IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Potentials Of Azotobacter Chroococcum Against Plant Pathogenic Fungi

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Abstract

In present study, five Azotobacter species were screened against A. alternata, A. niger and P. frequentans to check their antifungal activity. Azotobacter strains were tested for the antagonistic activity against pathogenic fungi. Secondary metabolites were isolated and characterized from the Azotobacter strains for the first time. Azotobacter strains were isolated from different cereal field soils by serial dilution agar plating method on Jenson's N-free media. The colonies were glistening, smooth, slimy, brown to black on Jenson's N-free agar plates and the cells were Gram negative rod shaped non motile. Biochemically, they were positive for indole acetic acid production, citrate, catalase and Voges Proskauer test. All the isolated species were tested for antifungal activity against different plant pathogens. The species of Azotobacter were able to inhibit the growth of three species plant pathogens viz., Aspergillus, Fusarium and Alternaria in two to four days of incubation period. Azotobacter chroococcum produced a maximum zone of inhibition against Fusarium and Alternaria species by producing antifungal metabolites in the media. Among all the tested pathogens A. niger and P. frequentans showed resistance against Azotobacter.

Key words: Azotobacter chroococcum, antifungal activity, antagonist, Aspergillus, Fusarium, Alternaria

Introduction:

Microbial antagonists are widely used for the biocontrol of fungal plant diseases (Prapagdee et al., 2008; Yang et al., 2007). Their multiple mechanisms in biocontrol include ability to produce wide variety of antibiotics, chitinolytic enzymes, siderophores and HCN (Banasco et al., 1998; Kumar et al., 2002). Azotobacter is free living, nitrogen fixing bacterium. Azotobacter is one which is found in most of the soils and established more profusely in the rhizosphere of the crop plants. There are many reports on beneficial effects of A. chroococcum on growth and yield of agriculturally important crops (Mali and Bodhankar, 2009). Primarily the function of Azotobacter is to fix molecular nitrogen, but the ability of A. chroococcum to synthesize auxins, vitamins, growth promoting substances and antifungal antibiotics. The beneficial effects of Azotobacter on growth and yield of various agriculturally important crop plants include their potential to fix N_2, produce vitamins and growth substances including indole acetic acid, gibberellins and cytokines (Verma et al.,2001) confer it with additional advantage (Brown, 1974 and Mishustin and Naumova, 1962). By virtue of these facts, Azotobacter chroococcum plays nutritional and stimulatory role for the benefit of the plants with its manifold action such as suppressing the infestation of plant pathogens, attributes the favorable effects on beneficial microorganisms in soil. Cereal crops like wheat, rice, sorghum and maize carry part of micro flora occur naturally with growing plants. Many researchers also reported antifungal activity of Azotobacter sp. isolated from different soil samples. Cavaglieri et al. (2005) studied the effects of Azotobacter sp. The most common genera of fungi found in the grains of cereal crops are Fusarium, Alternaria, Cladosporium, Helminthosporium, Aspergillus and Penicillium. Out of these Aspergillus and Penicillium are considered storage fungi and others are field fungi. These fungi cause the spoilage and destruction of food grains. The toxins produce by these fungi

are hazardous to human and animal health. In the foresaid investigation attempts are made to use the antagonistic substance produced by isolates of Azotobacter chroococcum against the pathogens of cereal crops. The aim of the present study was to test the antagonistic activities of A .chrococcum isolates against seed borne fungi of cereals in vitro and subsequently its influence particularly on sorghum in relation to effect on germination and yield of grains under field condition.

Materials and Methods:

Isolation and characterization of A. chroococcum:

Total 124 isolates of A. chroococcum were isolated from rhizosphere soil of cereal crops from different locations of Vidarbha region. The soil samples were inoculated on Jensen's medium. It has been reported that availability of nutritional requirements of organisms play an important role during metabolite synthesis process. Amongst various nutritional requirements, antifungal substance production has been known to be influenced by media components and cultural conditions, such as agitation, pH, temperature, carbon source and incubation time, which vary from organism to organism (Dahiya et al., 2006). Morphological, biochemical and cultural identifications were done by standard methods (Bergey's Manual of Systematic Bacteriology) (Holt, 1994).

Preparations of antagonistic fractions:

The isolates of A. chroococcum were inoculated in 100 ml of Ashby's broth medium in 250 ml conical flask and incubated at 28°C on shaker for four days. Four fractions were prepared from A5, M4, M7, M8 and S2 isolates of A. chroococcum. The broth culture of A. chroococcum, the broth culture was centrifuged and collected the supernatant. The cell mass obtained was suspended in minimal quantity of phosphate buffer of pH 4.6 and sonicated for 20 min. The sonicated cultures were centrifuged and collected the supernatant. The four fractions obtained were:

I - Culture broth containing cells in growth medium.

Il - Filtrate of culture broth (supernatant after centrifugation).

III - Sonicated culture and

IV - Filtrate of sonicated culture containing cell free extracThe antagonistic activity of each isolate was tested on PDA plates seeded with A. niger, P. frequentans and A. alternata, by standard cup plate method. The antagonistic test was carried out with 0.2ml from each fraction.

Ethanol Fractionation of Protein:

Ethanol fractionation was carried out with 8 ml of 20% chilled ethanol added in fraction III and shaken vigorously for 60 minute under cold environment. The precipitate obtained was separated by centrifugation for 30 minute at 4000 rpm, then washed twice with sterilize distilled water. This precipitate were suspended in 0.01M Phosphate buffer, 1 N HCl and 1 N NaOH. The suspensions were kept at 4°C prior to analysis.

Antagonistic Test of fractioned protein:

Antagonistic activity of 0.2 ml ethanol fractionated protein was evaluated by cup plate method at 28°C for 48 hrs, and the zone of inhibition was measured in mm. Beside the antagonistic activity of A. chroococcum against plant pathogenic fungi, studies were also conducted on determination and identification of location of antibiotic substance in A. chroococcum cell

Results and Discussion:

All the isolated species were tested for antifungal activity against different plant pathogens. The species of Azotobacter were inhibited the four species plant pathogens viz., Aspergillus, Fusarium, Penicillium and Alternaria in 48 to 96 hrs. of incubation period. Out of 124 samples of A. chroococcum, broth culture (Fraction 1) of A5, M4, M7, M8 and S2 recorded maximum inhibitory activity against plant pathogenic fungi (Fig 1). Agarwal and Singh (2002) also reported antifungal activity of Azotobacter sp. against F. oxysporum, Rhizoctonia solani and Aspergillus sp.The potentiality of individual isolates of A. chroococcum culture varied in antifungal activity is shown in table and graph. Amongst all isolates of A. chroococcum S2 showed maximum activity against all pathogenic fungi A potential of A. chroococcum isolates against different plant pathogens were tested with prepared fractions of II, III & IV. Filtrate of culture broth, sonicated culture and filtrate of sonicated culture containing cell free extract of five isolates of A. chroococcum were shown varied inhibitory effects on plant pathogens. The fraction III of all isolates was

proved superlative against P. frequentans (Fig :3) followed by fraction IV and II (Fig.2). Isolate M8 and S2 of A. chroococcum showed high potentials against fungi tested in this study. The study was also revealed that the A. niger remain unaffected by all fractions of A5, M4 and M7. The result of potential is shown in table 2. Similarly, the ethanol extracted moiety from A. chroococcum indicated that the extracted metabolites were fungistatic in nature. Dilutions of ethanol extracted moieties in 1 N HCI, IN NaOH and 0.01 M phosphate buffer respectively, showed different inhibitory values against the tested plant pathogens. The dilutions in 1 N NaOH showed no inhibitory actions while dilutions in 1 N HCl and 0.01M P buffer showed good inhibitory effects on plant pathogens. After collecting and testing the four fractions of A. chroococcum culture it is revealed that these metabolites located intracellularly. The maximum zone of inhibition was recorded in sonicated broth culture of fraction III. Similarly studies on determination of inhibitory metabolites indicated that the active inhibitory principal was protenicious and funfistatic in nature, It has been reported that A. chroococcum showed beneficial effects on agriculturally important crops. It advantageous for plants in various ways; like produces nitrogen source, vitamins, phytohormones and fungistatic substances. Fungistatic substances pays increasingly important role in improvement of agricultural crops thus bacterization with A. chroococcum may add economic feasibility in crop production programme as self generating source of inhibitors.

Isolates of A. chroococcum varied in their potential to inhibit the growth of plant pathogens as per results shown in Table 1. Similar antifungal action of A. chroococcum against Aspergillus sp. Penicillium sp. Fusarium sp. and Alternaria sp. have been reported in different studies (Laxmikumari et.al 1978 and Mishustin, E. N. 1966). The efficiency of inhibition depended upon the selection of appropriate isolates, the mode of application of inoculants and susceptibility of different phytopathogenic fungi to Azotobacter isolates (Meshram, S.U. and Shende, S. T. 1982). Our findings about the efficacy of selected isolates and their effectiveness against plant pathogenic fungi are coequal with Mishustin et al and Laxmikumari et al. Variation in disease suppressing potential of isolates of A. chroococcum may be due to their variable production of inhibitory metabolites in culture after the inoculation. Fraction III and IV of all isolates was found to be more antagonistic. This part consisted the intracellular moiety of the cells, indicated the presence of inhibitory substance within the cells. The present study was in tuned with Mishusthin et. al.1962 and Sardaryan 1967 In present investigation attempts were made to fractionate the antifungal metabolite by ethanol extraction followed by test of the efficiency of extract in different solutions. However, our findings are in agreement with the studies of Sardaryan 1967 and Pridachina, 1972, who isolated the antibiotic substance from bacterial mass (A. chroococcum) with ethanol extraction followed by purification with column and thin layer chromatography (Pridachina et.al.1982 and Sardaryan, E. O. 1972). They reported that it was totally new antibiotic moiety which was not described earlier.

Fig 1: Antifungal potential of different isolates of A. chroococcum

Table 1 : Antagonistic activity of A. chroococcum isolates (fraction I) against plant pathogens

Sr. No	Plant Pathogemic fungi	Isolates of A. chroococcum with zone of inhibition (mm)														
		A5			M4			M7			M8			S2		
		48 hrs.	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs	48 hrs	72 hrs	96 hrs
	Alternaria, alterneta	16	15	21	31	30	30	24	24	24	24	25	25	27	28	28
2	Aspergillus niger	00	00	00	00	00	00	00	00	00	27	27	27	28	29	32
3	Penicillium frequntans	42	42	42	27	27	36	24	23	23	19	19	23	25	25	30

Taken together our findings and data in the literature indicate that A. chroococcum isolates produces antifungal metabolites, have potential to restrict the growth of pathogenic fungi in vitro and in vivo conditions. According to Mishushtin et. al. (1968) the antifungal metabolite produce by A. chroococcum was totally new antibiotic moity which was not describe earlier They reported that antifungal substance produce by A. chroococcum belongs to the conactin groop of antibiotics which is closure to “Anisomycin” in action but differ from the later in its UV spectrum These isolates can be used as bio-control agents for control of different plant pathogens causing different plant diseases. These isolates are varied in the production of metabolites and their antagonistic potential. Further investigation is needed for isolation, separation, purification and identification of the antibiotics produce by A. chroococcum, in future which would be great source of help to the farmers in controlling the fungal infestation of cereal crops.

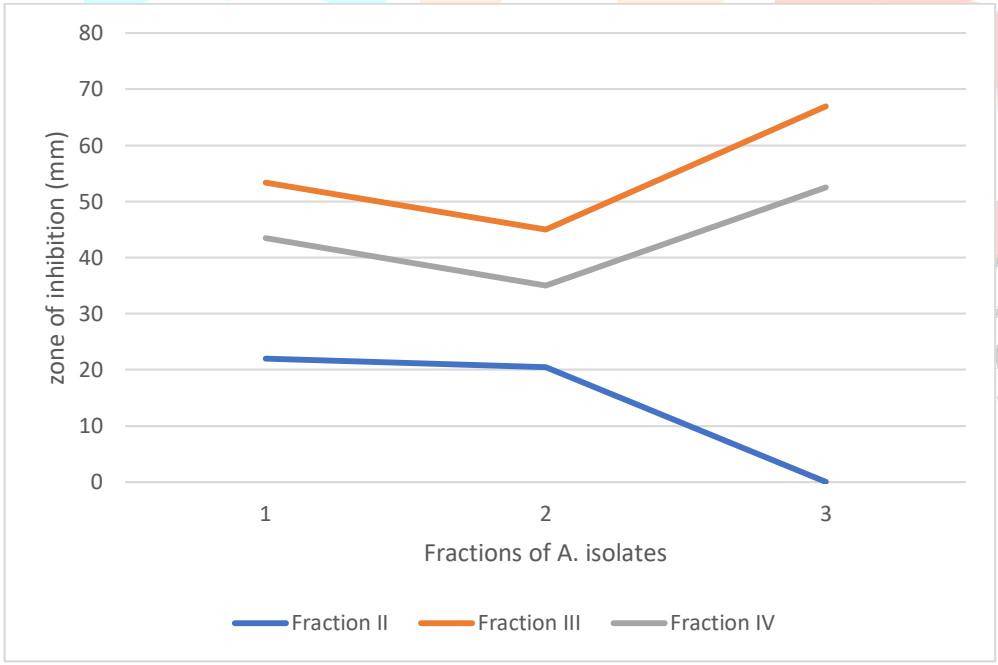

Fig 2: Antifungal potential of different fractions prepared from A. chroococcum

Table 2: Inhibitory effect of fractions II, III, IV of Azatobacter isolates against plant pathogens.

Sr. No.	Fungi on antagonistic activity tested	Fractions of A. chroococcum	A. chroococcum isolates and their Zone of inhibition in mm (Average)				
			A5	M4	M7	M8	S2
1	A.alterneta	II	12	17	10	11	22
2		III	41.8	43.7	33.6	38.3	53.4
3		IV	32	35	30.1	30.3	43.5
4	A.niger	II	00	00	00	20	20.5
5		III	00	00	00	35	45
6		IV	00	00	00	25	35
7	P. frequentans	II	18.8	20	11	20.5	00
8		III	52.9	51.4	52.1	52.1	67
9		IV	42.8	41.5	42.1	42.8	52.2

Fig 3: Antagonistic action of isolate S2 (fraction III and IV) against P. frequentans

Table 3: Inhibitory effect of ethanol freeze extracted metabolites of fraction IV from A. chroococcum on the growth of plant pathogens.

Sr. No.	Fungi on antagonistic activity tested	Fractions IV of A. chroococcum diluted in solvents	A. chroococcum isolates and their Zone of inhibition in mm (Average)				
			A5	M4	M7	M8	S2
1	A.alterneta	1 N HCl	20	22.5	16	20	33
2		1 N NaOH	00	00	00	00	00
3		0 01M P buffer	24	25	20	23	41
4	A.niger	IN HCl	00	00	00	24	26
5		1N NaOH	00	00	00	00	00
6		0.01M P buffer	00	00	00	29	32
7	P. frequentans	1 N HCl	29	23	16	26	23
8		1N NaOH	00	00	00	00	00
9		0.01 M P buffer	13	29	23	29	33

Acknowledgement: The author is grateful to ICAR, New Delhi for financial help, also thankful to the Principal Seth Kesariml Porwal College Kamptee for his constant support.

References:

1. Agarwal, N. and H.P. Singh: Antibiotic resistance and inhibitory effect of Azotobacter on soil Ann. Rev. Phytopathol., 26, 379-407 (2002).
2. Banasco, P., F.L. DeLa, G. Gaultieri, F. Noya, and A. Arrias: Fluorescent Pseudomonas spp. as biocontrol agents against forage legume root pathogenic fungi. Soil Biol. Biochem., 10, 1317-1323 (1998).
3. Brown, M.E. (1974). Ann. Rev. Phytopath. 12:181-97.
4. Cavaglieri, L.R., L. Andres, M. Ibanez and M.G. Etcheverry: Rhizobacteria and their potential to control Fusarium verticillioides: Effect of maize bacterisation and inoculum density. Antonie Van Leeuwenhoek, 87, 179-187 (2005).
5. Dahiya, N., R. Tewari and G.S. Hoondal: Biotechnological aspects of chitinolytic enzymes. Appl. Microbiol. Biotechnol., 71, 773- 782 (2006).
6. Holt, J.G. (1994). Bergy's manual of determinative bacteriology-9th edition. Pp 77.
7. Laxmikumari, M., Vijayalakshmi, M and Subha Rao,N.S.(1972). Phytopath. 75:27-30.
8. Mali, G.V. and Bodhankar, M. G. (2009). Asian J. Exp. Sci. 23 (1): 293-297.
9. Meshram, S.U.and Shende, S. T. (1982). Plant and Soil. 69: 265-273.
10. Mishustin, E. N., Naumova, A.N. Khokhlova, Yu. M., Ovshtoper, S. N. and Smirnova, G.A. (1969). Mikrobiologia 38 (1): 71-73.
11. Mishustin, E. N. (1966). Ann. Inst. Pasteur Paris III.: Suppl. 3: 121-35.
12. Mishustin, E.N., and Neumova, A.N., (1962) Mikrobiologia 31:543-555.
13. Prapagdee, B., C. Kuekulvong and S. Mongkolsuk: Antifungal potential of extracellular
14. metabolites produced by S. hygroscopicus against phytopathogenic fungi. Int. J. Biol. Sci., 4, 330-337 (2008).
15. Pridachina, N. N., Novogrudskaia, E. D.,Krugliak, E. B., Chekasina, E. V., Korchak, T. S.(1982). Antibiotiki. 27 (1):3-6.
16. Sardaryan, E. O. (1972), Biol. Zn. Arm. 25 (4): 31-35.