungal pathogen *Sclerotium rolfsii*. Similarly, 4 endophytic bacteria isolated from maize plant niche characterized successfully and found have the potential to be developed as biopesticides to control maize disease, especially *R. solani* and *Pantoea* sp. (Prihatiningsih and Soesanto, 2020). Soybean root endophytes *Pseudomonas otitidis*, *Pseudomonas* sp and *Bacillus cereus* exhibited antagonistic activity against *F. oxysporum*, inhibiting fungal growth by 97%, 98% and 98.5% respectively (Dubey *et al.,* 2021). Several maize endophytic bacteria were reported previously for having antagonistic properties against pathogenic fungi (Naveed *et al.,* 2014; Bodhankar *et al.,* 2017; Ali *et al.,* 2018; Rana *et al.,* 2021).

**Seed vigor index:** Many of the isolates from current investigation have improved seedling length, % germination and seed vigor index compared to control. Maximum root length was obtained in seedlings treated with the bacterial isolates *Pantoea dispersa* (KS3E1) measuring 38.33 cm and *Priestia megaterium* (PdS3E1) measuring 38.27 Cm of root length which are significantly higher than control (no inoculum) having root length of 30.47 cm. significantly higher shoot length was obtained in seedlings treated with *Pantoea dispersa* (KS3E1) measuring 19.83 Cm followed by *Methylorubrum populi* (LL3E1) with 19.40 cm of shoot length. Maximum germination per cent was shown by *Bacillus licheniformis* (VaR3E1) recording 95.33% followed by *Priestia megaterium* (PdS3E1) and *Klebsiella pneumonia* (RgL3E4) recording 94.67% which are significantly higher than the control (88%). The seed vigor index is significantly higher for *Pantoea dispersa* (5390.8) followed by *Priestia megaterium* (5334.9) and *Bacillius licheniformis* (5265.2) (Table 5). Thus inoculation of few efficient strains has improved seed germination percentage and seedling length which may be due to the accumulation of nitrogen and IAA production by endophytic bacteria.

Supportive results were observed in studies of Ullah *et al.,* (2017) that root length and plant density was observed to increase when inoculated with endophytic bacteria via various mechanisms including the production of plant hormones, ammonia, making bioavailability of nutrients and antagonistic action to phytopathogens. Similarly, inoculation of endophytic

bacteria has reported to increase germination rate by 20-40% in maize seeds and highest of 40% increase was given by *Enterobacter* sp. FD 17 (Naveed *et al.*, 2014).

Seedling vigor, germination percentage and plant biomass were reported to get enhanced when the seeds were treated with *B. amyloliquefaciens* EPP90 (Kushwaha *et al.*, 2019). Inoculation of endophytic bacterium *Herbaspirillum* spp at the rate of 10 to 20% (v/v) increased seed vigor rate and total seed germination rate 80-95% and 90-100%, respectively (Briatia *et al.*, 2016). The increased seedling length and germination per cent may be due to the production of IAA by endophytic bacteria. Though many isolates from our study improved seedling length and % germination but further investigation is needed for tracking influence of isolates under given water potential against seedling growth.

**Table 6: Antagonistic activity of endopytic bacterial isolates against maize fungal pathogens.**

| Sr.No. | Isolate code | Isolate Name | % inhibition of pathogenic fungal growth | | |
|---|---|---|---|---|---|
| | | | *Rhizoctonia solani* | *Fusarium oxysporium* | *Exserohilum turcicum* |
| 1. | AR3E2 | *Gordonia hongkongensis* | 8 | 12 | 10 |
| 2. | VR1E1 | *Microbacterium hydrothermale* | 10 | 6 | 7 |
| 3. | NL3E3 | *Kosakonia radicincitans* | 96 | 68 | 8 |
| 4. | NC3E2 | *Kosakonia cowanii* | 75 | 72 | 16 |
| 5. | PdS3E1 | *Priestia megaterium* | 82 | 7 | 12 |
| 6. | PdS3E2 | *Priestia aryabhattai* | 76 | 6 | 10 |
| 7. | RgL3E4 | *Klebsiella pneumoniae* | 95 | 15 | 54 |
| 8. | JC3E2 | *Priestia megaterium* | 74 | 12 | 10 |
| 9. | PL3E2 | *Priestia aryabhattai* | 72 | 10 | 12 |
| 10. | CC3E3 | *Cellulosimicrobium funkei* | 10 | 8 | 6 |
| 11. | VaR3E1 | *Bacillus licheniformis* | 94 | 89 | 84 |
| 12. | KL3E1 | *Kosakonia cowanii* | 92 | 74 | 15 |
| 13. | KS3E1 | *Pantoea dispersa* | 12 | 10 | 8 |
| 14. | LS3E1 | *Pseudomonas aeruginosa* | 96 | 92 | 56 |
| 15. | LS3E3 | *Klebsiella pneumoniae* | 94 | 17 | 62 |
| 16. | LL3E1 | *Methylorubrum populi* | 71 | 12 | 23 |
| 17. | Control | No inoculum | 0 | 0 | 0 |

**Intrinsic antibiotic sensitivity:** Out of all (16) studied endophytic bacteria many were found resistant to 2 antibiotics Ampicillin and Penicillin G out of 6 tested (Table 7). Endophytic bacterial FTR isolated from maize niche was resistant to eight antibiotics out of 19 tested and reported as the most potential endophyte to compete against other microbes. (Sandhya *et al.*, 2017). In vitro screening of maize endophytic bacteria *Enterobacter cloacae* showed resistance against 15 different antibiotics (Maqbool *et al.*, 2021). Broad spectrum resistant strains have been reported from the palm tree endophytes *Enterobacter cloacae* subsp. *Cloacae* and *Acinetobacter pitti* against many antibiotics (Yaish *et al.*, 2015). Thus, *Kosakonia* strains of maize endophytes studied in present research *K. radicincitans* and *K. cowanii* were assumed as the potential isolates to compete against other microbes as the antibiotic resistant strains have the ability to compete with other strains.

**Table 7: Assay of intrinsic antibiotic sensitivity of endophytic isolates.**

| Sr.No. | Isolate code | Ampicillin (10 mcg) | Chloramphenicol (25 mcg) | Penicillin-G (1 unit) | Streptomycin (10 mcg) | Sulphatriad (300 mcg) | Tetracyclin (25 mcg) |
|---|---|---|---|---|---|---|---|
| 1. | AR3E2 | S | S | R | S | S | S |
| 2. | VR1E1 | R | S | R | S | S | S |
| 3. | NL3E3 | R | S | R | S | R | S |
| 4. | NC3E2 | R | S | R | S | R | S |
| 5. | PdS3E1 | R | S | S | S | S | S |
| 6. | PdS3E2 | R | S | S | S | S | S |
| 7. | RgL3E4 | R | S | R | S | S | S |
| 8. | JC3E2 | R | R | R | S | S | S |
| 9. | PL3E2 | R | S | S | S | S | S |
| 10. | CC3E3 | R | S | R | S | S | S |
| 11. | VaR3E1 | R | S | R | S | S | S |
| 12. | KL3E1 | R | S | R | S | R | S |
| 13. | KS3E1 | R | S | R | S | S | S |
| 14. | LS3E1 | S | S | S | R | S | R |
| 15. | LS3E3 | R | S | R | S | S | S |
| 16. | LL3E1 | S | S | S | R | S | R |

R- Resistant (no zone formation); S- Sensitive (zone formed).

## CONCLUSION

To cope with the biotic and abiotic stress against plant growth various approaches have been under consideration including plant-microbe interactions. The endophytic bacteria with less per cent of decreased growth at all treated water potential are found to be *Kosakonia radicincitans, Priestia aryabhattai* and *Bacillus licheniformis*. For all the three (*Rhizoctonia solani, Fusarium oxysporium* and *Exserohilum turcicum*) tested pathogenic fungi *Bacillus licheniformis* and *Pseudomonas aeruginosa* were found to inhibit the fungal mycelium growth at high rate. And many isolates have improved seedling growth and germination percentage significantly. From our in vitro results, we can conclude that plants may survive and grow even under moisture deficit stress and biotic stress as endophytes have adapted to function in effective way under stress conditions. But it has become essential to evaluate true efficiency of isolates under stressed environment. Thus, influence of isolates under field conditions either as single inoculum or as a consortium should be investigated.

## FUTURE SCOPE

A deep insight of the symbiotic interaction among the host plant and endophytes is essential for optimal growth and development of plants under biotic and abiotic stress comdtions. Future omics research is needed to elucidate the exact mechanism of their action in order to harness their potential in other crops and achieve the goal of sustainable crop production. The endophytes used in this study may be used for the development of bioinoculants under drought stress conditions

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