Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 2024-2035

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.242

Isolation of Biocontrol (Bacterial) Agents from Chickpea (*Cicer arietinum linnaeus*)

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Keywords

Antagonistic, Bacterial isolates, Bio-control agent, Chick pea and Soil born

Article Info

Accepted:
17 January 2018
Available Online:
10 February 2018

A B S T R A C T

The present study entitled "Isolation and identification of Biocontrol (bacterial) agents from the 50 soil samples which were collected from rhizosphere of infected Chick pea (*Cicer arietinum* L.)"crop were found to have twenty nine bacterial isolates *Pseudomonas spp*, *Bacillus spp* and *Paenibacillus spp* are (0.985%). *Halobacterium spp* and *Planococcus spp* are (0.015%). *Micrococcus spp* (0.188%), *Enterobacter spp* (0.462%) and *Staphylococcus spp* are (1.53%). By characterizations test, biochemical tests and sugar fermentations we identified the bacterial isolates. From this isolated species, some bacteria like *Pseudomonas spp* and *Bacillus spp* showing antagonistic property against soil borne pathogens like *Alternaria spp*, *Curvularia spp*, *Aspergillus spp* etc., which causes diseases in Chick pea

Introduction

Gram or Chickpea (*Cicer arietinum Linnaeus*), a member of family Fabaceae, is an ancient leguminous crop which is self pollinated, diploid annual (2N=16 chromosomes) grown since 7000BC, in different area of the world but major cultivation is concentrated in semi-arid environments of different areas of the world. It is ranked 3rd after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum*) and known with different regional names like Bengal gram and locally called 'Chana' (Tekeoglu *et al.*, 2000).

Area, production and productivity of chick pea crop in India is about 73.7lakhs/ha in area 88.9lakhs tonnes in production and productivity of chick pea are 495051 (ha), 440572mt, 890 (q/ha). Historically the name of chick pea back to French 'Chiche' and to Latin *Cicer* but Oxford English Dictionary lists a 1548 citation indicate "*Cicer* may be named in English Cich after the French tongue." Grain legumes are members of sub family papillionoidae, and Chick pea was placed in the tribe Vicieae that includes vetch, lentil and faba bean mainly due to the pollen morphology and vascular anatomy. After this tribe Ciceraceae was subsequently classified

separately from the members of the Vicieae. There are 43 species so far reported for the genus *Cicer*, 9 annual (including the cultivated *Cicer arietinum*), 33 perennial and 1 unspecified. Out of these, three species of *Cicer*, viz., *Cicer bijugum, Reck., Cicer echinospermum* Davis and *Cicer reticulatum* are closely related to chickpea that grow wild in nature (Ladizinsky,1976).

Chick pea is grown in mainly topical, subtropical and temperate regions of the world and this is one of the most important pulse crops of Pakistan and India due to its multiple functions in the traditional farming system (Saxena and Sing, 1987).

There are two types of gram, one is the 'Kabuli' type of Chick pea is grown in temperate regions while the desi type chick pea is grown in the semi-arid tropics (Malhotra *et al.,* 1987). It was grown on area of 1029 thousand hectares in Pakistan with a yield of 480 kg per hectare during 2006.

More than fifty pathogens have, so far been reported on Chich pea from different parts of the world. Only a few of the pathogens have the ability to devastate the chick pea crop and hamper its overall yield. One of the reasons for low yield of chick pea is the damage by various diseases. The most common diseases in chick pea are blight, wilt and root rot diseases (Nene *et al*, 1978).

By using of biological control agents, like plant growth promoting rhizobacteria (PGPR), can be a suitable approach in control of disease (Schmidt *et al.*, 2004).

By use of microorganisms for biocontrol purposes has become an effective to control plant pathogens. There are many bacterial and fungal strains for biocontrol application. As bacterial biocontrol agents *Agrobacterim, Pseudomonas, Alcaligiens, Bacillus, Streptomyces* and others have been reported. The *Pseudomonas* exhibits chitinolytic activity and cellulolytic activity have occasionally reported among Fluorescent pseudomonas antagonistic to fungal pathogens promotes the formation of nodulation by *Mesorhizobium spp. Cicer* in Chick pea (Sindhu *et al.,* 2001).

The use of antagonistic microorganisms against the soil born fungi like *Rhizoctonia solani, Fusarium oxysporium, Sclerotium sclerotiarum* have been investigated as one of the alternative control methods. *Trichoderma spp* and *Bacillus Spp* are wide spread throughout the world and have been recognised as the most successful biocontrol agents for controlling the soil borne pathogens (Bernal *et al.,* 2002).

Plant growth promoting Rhizobacteria (PGPR), such as pseudomonas and bacillus strains, are the major root colonizers, and can elicit plant diseases, pseudomonas and bacillus strains have great potential in control the diseases of Chickpea. Chick pea Fusarium wilt was controlled by using *Arbuscular mycorrhizal* fungai, *Glomus hoi, Glomus fasciculatum* which are the important members of rhizosphere and biological control agents. Seven biocontrol agent namely *Bacillus subtilis, B. megaterium, B.cerus, Trichoderma viride, T. harzianum, Aspergillus spp, Pencillium spp*. isolated from Chick pea rhizosphere, were tested for their antagonistic action against the tested pathogens. *T. viride* and *B. megaterium* proved to be the most effective isolates for controlling the diseases (Montaser, 2008). The seed treatments with *T. harzianum* and *P. chlamydosporia* effectively controlled the wilt and root knot on Chick pea and Pigeon pea and greatly reduced the soil population of pathogens (Mueebur *et al.,* 2011). Chickpea is eaten directly and for preparation of food materials by the human beings. To control the

diseases in Chick pea the farmers using toxicant pesticides which are harmful to human beings. To avoid this, use of bi - control agents are safer to human beings and also to control the diseases. Keeping this in view the present study entitled 'isolation and identification of Biocontrol (bacterial) agents from rhizospheric soil of Chick pea (*Cicer arieninum* L.) was conducted.

Materials and Methods

Isolation of biocontrol (bacterial) biocontrol agents from the rhizosphere soil

The Soil from the Chick pea field was collected and the soil which is adhering to the Rhizosphere was collected and the composite mixture of the soil is made. For the Bacterial isolation the serial dilution technique were done and King's B media were used for the isolation of bacterial bio control agents.

Place of work

Present study entitled Isolation and identification of the Biocontrol (bacterial) agents from rhizosphere soil of Chick pea crop" was conducted at Department of Microbiology and Fermentation Technology, Post graduate laboratory of Sam Higgins bottom Institute of Agriculture, Technology and Sciences, Deemed to be university During 2014-15.

Sample collection

Total Fifty soil samples were collected from rhizosphere of Chick pea crop from the growing areas of Ganga par and Jamuna par in Allahabad region. Soil samples (50g each) were collected from four corners and the center of the field. The samples were collected in polythene bags with the help of auger and samples were sun dried. Before the collection of samples auger was cleaned with sterilized water. Total soil samples were collected from rhizosphere of local chick pea fields. For rhizosphere soil, plants were gently uprooted, soil tightly adhered the roots is collected and mixed and composite mixture of soil of the region was obtained. The healthy plants in the vicinity of sick plants was collected by uprooting the plants; around 60-100g of rhizosphere soil collected by uprooting with the roots. Then 10g of rhizosphere soil with roots transferred into 250ml of conical flask containing 100ml of sterile distilled water, and shall be kept in shaker for about 24 h then 1ml of this suspension was made into serial dilution up to 10^{-6} and about 0.1ml of this was placed nutrient agar media to isolate the cultures or the colonies of bacteria. The plates were incubated at 28^{o} C for 48 h (Manjunatha *et al.*, 2012).

Characterization tests of Biocontrol (bacterial) agents

HCN production

By using t6h method of solution of 0.5% picric acid and 1% sodium carbonate is prepared in a sterilized flask. One ml of test culture organism is inoculated in nutrient agar plate except one, which served as control. Whatsman filter paper strip impregnated with alkaline picric acid solution is placed in the upper lids of inoculated petriplates under aseptic condition. The plates are incubated for 24 hours at 28^{O}C. When Whatsman filter paper impregnated with alkaline picric acid a colour change from yellow to orange is observed indicating HCN production by isolates.

Production of siderophores

The chrome Azurol S (CAS) method is used to determine siderphores production by the soil rhizosphere isolates. An orange yellow

colour indicates siderophores production and a blue colour indicates no siderophore production. The rhizosphere isolates are grown for 48 hour in rhizosphere. This low iron containing medium encourages the production of siderophore and has sufficient carbon to promote growth. Aliquots of the isolates are transferred into the fresh RSM and the color indicator CAS color change is recorded after 48 hours. (Schwyn and Neiland, 1987)

Chitinase production

Chitinase production is investigated by using chitin agar medium the bacterial Isolates are prepared by cultivating on king s medium far 24h on shaking incubator About 1 ml suspension of bacteria is dropped in to surface of chitin agar medium Chitinolytic activity is determined by the development of clear zone around the bacterial colony (Cattelan *et al.*, 1999).

Anti bacterial activities

The antagonistic effects of all bacterial isolates were tested against fungal pathogens of Chick pea. For this the bacterial isolates were streaked at a distance of 3.5 cm from rim of individual petriplate containing Potato dextrose agar medium 6 mm mycelia disc from a 7 day old Potato dextrose agar medium culture of fungal pathogens were then placed on the other side of Ale Petridish and the plates were incubated at 28°C for 4-7 days.

Identification methods

The isolates were further characterized by Gram staining (Table 1) and biochemical tests (Table 2) as per methodology described by Krieg and The various tests performed were Oxidase MR-VP Indole Citrate, Urease, Nitrate reduction and fermentation of various sugars (Table 3).

The isolates which showed maximum PGPR activity were further characterized by Gram staining and biochemical tests as per methodology described by Krieg and the various tests performed were Oxidase, MRVP, Indole Citrate, Urease, Nitrate reduction and fermentation of various sugars like galactose, trehalose, lactose, arabinose, rhamnose, fructose, glucose, monitol, sorbitol, xylose, sucrose.

The bacteria isolated from the soil sample is identified on the basis of Cultural, morphological and biochemical methods (Table 4) explained in Bergey's manual of systematic bacteriology (Simon, 2013).

Cultural characterization

The bacteria are examined under UV light and colonies with yellow green color Pigmentation are marked and recorded. The individual colonies are observed and are being picked up with the help of sterilized loop and incubated on Kings B agar Medium by quadrangular streaking Later on, previously incubated culture are transferred on slant and kept in refrigerator at 40°C for further characterization of isolates.

Morphological characterization

1. Cell shape and arrangement

The young cultures are Gram stained (Table 1) by smearing loop full culture on grease free clean slides stained accordingly and examined microscopically for shape, arrangement and Gram reaction.

2. Gram staining

A thin smear of bacteria is made in a separate glass slide and heat fixed. The smear is covered with crystal violet for 30 seconds. The slide is washed with did wader for few

seconds by using wash bottle the smear is stained with iodine solution for 60 seconds. The iodine solution is washed with 95% ethyl alcohol. Ethyl alcohol is added drop by drop until no more colors of flows from the smear. The slides are washed with distil water and drained. Safrranin is applied to srnear for 30 seconds and washed with distil water and blot dried with absorbent paper. The slide is examined under oil immersion objective.

3. Colony morphology

Pure culture is streaked on Kings B agar medium and visual sedation is made after 48 hours of incubation at 28°C.

4. Biochemical characterization

Gelatin hydrolysis

The gelatin broth is sterilized by autoclaving in culture tube are inoculated with test isolates and incubated at 28°C for 48 hours. After inoculation the tubes are chilled in ice bath. Control tube without any inoculation solidified and tubes are gelatin is hydrolyzed remained in liquid stages, which suggest a positive reaction (Aneja, 2009).

Urease test

Urease agar petri dishes are inoculated with test isolates and one with uninoculated, which acted as control. The Petri dishes are incubated for 24-48 hours at 28^{o}C. Production of deep pink coloration of the medium showing positive reaction for the urea by means of the production of an enzyme urease (Aneja, 2009).

Ammonification

Sand Petri dishes containing 4% peptone solution is inoculated with test isolates and incubated at 28°C for 48 hours. A drop of Nessler‘s reagent is placed on Petri dishes along with test isolates sample, change of yellow color indication 0 presence of Ammonia (Aneja, 2009).

Indole production and hydrogen sulphide test

Taking 5 ml of 1% trypton broth in culture tube after autoclaving incubated the culture tube with isolate and one remained un inoculated which acts as control. It is incubated at 35°C for 48 hours. After incubation added one ml of Kovac’s reagent added to each tube including control. Development of a cherry (deep) red color in the top layer of the tube is a positive test for indole production. In hydrogen sulphide test stab the SIM media with inoculating needle. SIM media contains the sulphur containing amino acid, cysteine, sodium thio-sulphate and peptonised iron or ferrous sulphate. Hydrogen sulphate reacts with iron or ferrous sulphate and produce black precipitate it is positive result for hydrogen sulphide test (Aneja, 2009).

Starch hydrolysis

Melt the starch agar medium, cooled to 45°C and poured into the sterile Petri plates. After solidification the isolates are single streaked into the centre of starch agar plate and incubated for 48 hours at 37°C in an inverted position. The plates are then flooded with Gram's iodine and observed for blue color zone around the bacterial growth Amylase producing isolates identified by blue colored zones surrounding them (Aneja, 2009).

Carbohydrate fermentation

Fermentative degradation of various carbohydrates such as glucose (Monosaccharide), sucrose (disaccharide), cellulose (polysaccharide) by microorganisms, under anaerobic condition is carried out in

fermentation tube. A fermentation tube is a culture tube that contains a Durham tube for detection d gas production, as the end product of metabolisrn. The fermentation broth contains ingredients of nutrient broth, a specific carbohydrate (Glucose, lactose, maltose, sucrose or mannitol) and p^H indicator (phenol red), which is red at neutral pH (7.0) and turns yellow at or below a pH 6.8 due to the production of organic acid (Aneja, 2009).

Methyl red and Voges-Proskauer test

The Methyl red and Voges-Proskauer test are used to differentiate two major types of facultative anaerobic enteric bacteria that produce large amount of acid and those that produce neutral product acetone as end product. If an organism produces large amount of organic acids (i e Formic, acetic, lactic, succinic) as end product from glucose, the medium remained red (a positive test) after addition of methyl red a pH indicator (i.e. pH remains below 4.4) in other organisms methyl red was turned into yellow (a negative test) due to elevation of pH above 6.0 because of enzymatic conversion of the organic acids (produced during the glucose fermentation) to non acidic end product such as ethanol and acetone (acetyl methyl carbinol). Prepare MRVP broth (peptone - 7.0g, dextrose - 5.0 g, potassium phosphate - 5.0g, distilled water1000 ml, pH 6.9) tubes by pouring 5 ml broth in each tube and sterilize by autoclaving. Inoculate MRVP tubes with bacterial isolate and keep one tube as uninoculated comparative control. Incubate all tubes at 35°C for 48 hours. Add five drops of methyl red indicator to each tube and record observation (Aneja, 2009).

Citrate utilization test

The citrate test is performed by inoculating microorganisms into an organs synthetic medium, Simmon's citrate agar, where sodium citrate is only source of carbon and energy Bromothymol blue is used as an indicator (Bromothymol blue is green when acidic (p 6.8 and below) and blue when alkaline (pH 7.6 and higher). When the citric acid metabolized, the CO_2 generated and combined with sodium and water to form sodium carbonate an alkaline product, which changes the color of the indicator from green to blue and constitutes a positive test (Aneja, 2009).

Results and Discussion

Catalase test

During anaerobic respiration in the present of oxygen, microorganism produce hydrogen peroxide (H_2O_2) which is lethal to the cell The enzyme catalase present in some used. Microorganisms break down hydrogen peroxide to water and oxygen. Preparation of trypticase soya agar (pH 7.3) slant of the following composition. ^Trypticase-15.0 g. Phytone - 5.0g, Sodium chloride- 5.0 g, Agar-15 0 g. Distilled water- 1000.0 ml. Pour the medium in culture tubes and sterilize by autoclaving at 15 Lbs. pressure for 15 minutes. Inoculate the trypticase soya agar slants, one with bacterial isolate. Keep an uninoculated trypticase soy agar slant as control. Incubate the culture at 35°C for 24-28 hours. While holding the inoculated tube at an angle. Allow 3-4 drops of hydrogen peroxide to flow over the growth of each slant culture (Aneja, 2009).

Oxidase test

Oxidase enzyme plays important role in the operation of the elected tray system during aerobic respiration. The ability of bacteria to produce cytochrome oxidase can be determined by the addition of the test reagent, p- aminodimethylaniline oxalate, to colonies grown on a plate medium. This light pink

reagent serves as an artificial substrate, donating electors there by becoming oxidized to blackish compound in the presence of oxidizer and free oxygen. No color change or light pink colouration on the colonies is indicative of the absence of oxidase activity. Trypticase soy agar medium was used detection of oxidize activity. Streak the plate and prepare culture selected bacterial isolates. Place a drop of oxidize regent Dimethylene diaminehydrichloride covering the colonies.

Results and Discussion

Isolation of biocontrol (bacterial) agents from Chick pea crop. From the fifty soil samples were collected from rhizosphere of Chick pea crop were four to have 58% bacterial isolates (Table 1) in the selective isolation process on selective media Nutrient Agar medium Among twenty nine isolates, *Pseudomonas spp* (0.983%), *Bacillus spp* (0.983%), *Paenibacillus spp* (0.983%), *Klebsiella spp* (1.530%), *Staphylococcus spp* (1 530%), *Micrococcus spp* (0.188%), *Enterobacter spp* (0.462%), *Planococcus spp* (0 155%) *Pseudomonas spp* (0.983%), *Bacillus spp* (0.983%) found to have highest isolates and showed antifungal activity against *Fusarium spp* on the Chick pea crop when Compared to remaining isolates. The difference was found to be statistically non-significant (P<0.05) (Table 1).

Identification of individual bacterial isolates

identification of individual bacterial isolates (Fig. 1) on the basis of characterization tests, biochemical tests, sugar fermentation tests revealed that presence of *Halobacterium vallismortis* (0.3%), *Halobacterium pharaonis* (0.3%), *Pseudomonas fuscovaginae* (0.3%), *Pseudomonas tuomeurensis* (0.3%), *Staphylococcus aureus* (0.3%), *Paenibacillus siamensis* (0.9%), *Klebsielta oxytoca* (%), *Paenibacillus apiaricus* (0.3%), *Paenibacillus nematophilus* (0.3%), *Bacillus subterraneus* (0.3%),*Bacillus aidengensis* (0.3%), *Bacillus farraginis* (0.3%), *Bacillus cereus* (0.3%), *Bacillus polymyxa* (0.3%), *Micrococcus Iuteus* (0.6%), *Micrococcus halbius* (0 3%), *Pseudomonas alcaligenes* (0.3%), *Pseudomonas putida* (0.3%), *Enterobacter diversus* (0.3%), *Enterobacter turda* (0.3%), *Planococcus citreus* (0.9%).

Table.1 Isolation of bacterial species from rhizospheric soil of Chick pea

Total number of Soil sample	Number of isolates isolated	Percentage								
N= 50	9(58%)	*Halobacterium spp*	*Pseudomonas spp*	*taphylococcus spp*	*Paenibacillu s spp*	*Klebsiella spp*	*Bacillus spp*	*Micrcoccuss spp*	*Enterobacter spp*	*Planococcus spp*
		3(0.015)	5(0.983)	1(1.530)	5(0.983)	1 (1.530)	5(0.983)	4(0.188)	2(0.462)	3(0.015)

Value in parenthesis indicates the incidence of bacterial pathogen on Chick pea
X^2_{cal}=6.694< X^2_{tab}=15.507(5%), Non significant

Table.2 Biochemical tests

	Colony morphology		Gram staining		Gelatin hydrolysis test	Urease test	Ammonification test	Starch test	Oxidase test	Catalase test	Hydrogen sulphide test	Indole test	Methyl red test	Voges-proskuer test	Citrate test
	colour	texture	staining	shape											
1	White	Spongy		Rod	+	+	+	+		+	+	+	+		+
2	White	Transulucent		Rod		+	+	+	+			+			
3	Light brown	Smooth		Rod	+			+	+	+		+	+		
4	White	Spongy		Rod			+	+	+	+		+	+		+
5	Red (or) Purple	Spongy		Rod	+				+		+	+		+	
6	Light yellow			Rod								+			
7	Light yellow	Opaque	+	Cocci		+	+			+		+		+	+
8	White yellow	Clusters	+	Cocci		+	+		+	+	+	+		+	
9	White to light yellow	Mucoid		Rod	+	+				+	+	+		+	+
10	Yellow	Opaque		Rod		+		+		+	+	+	+		+
11	yellowish			Rod				+	+	+	+	+	+	+	
12	Cream	Thick opaque		Rod			+	+				+	+	+	+
13	White	Opaque		Rod	+		+	+		+		+			
14	Yellow	irregular edges	-	Long rod		+		+	+	+	+	+		+	
15	Light whitecolour	Opaque		Rod	+					+		+	+	+	
16	Light white colour	Opaque		Rod	- ve	- ve	+ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve	+ ve
17	Light white colour	Opaque	+	Rod	+	+				+		+		+	
18	Light white colour	Thin amoeboid	+	Rod	+		+	+	+	+	+		+	+	
19	Light yellow	Opaque	+	Cocci			+	+	+	+	+		+		
20	colourless	Smooth	+	Cocci		+									
21	Light yellow	Opaque	+	Cocci	+			+	+	+	+				
22	Light yellow	Flat or mucous		Rod	+		+							+	+
23	Yellow	Mucoid		Rod	+	+		+	+	+	+		+	+	
24	White	Transulent	+	Rod				+	+	+	+		+	+	+
25	Yellow	Irreregular edges		Rod	+	+	+		+		+		+	+	
26	Slightly yellow	smooth cirular	+	Cocci	+	+	+						+	+	+
27	White to yellowish	Irregular margins	+	Rod	+	+	+	+		+			+		
28	Yellow	Opaque	+	Cocci					+		+		+	+	+
29	Light yellow	opaque	+	Cocci	+			+		+				+	
30	Light yellow	opaque		Rod			+				+		+		

Table.3 Sugar fermentation test for isolates

Iso.no	Galactose	Lactose	Sucrose	Arabinose	Rhamnaose	Fructose	Glucose	Mannitol	Trehalose	Sorbitol	Xylose
1	A+ G-	A- G-	A- G-	A+ G-	A- G-	A - G+	A- G-	A- G-	A + G-	A- G-	A- G-
2	A- G-	A+ G-	A- G-	A- G+	A + G-	A - G-	A- G-	A - G-	A - G+	A- G-	A+ G-
3	A+ G-	A- G-	A- G-	A+ G-	A G-	A G-	A- G	A G+	A G-	A G-	A G+
4	A G-	A G-	A- G	A G-	A G-	A- G-	A G-	A G-	A G-	A G-	A+ G-
5	A + G-	A G-	A G+	A G-	A+ G	A G+	A G-	A+ G	A- G	A G-	A+ G
6	A G-	A+ G	A G-	A G-	A+ G+	A- G-	A- G-	A- G-	A+ G	A- G+	A - G-
7	A - G-	A- G-	A+ G-	A- G-	A- G-	A+ G	A- G-	A- G+	A- G-	A+ G+	A - G-
8	A + G+	A- G-	A- G-	A- G+	A- G-	A- G-	A- G-	A - G-	A+ G-	A- G-	A+ G-
9	A + G+	A- G-	A + G+	A + G+	A + G+	A + G+	A + G+	A- G-	A- G-	A+ G-	A+ G-
10	A+ G-	A- G-	A- G-	A+ G-	A- G-	A- G-	A- G-	A- G-	A+ G-	A- G-	A- G-
11	A+ G-	A- G-	A+ G-	A + G+	A+ G-	A + G+	A+ G-	A- G-	A + G+	A+ G-	A+ G-
12	A- G-	A+ G-	A+ G-	A- G-	A- G-	A + G+	A+ G-	A- G-	A- G-	A+ G-	A + G+
13	A+ G-	A- G-	A- G-	A+ G-	A- G-	A- G-	A- G-	A- G-	A+ G-	A- G-	A- G-
14	A + G+	A + G+	A + G+	A- G-	A+ G-	A+ G-	A+ G-	A- G-	A- G-	A + G+	A + G+
15	A + G+	A + G+	A + G+	A- G-	A+ G-	A+ G-	A+ G-	A- G-	A- G-	A + G+	A + G+
16	A + G+	A- G-	A + G+	A + G+	A + G+	A + G+	A + G+	A- G-	A- G-	A+ G-	A+ G-
17	A+ G-	A- G-	A- G-	A+ G-	A- G-	A- G-	A- G-	A- G-	A+ G-	A- G-	A- G-
18	A+ G-	A- G-	A + G+	A+ G-	A- G-	A- G-	A + G+	A+ G-	A+ G-	A- G-	A+ G-
19	A+ G-	A- G-	A- G-	A+ G-	A+ G-	A+ G-	A+ G-	A+ G-	A- G-	A- G-	A+ G-
20	A+ G-	A + G+	A+ G-	A- G-	A- G-	A+ G-	A+ G-	A + G+	A+ G-	A- G-	A- G-
21	A- G-	A + G+	A- G-	A- G-	A+ G-	A+ G-	A+ G-	A- G-	A- G-	A + G+	A+ G-
22	A+ G-	A+ G-	A + G+	A+ G-	A + G+	A + G+	A+ G-	A+ G-	A- G-	A+ G-	A+ G-
23	A+ G-	A + G+	A+ G-	A- G-	A + G+	A- G-	A- G-	A- G-	A + G+	A+ G-	A+ G-
24	A- G-	A- G-	A+ G-	A- G-	A- G-	A+ G-	A- G-	A + G+	A- G-	A + G+	A- G-
25	A + G+	A- G-	A- G-	A+ G-	A+ G-	A- G-	A- G-	A- G-	A+ G-	A- G-	A+ G-
26	A+ G-	A+ G-	A + G+	A- G-	A- G-	A- G-	A+ G-	A + G+	A- G-	A+ G-	A- G-
27	A + G+	A- G-	A- G-	A- G-	A+ G-	A- G-	A- G-	A- G-	A- G-	A + G+	A- G-
28	A+ G-	A- G-	A+ G-	A- G-	A- G-	A + G+	A- G-	A- G-	A + G+	A+ G-	A+ G-
29	A- G-	A+ G-	A+ G-	A + G+	A- G-	A + G+	A+ G-	A- G-	A- G-	A+ G-	A- G-
30	A + G+	A- G-	A+ G-	A+ G-	A- G-	A- G-	A- G-	A- G-	A + G+	A- G-	A+ G-
31	A- G-	A+ G-	A+ G-	A- G-	A + G+	A- G-	A- G-	A+ G-	A+ G-	A+ G-	A- G-

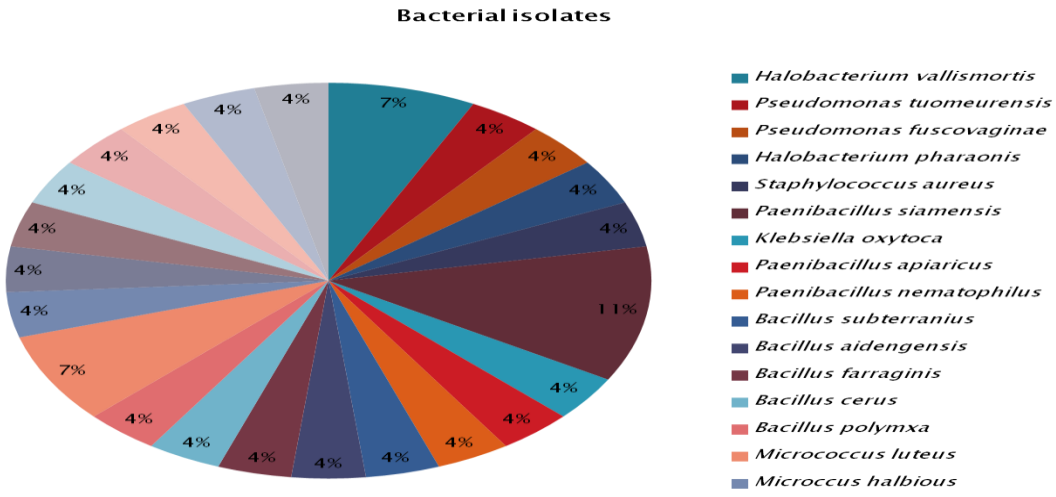
Table.4 Cultural, microscopic and characterization of bio control agent

Isolate number	Colony characteristics		Microscopic observation		Characterization of biocontrol agents				
	Color	Texture	Gram staining	Shape	HCN Production	Siderophore production	Chitinase production	Antibacterial activity	Organism
1	White	clusters		Rod		+			*Halobacterium vallismortis*
2	White	Translucent		Rod	+	+	+		*Pseudomonas tuomuerensis*
3	Light Cream	opaque		Rod			+		*Pseudomonas fuscovaginae*
4	White to yellowish	opaque		Rod					*Halobacterium vallismortis*
5	purple	opaque		Rod					*Halobacterium pharaonis*
6	Light yellow	mucoid		Rod		+	+	+	*Pseudomonas graminis*
7	Light yellow	Opaque	+	Cocci	+		+		*Staphylococcus aureus*
8	White yellow	Clusters	+	Cocci			+		*Paenibacillus siamensis*
9	White to light yellow	Mucoid		Rod		+	+		*Klebsiella oxytoca*
10	Yellow	Opaque		Rod	+			+	*Paenibacillus apiaricus*
11	yellowish			Rod		+	+		*Paenibacillus siamensis*
12	Cream	Thick opaque		Rod			+		*Paenibacillus nematophilus*
13	White	Opaque		Rod	+	+	+	+	*Bacillus subterranius*
14	Yellow	Irregular edges		Rod					*Paenibacillus siamensis*
15	Light whitecolour	Opaque		Rod					*Bacillus aidengensis*
16	Light white colour	Opaque		Rod	++	+		+	*Bacillus farraginis*
17	Light white colour	Opaque	+	Rod		+		+	*Bacillus cerus*
18	Light white colour	Thin amoeboid	+	Rod		+		+	*Bacillus polymxa*
19	Light yellow	Opaque	+	Cocci		+	+		*Micrococcus luteus*
20	Light yellow	Flat or mucous		Rod					*Pseudomonas alcaligens*
21	Light yellow	Opaque	+	Cocci			+	+	*Micrococus luteus*
22	colourless	Smooth	+	Cocci		+	+		*Micrococus halbious*
23.	Yellow	Irreregular edges		Rod					*Enterobacter turda*
24.	White	Transulent	+	Rod		+		+	*Clostoridum herbivore*
25.	Yellow	Mucoid		Rod	+		+	+	*Pseudomonas putida*
26.	Light white	Opaque		Yellow					*Planococcus citrens*
27.	Slightly yellow	smooth cirular	+	Cocci	++		+		*Planococcus citrens*
28.	Light yellow	Opaque	+	Cocci					*Planococcus citrens*
29.	White	Opaque		Yellow					*Enterobacter diversa*
30.	Light yellow	Opaque	+	Cocci	++		+	+	*Micrococcus luteus*

Table.5 Antifungal activity of isolates bacterial species on the pathogens

S.NO	Isolated bacterial spp showing antagonistic property with	Against the pathogens			
		Aspergillus spp	*Alternaria spp*	*Curvularia spp*	*Fusarium spp*
1	*Pseudomonas graminis*	+	+	-	+
2	*Bacillus cereus*	+	+	+	+
3	*Bacillus subterraneus*	-	+	+	+
4	*Micrococcus luteus*	-	+	-	+

Fig.1 Bacterial isolates



Summary and Conclusion

The present study entitled "Isolation and identification of Biocontrol (bacterial) agents from Chick pea (*Cicer arietinum* L.)" was conducted at Department of Microbiology and Fermentation Technology, Post graduate laboratory of Sam Higgin bottom Institute of Agriculture, Technology and Sciences, Deemed to be university. Fifty soil samples were colleted from rhizosphere of chickpea crop were performed selective isolation process on selective media like Nutrient agar media to study isolation and identification of bacterial spp. 10g of rhizosphere soil with roots transferred into 250ml of conical flask containing 100 ml of sterile distilled water and kept in shaker for about 24 hr. Then 1 ml of this suspension was made into serial dilution up to 10^{-6} and about 0. 1ml of this was placed in the King s B media and Nutrient agar media to isolate the cultures or the colonies of bacteria. The plates were incubated at 28°C for 48 hr.

From Chick pea crop characteristics Bacterial spp are identified on the basis of characterizations, biochemical tests and sugar fermentations. The significance of bacterial isolates were tested by using chi-square test and interpreted following is the summary of experimental results and the conclusion there by:

Twenty nine isolates of bacterial with (58%) of isolates were recorded. Among twenty nine isolates, five isolates of Pseudomonas spp, *Bacillus spp, Paenibacillus Spp* (2.489%). Remaining isolates were *Micrococcus spp, Halobactenum spp, Staphylococcus spp, Klebsiella spp, Planococcus spp* were (3.278%) identified. From the isolated organisms, *Pseudomonas graminis, Bacillus cerus, Bacillus subterranius, Micrococcus luteus* showed biocontrol effect on soil borne pathogens. By using them we controlled effectively the *Fusarium oxysporium f. sp ciceris, Alternaria alternata, Curvularia lunata and Aspergillus spp*. More emphasis is needed to disease management of this crop as this feild lacks attention in spite of the importance it renders to the masses. To compact such problems there is need of proper disease diagnosis programme.

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How to cite this article:

Vijaya Kumar, S. and Sandhya, N. 2018. Isolation of Biocontrol (Bacterial) Agents from Chickpea (*Cicer arietinum linnaeus*). *Int.J.Curr.Microbiol.App.Sci.* 7(02): 2024-2035.
doi: https://doi.org/10.20546/ijcmas.2018.702.242