Isolation & characterization of potent phosphate solubilizing bacterial strain from sheep manure

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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CHAPTER 1.0
INTRODUCTION

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1.0 INTRODUCTION

Bio-fertilizers are living microorganisms or their metabolites that promote plant growth. improve soil fertility and increase crop yields They are natural, eco-friendly and sustainable alternatives to chemical fertilizer.

Bio-fertilizers are an innovative, eco-friendly approach to enhancing soil fertility and promoting sustainable agricultural practices. These natural products contain living microorganisms that help improve nutrient availability to plants, boost soil health, and increase crop yield. A key element in the development of bio-fertilizers is the carrier material, which is essential for the survival and effective application of these microorganisms. Sheep manure, being rich in organic matter and nutrients, serves as an excellent carrier material for bio-fertilizer production.

Sheep manure is known for its high nutrient content, including essential macro- nutrients such as nitrogen, phosphorus, and potassium, along with micro-nutrients and organic matter that contribute to soil fertility. Additionally, sheep manure provides a favourable environment for the growth of beneficial microbes, such as nitrogen-fixing bacteria (Rhizobium), phosphate solubilizing bacteria, and other beneficial microorganisms that are integral to bio-fertilizer formulations (Singh et al., 2015). These microorganisms help in converting complex organic matter into plant-available nutrients, thereby improving soil health and promoting plant growth.

There are two types of the bio-fertilizers

A] Liquid bio-fertilizer

B] Solid bio-fertilizer

A]Liquid bio-fertilizer-

Liquid bio-fertilizer are microorganism-based solutions that promote plant growth and soil fertility

They include liquid microbial inoculum, Bacterial suspension, fungal suspension, algal extracts fermented plant extract

They are easy to apply.

B] Solid bio-fertilizer -

Solid bio-fertilizers are microorganisms-based products that improve soil fertility and plant growth. Solid biofertilizers include carrier material such as peat, lignite, charcoal, farm yard manure, etc.

Sheep manure-based bio-fertilizer is a sustainable solution for enhanced crop productivity. Sheep manure, a readily available and renewable resource, can be transformed into a valuable bio-fertilizer. It is rich in nutrients, microorganisms and organic matter Sheep manure-based bio-fertilizers offer sustainable alternative to chemical fertilizers. Very little research has been done till date on sheep manure as carrier Material for production of biofertilizer hence research was undertaken to isolate a potent phosphate solubilizing bacterial strain from sheep manure to characterize it for other plant growth promotion activities.

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CHAPTER 2.0

REVIEW OF LITERATURE

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2.0 REVIEW OF LITERATURE

Bio-fertilizers come in solid and liquid forms, each tailored to different application methods and farming systems. These formulations ensure the survival and functionality of the microorganisms they contain, depending on environmental conditions and storage requirements. Below is an overview of the two types, their characteristics, advantages, and examples.

Sheep manure is a valuable organic fertilizer due to its balanced nutrient composition and relatively low contamination risk, making it particularly suited for bio fertilizer production (Agboola et al., 2019). Studies indicate that sheep manure contains a significant amount of nitrogen (N), phosphorus (P), potassium (K), and organic matter, all essential for plant growth (Scharf et al., 2020).Macro-nutrients and Micro-nutrients Research indicates sheep manure generally has about 0.5-1% nitrogen, 0.3-0.5% phosphorus, and 0.4-0.7% potassium, although these amounts vary based on diet and manure management practices (Singh et al., 2018).Organic Matter:

The organic matter content in sheep manure helps improve soil structure, aeration, and moisture retention, benefiting plant root growth and nutrient availability (Meng et al., 2021). Diverse Microbial Population: Sheep manure contains various beneficial microorganisms, including bacteria and fungi that contribute to nutrient cycling and plant growth (Das et al., 2022). Nitrogen Fixation and Phosphate Solubilization

Sheep manure contains microbes capable of nitrogen fixation and phosphate solubilization, making these nutrients more readily available to plants (Khan et al., 2019). Disease Suppression: Beneficial microbes in sheep manure help suppress harmful pathogens in the soil, reducing the incidence of soil-borne plant diseases (Okur et al., 2020).

Composting stabilizes the nutrients in sheep manure, reduces pathogens, and improves the material's handling properties. The composted manure also provides a more balanced nutrient supply (Agboola et al., 2019).

Direct Application and Compost Tea: Fresh or composted sheep manure can be applied to soil, while compost tea, made by steeping manure in water, provides a foliar nutrient source (Khan et al., 2019).

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Positive Effects on Crop Yield: Studies have shown improved growth and yield in crops such as wheat, tomatoes, and maize when using sheep manure bio-fertilizers, attributed to increased nutrient availability and microbial activity (Das et al., 2022; Singh et al., 2018).

Reduction in Chemical Fertilizer Use: By supplying essential nutrients, sheep manure bio-fertilizers reduce reliance on synthetic fertilizers, which can lead to cost savings and lower environmental impact (Scharf et al., 2020).

Soil Health Improvement: The long-term application of sheep manure enhances organic carbon in the soil, promoting soil health and carbon sequestration (Okur et al., 2020).

Pathogen and Weed Seed Contamination: Fresh sheep manure can carry pathogens and weed seeds, although composting or other treatments can mitigate this risk (Kale et al., 2021).

The nutrient content and microbial act…Bio-fertilizers come in solid and liquid forms, each tailored to different application methods and farming systems. These formulations ensure the survival and functionality of the microorganisms they contain, depending on environmental conditions and storage requirements. Below is an overview of the two types, their characteristics, advantages, and examples.

1. Solid Bio-fertilizers

Solid bio-fertilizers are carrier-based formulations where microorganisms are embedded in solid materials like peat, lignite, or compost.

2. Liquid Bio-fertilizers

Liquid bio-fertilizers are aqueous formulations containing live microorganisms suspended in a liquid medium, often fortified with stabilizers and nutrients to enhance microbial survival.

CHAPTER 3.0

MATERIAL AND METHOD

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3.0 MATERIAL AND METHOD

3.1 Collection of sheep manure

Sheep manure sample was collected from sheep farm of Kolhapur district. The sheep manure was collected in sterile polythene bags, bags were packed and immediately brought to the laboratory.

Fig. 1 Sheep mannure

3.2 Isolation of bacterial isolates from sheep manure on the basis of phosphate solubilization ability

Two methods were applied for isolation of phosphate solubilizing bacteria

A) Serial dilution

B) Enrichment

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A) Serial dilution

Collected sheep manure was converted into fine powder using mortar and pestle. The powdered sheep manure was then serially diluted using sterile distilled water blank (10^{-1}, 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10}). Each dilution was spread on sterile KB agar plates

KB agar composition: -
For 100 ml:

Component	**Amount**
Glucose	1.0 gm
KH_2PO_4	5.0 ml
$CaCl_2$	10.0 ml
Agar	2.0 gm
Soli extract	100 ml
PH	7.0

All plates were labelled properly and incubated at room temperature for 24 to 72 hrs. After incubation plates were observed for bacterial colonies showing clear zone around the colony. The selected colonies were stored on nutrient agar slant and all culture were coded as isolate A1, B1, ……, Y1, Z1, A2, B2, ……

A) Enrichment

Collected sheep manure was powdered and inoculated into pk broth. The flask was labelled properly incubated at room temperature for 3 days. After incubation a loopful of enriched broth was inoculated on sterile KB agar plates. The cultures showing clear zone were labelled as above.

3.3 Selection of potent phosphate solubilizing bacterial strain

All isolated strains by serial dilution technique and enrichment technique. were studied for Gram staining characteristics. The impure cultures were purified by repeated transfer on sterile nutrient agar plate. Finally, all purified cultures were studied for phosphate solubilization ability. For this equal amount of culture was spot inoculated on sterile KB agar plates. Plates were labelled properly and incubated at room temperature for 3 days. After incubation diameter of colony and diameter of clear zone were measured and solubilization index of phosphate solubilization of each isolate was calculated with the help of following formula.

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Formula: -

SI index = B/A

B= Colony zone diameter

A= Colony diameter

3.4 Identification of potent isolates

On the basis of phosphate solubilization potent isolates were identified.

3.4.1 Identification by morphological characters

Potent isolates labelled as A1 and F2 were Gram stained to study their morphological characters. The motility of both cultures was studied by hanging drop method. This spore development ability of culture was detected by Dorner's Staining method.

3.4.2 Identification by biochemical characterization

The potent strains A1 and F2 were further studied for their biochemical characteristics.

3.4.2.1 Sugar fermentation test

Utilization of different sugars such as Lactose, Maltose, Sucrose, Arabinose, Xylose Mannitol and Glucose were tested by inoculating fresh culture into Peptone water broth medium containing sugar PH indicator and inverted Durham's tube. Sugar fermentation was detected by change in colour of medium.

3.4.2.2 H_2S production

Each culture was inoculated into Peptone broth medium containing lead acetate paper. After incubation H_2S production was detected by blacking of lead acetate paper.

3.4.2.3 Indole production

Peptone broth inoculated cultures after incubation was added with xylene and convex reagent to detect production of Indole

3.4.2.4 Gelatinase activity

The cultures were streaked on sterile Gelatine agar plate. After incubation Gelatinase activity was identified by addition of Frazier's reagent.

3.4.2.5 Catalase test

A loopful fresh culture was added into H_2O_2 solution to observe catalase production ability.

3.4.2.6 Oxidase test

A loopful of fresh culture was placed on Whatman filter paper, soaked in oxidase reagent, N.N- tetramethyl phenylenediamine dihydrochloride to identify oxidase production ability.

3.5 STUDY OF AUXILLARY CHARACTERISTICS OF POTENT ISOLATES

3.5.1 Study of nitrogen fixation ability

Plates of Ashby's mannitol agar were prepared. The potent isolates A1 and F2 were streaked on Ashby's agar medium and plates were labelled properly and kept for incubation for 24 hrs at room temperature to check ability of nitrogen fixation.

3.5.2 Study of IAA production ability

3.5.2.1 Qualitative study

A nutrient broth with tryptophan was prepared and sterilized. A loopful A1 and F2 was inoculated into separate nutrient broth with tryptophan media. Flasks were labelled properly and incubated at room temperature. Everyday 5ml of broth was removed from each flask and centrifuged at 5000 rpm for 20 min. The supernatant was collected and was added with orthophosphoric acid and Salkowski reagent. The tubes were incubated at room temperature in dark for half an hour. The development of pink colour in the tubes indicate IAA production.

3.5.2.2 Quantitative study

IAA produced by isolates A1 and F2 was estimated with standard graph of IAA (Table 1)

Table 1: - Standard graph of IAA

Sr No.	Standard IAA (ml)	D/W (ml)	Conc of IAA (µg/ml)	Orthophosphoric acid (ml)	Salkowski Reagent (ml)	Incubation at room temperature for 15 min in dark
1	0.2	0.8	20	2	2	
2	0.4	0.6	40	2	2	
3	0.6	0.4	60	2	2	
4	0.8	0.2	80	2	2	
5	1.0	-	100	2	2	

3.5.3 Study of enzyme activity

3.5.3.1 Study of amylase activity (starch hydrolysis)

Amylase activity of isolate A1 and isolate F2 was studied using starch agar medium. Bacterial isolates A1 and F2 were streaked by cross streaking method on starch agar medium.

The plates were incubated at room temperature for 24hrs. After incubation the plates were flooded with iodine solution to observe amylase activity.

3.5.3.2 Study of proteases activity

Caseinase activity of isolate A1 and isolate F2 was studied by steaked by cross streaking isolates on milk agar medium. The plates were incubated at room temperature for 24hrs. Plates were observed for development of clear zone around growth.

3.5.4 Study of ammonia production

Peptone water was prepared inoculated with isolate A1 & F2 separately. The tubes were labelled properly and inoculated at RT for 24 hrs. After incubation Nessler's, reagent added to detect ammonia production.

Nessler's reagent composition -

Component	Amount
Potassium iodide	50.0 gm
Ammonia free distilled water	35.0 ml
Mercuric chloride solution	35.0 ml
Potassium hydroxide	400 ml
Distilled water	530 ml

3.6 Study of salt tolerance ability

3.6.1 Study of NaCl tolerance ability

Nutrient broth tubes with varying NaCl concentrations (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%) were prepared. A loopful of a fresh suspension of isolates A1& F2 was inoculated into each concentration of NaCl separately. The

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tubes were incubated at room temperature for 24 hours. After incubation, the tubes were examined for growth, indicated by turbidity.

3.6.2 Study of KCl tolerance ability

Nutrient broth tubes with different KCl concentrations (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%) were prepared. Isolates A1 and F2 were inoculated into each concentration and incubated at room temperature for 24 hours. After incubation, the tubes were examined for growth.

3.6.3 Study of Na_2CO_3 tolerance ability

Nutrient broth tubes with different sodium carbonate concentrations (0.1%, 0.3%, 0.5%, 0.7%, 0.9%, and 1%) were prepared. Isolates A1 and F2 were inoculated into each concentration and incubated at room temperature for 24 hours. After incubation, the tubes were examined for growth.

3.7 STUDY OF ANTIBIOTIC SENSITIVITY

The bacterial isolates A1 & F2 were evaluated for antibiotic sensitivity. Nutrient agar plates were prepared, after solidification of agar medium 0.1 ml suspension of each isolate was spread on NA media in aseptic condition. Discs of Clindamycin, Teicoplanin, Lomefloxacin, Moxifloxacin, Ampicillin, Sulbactam, Cefaclor, Cefadroxil, Roxithromycin, Clarithromycin, linocomycin, Sparfloxacin, Gemifloxiacin, Linezolid and vancomycin were placed over seeded NA plates. Plates were kept for incubation for 24 hrs at room temperature and after incubation plates were observed for inhibitory zone. The diameter of inhibition zone for each antibiotic was measured.

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CHAPTER 4.0
RESULT AND DISCUSSION

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4.0 RESULT AND DISCUSSION

4.1 Isolation of bacterial isolates from sheep manure

A total 4 bacterial colonies were isolated on KB agar plates by serial dilution technique and out of four colonies only one colony was showing clear zone thus indicating phosphate solubilization.

Nearly 28 colonies were obtained by enrichment technique. Out of 28 isolates, 6 colonies were showing phosphate solubilization.

Thus total 7 bacterial isolates having phosphate solubilization ability obtained from sheep manure (Table 2)

Table 2: - Isolation of phosphate solubilizing bacteria from sheep manure

Sr. No.	**Isolate name**	**Phosphate solubilization ability**
1	A1	Positive
2	B1	Negative
3	C1	Negative
4	D1	Negative
5	E1	Negative
6	F1	Negative
7	G1	Positive
8	H1	Positive
9	I1	Negative
10	J1	Negative
11	K1	Negative
12	L1	Negative
13	M1	Negative
14	N1	Positive

15	O1	Positive
16	P1	Positive
17	Q1	Positive
18	R1	Negative
19	S1	Positive
20	T1	Negative
21	U1	Positive
22	V1	Positive
23	W1	Negative
24	X1	Negative
25	Y1	Negative
26	Z1	Negative
27	A2	Negative
28	B2	Negative
29	C2	Negative
30	D2	Negative
31	E2	Positive
32	F2	Positive

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4.2 Selection of potent Phosphate solubilizing bacterial strain

Phosphate solubilization ability of all purified isolates (A1, G1, H1, U1, V1, E2, F2) represented in the form of solubilization index is shown in (Table 3)

Table 3: - Solubilization index of all isolates

Sr. no	Isolate name	Diameter of colony zone (mm)			Diameter of colony zone (mm)			SI		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	A1	4.5	4.5	5.3	8.5	10	12	1.88	2.22	2.26
2	G1	8.5	8.5	8	18	20	20	2.11	2.50	2.22
3	H1	6	6	7	10	12	13	1.66	2.0	1.85
4	U1	9	9	10	14	16.5	19.5	1.50	1.83	1.95
5	V1	6.5	7	7.5	7.5	10	11	1.1`5	1.42	1.60
6	E2	5.5	6	6	9	9.5	10	1.63	1.58	1.66
7	F2	6.5	6	7	19	19.5	21	2.90	2.80	3.0

According to result shown in Table 3 isolate F2 was seen most effective phosphate solubilizer. After F2, isolates A1 was recorded as effective phosphate solubilizer, while V1 showed least solubilization.

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4.3 Identification of A1 & F2

4.3.1 Identification by morphological characters

Table 4: - Colony characters of F2 grown on NA plates incubated at room temperature for 24 hrs

size	shape	colour	opacity
2mm	circular	Creamy white	opaque

Margine	Elevation	Surface	Consistency
entire	convex	smooth	moist

Table 5 Colony characters of A1 grown on NA plates incubated at room temperature for 24 hrs.

Size	Shape	Colour	Opacity
1mm	circular	white	opaque

Margine	Elevation	Surface	Consistency
entire	convex	smooth	moist

4.3.2 Identification by gram characteristics

Microscopic observation of F2 and A1 revelled that organisms are Gram negative short thick rods arranged signally.

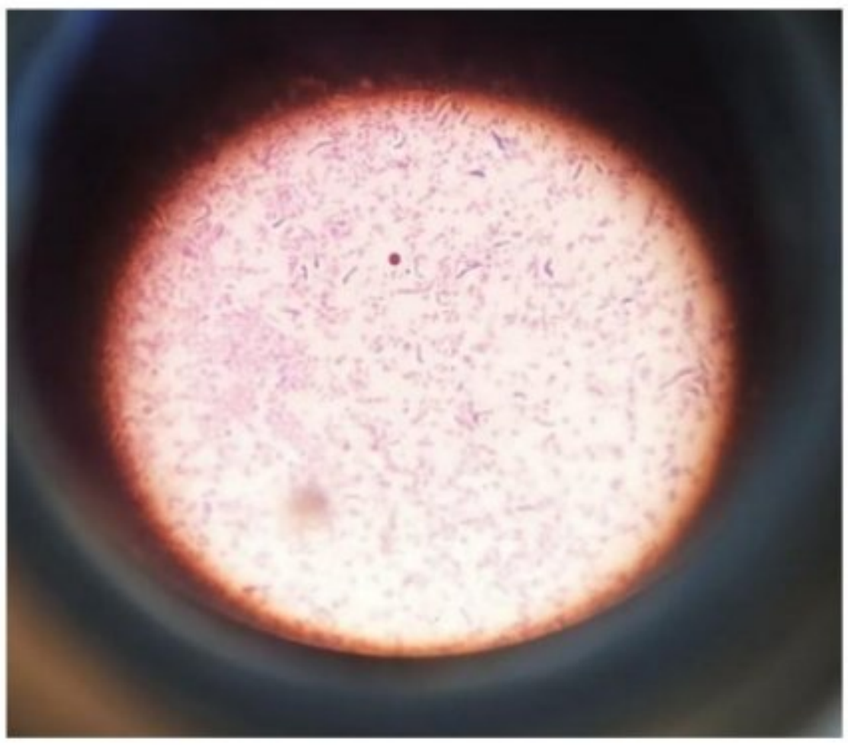

Fig. 2- A1

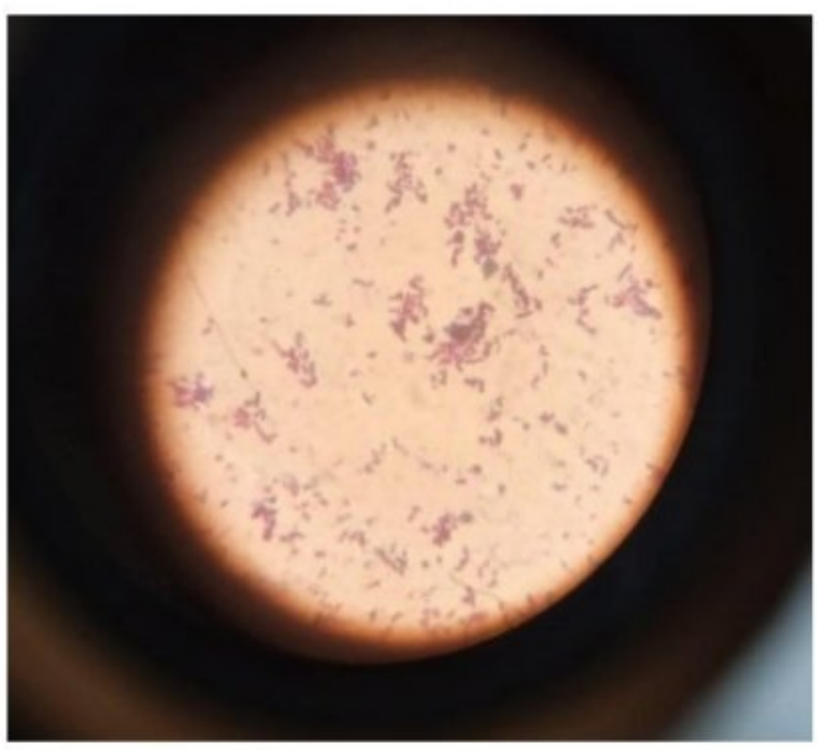

Fig.3-F2

Table 6 Morphological study

Sr.no	Characteristics	Name of isolates	
		F2	A1
1	Grame nature	Gram negative	Gram negative
2	Motility	Motile	Non-motile
3	Spore staining	Sporulating	Non-sporulating

4.3.3 Identification by biochemical characterization

4.3.3.1 Sugar fermentation test

Table 7: - Sugar fermentations

Sr.no	characteristics	Name of isolates	
		F2	A1
1	Glucose fermentation	Positive	Weakly positive
2	Maltose fermentation	Positive	Weakly positive
3	Mannitol fermentation	Positive	Weakly positive
4	Sucrose fermentation	Positive	Weakly positive
5	Arabinose fermentation	Positive	Weakly positive
6	Xylose fermentation	Positive	Weakly positive
7	Lactose fermentation	Positive	Weakly positive

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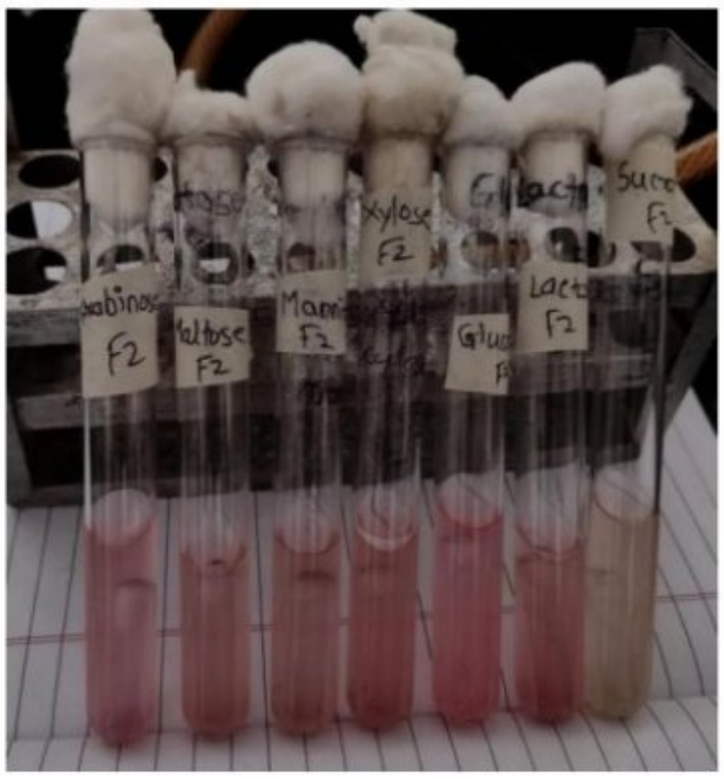

Fig.4 -F2

Fig.5-A1

4.3.3.2 H_2 S production

As no blacking of lead acetate paper was seen in F2 &A1 inoculated peptone water tube isolate F2 & A1 have no H_2S production ability.

4.3.3.3 Indole production

Isolate F2 and A1 showed negative indole production activity.

4.3.3.4 Gelatinase activity

After addition of Fraizers reagent in gelatinase agar plate in case of A1 clear zone was observed around the growth. So, gelatinase activity is positive for A1.

I n case of F2 no clear zone was observed thus gelatinase activity is negative.

4.3.3.5 Catalase test

Isolate F2 and A1 showed positive catalase activity.

4.3.3.6 Oxidase test

F2 and A1 showed Isolate positive oxidase test.

4.4 Study of auxiliary characteristics of potent isolates

4.4.1 Study of nitrogen fixation ability

On Ashby's mannitol agar medium both F2 & A1 isolates showed growth, thus indicating that both isolates have nitrogen fixation ability.

4.4.2 Study of Indole acetic acid production ability

4.4.2.1 Qualitative study

After incubation of cultures in nutrient broth containing tryptophan and after addition of orthophosphoric acid and Salkowski reagent pink colour was observed in both culture tubes. Thus, it indicated that both F2 and A1 have indole acetic acid production ability.

4.4.2.2 Quantitative study

The IAA produced by isolate A1 & F2 was quantified with the help of standard graph (Table8, 9) (Fig. 6)

Table 8: - Standard graph of IAA
Stock IAA – 1000 µg/ml

Conc. Of IAA (µg/ml)	**Optical density at 540 nm**
Blank	0.0
Unknown	-
20	0.19
40	0.39
60	0.44
80	048
100	0.49

Table 9: - Quantitative estimation of IAA
As per shown in Table 9 A1 was seen producing more quality of IAA than F2.

Sr. No.	isolates	Distilled water (ml)	Orthophosphoric acid	Salkowski reagent		O. D at 530 nm		Conc. Of IAA	
					Incubation at room temperature for 15 min in dark	1 day	6 days	1 day	6 days
1	F2	1	2	2		0.04	0.39	4	44
2	A1	1	2	2		0.04	0.44	4	40

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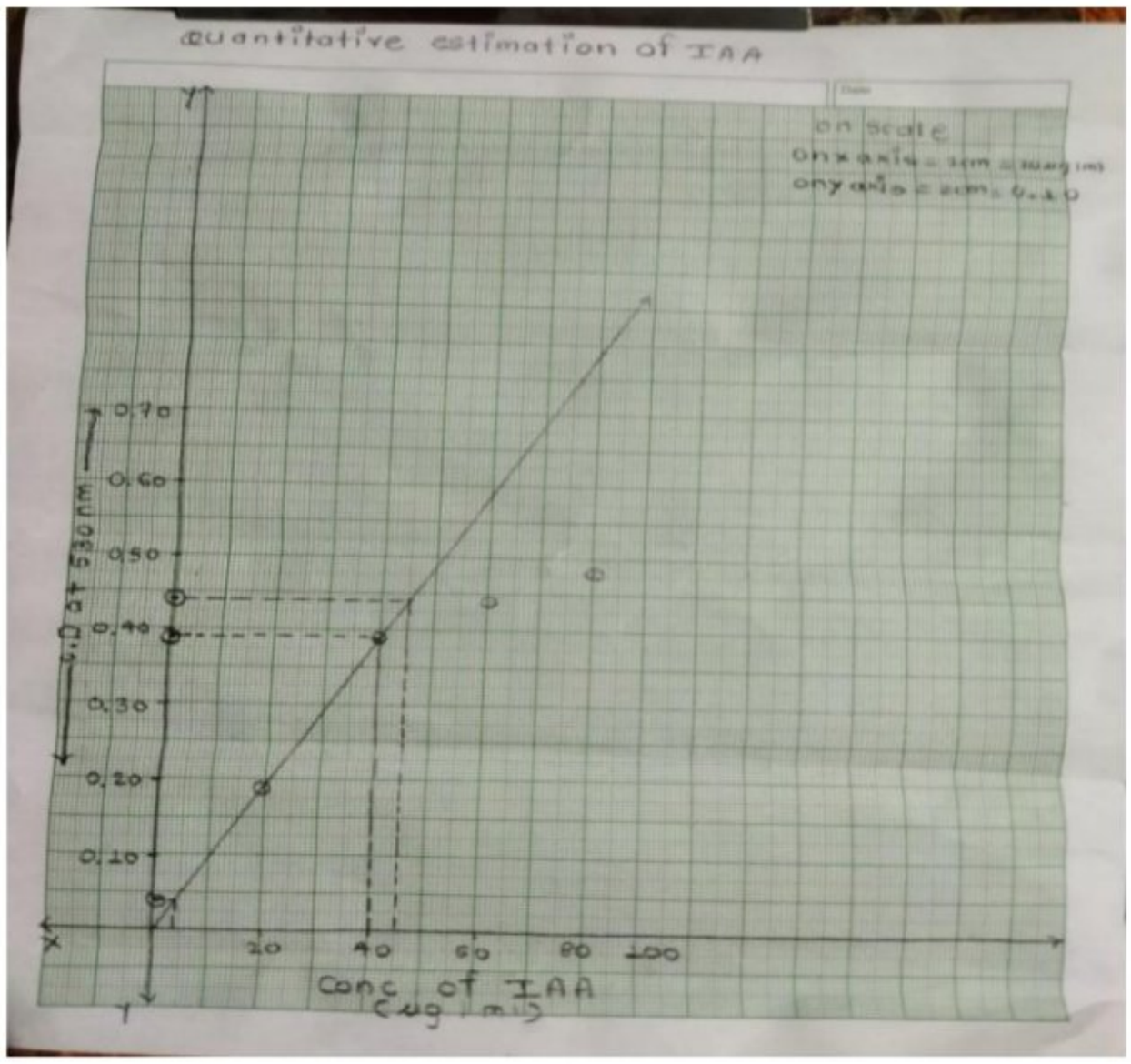

Fig. 6 standard graph of IAA

4.4.3 Study of ammonia production ability

After addition of Nessler's reagent, production of brown discolouration was observed for both strains indicating production of ammonia by both F2 & A1. (Fig.7, 8)

The ammonia useful for plant directly or indirectly. Ammonia production by the bacteria helps to influence plant growth indirectly.

Fig.7 Before addition of Nessler's reagent

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Fig.8 After addition of Nessler's reagent

4.4 Study of enzyme activity

4.4.1 Study of amylase (starch hydrolysis) activity.

Amylase production activity was checked for isolates F2 & A1 by addition of iodine Both the isolates showed amylase production ability. Amylase activity is the ability of the enzyme amylase to hydrolyse starches into maltose and other sugars.

4.4.2 Study of protease activity

Protease activity was checked for isolates by using milk agar. After incubation the plate showed no clear zone around the growth, thus indicating that isolates do not carry out production of caseinase. (Fig.9) Both the cultures may have other proteolytic activity.

Fig. 9 caseinase activity

4.5 Study of salt tolerance ability

4.5.1 study of NaCl tolerance ability

Isolate F2 &A1 showed strong NaCl tolerance ability. Turbidity was observed till 5% & 6% NaCl concentration respectively (Table 10). This indicates that, these isolates have potential to grow and flourish its life cycle in high saline condition.

Table 10: - Study of NaCl tolerance

Isolates	NaCl concentration (gm %)									
	1	2	3	4	5	6	7	8	9	10
F2	+++	+++	++	++	+	+	-	-	-	-
A1	+++	++	++	++	+	-	-	-	-	-

Fig. 10 NaCl tolerance of isolate F2

Fig.11 NaCl tolerance of isolate A1

4.5.2 Study of KCL tolerance ability

In case of potassium chloride effect, maximum turbidity was observed of isolate F2 & A1 till 8% & 7% of KCl concentration respectively. This indicates that these isolates are resistant to high KCl concentration. (Table 11)

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Table 11: - Study of KCl tolerance

Isolates	KCl concentration (gm %)									
	1%	2%	3%	4 %	5 %	6 %	7 %	8 %	9 %	10 %
F2	+++	+++	+++	++	++	+	+	+		
A1	+++	+++	++	++	++	+	+			

Fig.12 KCl tolerance of isolate F2

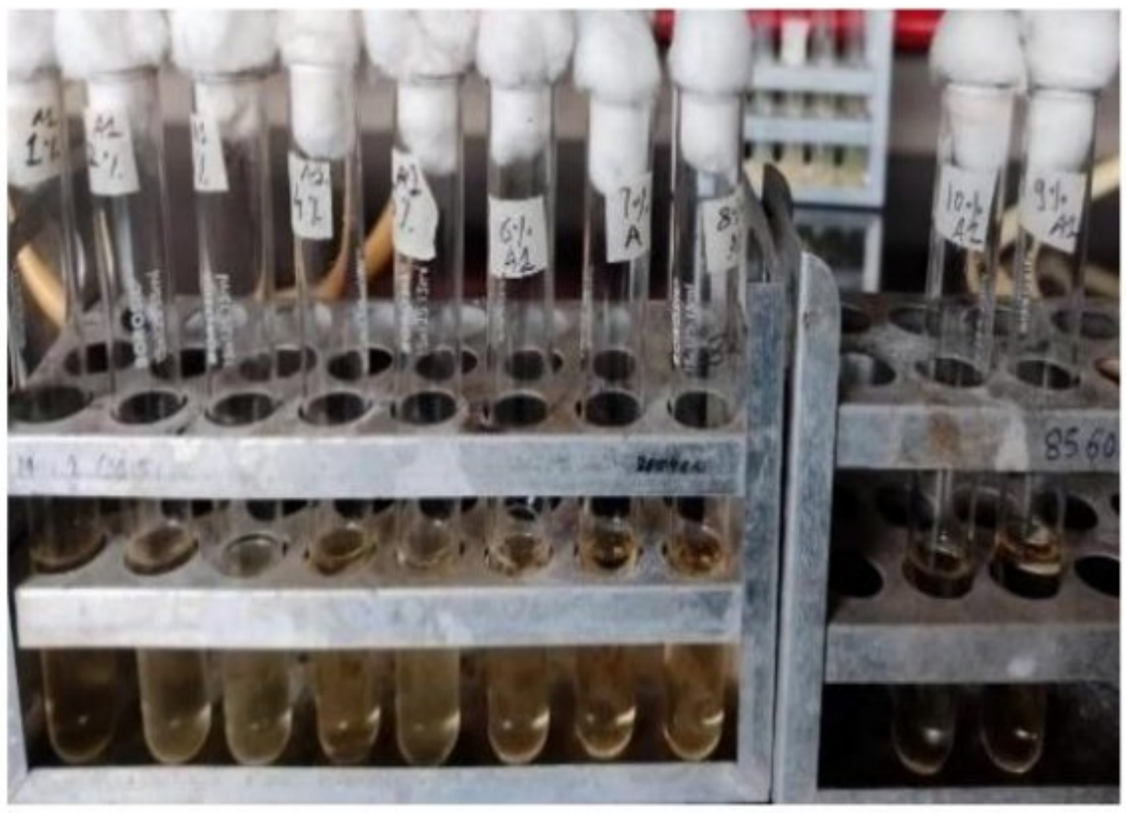

Fig.13 KCl tolerance of isolate A1

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4.5.3 Study of Na_2CO_3 tolerance ability

In case of sodium carbonate tolerance, maximum turbidity was observed of isolate F2 & A1 till 7% & 6% concentration respectively. This indicates that these isolates are resistant to high sodium carbonate concentration, (Table 12).

Table 12: - Study of Na_2CO_3 tolerance

Isolates	Na_2CO_3 concentration (gm %)					
	0.1	03	0.5	0.7	0.9	1
F2	+++	++	+	+	+	-
A1	+++	++	+	+	-	-

Fig.14 Na_2CO_3 tolerance of isolate F2

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Fig. 14 Na_2CO_3 tolerance of isolate A1

4.6 Study of antibiotic sensitivity

Isolate A1 & F2 were studied for antibiotic-sensitivity. In this study, out of 14 antibiotics used isolate F2 showed sensitivity towards nearly 13 antibiotics. Thus, F2 is very safe to use it as bioinoculant (Table 13)

Isolate A1 showed sensitivity towards only 5 antibiotics, out of 14 antibiotics use in the experiment (Table 14).

Table 13: - Antibiotic sensitivity testing of isolate F2

Name of antibiotics	**Short forms**	**Inhibitory zone**
Clindamycin	CM/2	3.8
Teicoplanin	TP/30	3.8
lomefloxacin	LOM/10	4.1
moxifloxacin	MXF/5	3.5
Ampicillin + sulbactam	SAM	No clear zone
Cefaclor	CEC/30	3.2
Cefadroxil	CFR/30	3
Roxithromycin	RO/30	4
Clarithromycin	CLM/15	4
lincomycin	LN/15	3.1
Sparfloxacin	SO/5	2.6
Gemifloxacin	GEM/5	3.5
Linezolid	LZ/30	4.1
vancomycin	VA/30	3.7

Table 14: - Antibiotic sensitivity testing of isolate A1

Name of antibiotics	**Short forms**	**Inhibitory zone**
Clindamycin	CM/2	No clear zone
Teicoplanin	TP/30	2.1
lomefloxacin	LOM/10	3.45
moxifloxacin	MXF/5	2.7
Ampicillin + sulbactam	SAM	No clear zone
Cefaclor	CEC/30	No clear zone
Cefadroxil	CFR/30	No clear zone
Roxithromycin	RO/30	No clear zone
Clarithromycin	CLM/15	No clear zone
lincomycin	LN/15	No clear zone
Sparfloxacin	SO/5	3.5
Gemifloxacin	GEM/5	3.9
Linezolid	LZ/30	No clear zone
vancomycin	VA/30	No clear zone

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CHAPTER 5.0
SUMMARY AND CONCLUSION

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5..0 Summary and conclusion

Thirty-two isolates were isolated on K8 agar plates from sheep manure. Out of 32, twelve (A1, G1, H1, N1, O1, P1, Q1, S1, U1, V1, E2, F2) isolates were showed clear zone around colonies. Only 7 seven isolates coded as (A1, G1, H1, U1, V1, E2, F2) showed measurable clear zone. Five isolates were showing very minute clear zone.

Seven isolates when again evaluated for phosphate solubilization, two isolates Pea labelled as F2 & Al showed potent phosphate solubilization ability.

Besides phosphate solubilization, isolates also have nitrogen fixation, IAA production & ammonia production abilities.

The salt tolerance ability of isolates F2 & A1 indicated that these isolates can survive & thrive in high salt conditions. Thus, these isolates could be useful as bio-inoculant for saline soils.

Sodium hydrogen carbonate was tested to evaluate their potential to survive and thrive in high-salt conditions sensitivity.

Antibiotic sensitivity was analysed using various antibiotics to test the response of potent isolates. The promising results revealed that the isolates were sensitive to the antibiotics, enabling safe usage of isolates as bio-inoculant.

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CHAPTER 6.0

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6.0 BIBLIOGRAPHY

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