CHAPTER 4

RESULTS

4. RESULTS

The present study describes the isolation and characterization of endophytes of *Achyranthes aspera* L. A total of 73 endophytic bacteria were isolated, out of these four potential isolates were analysed for their growth stimulatory effect on plant, the results are presented hereunder.

4.1. Isolation of bacterial endophytes from *Achyranthes aspera* L. plants:

A total of 73 isolates were isolated from 5 different district of Manipur as endophytic bacteria from the leaves and stems of *A. aspera* L. The following isolates were collected according to their selected study site. 11 isolates were isolated from the leaves collected from Imphal West, 21 isolates from Imphal East, 15 isolates from Thoubal site, 13 isolates from Bishnupur site and 13 isolates from Senapati district. The isolates were cataloqued according to the site of isolation (Figure 1)

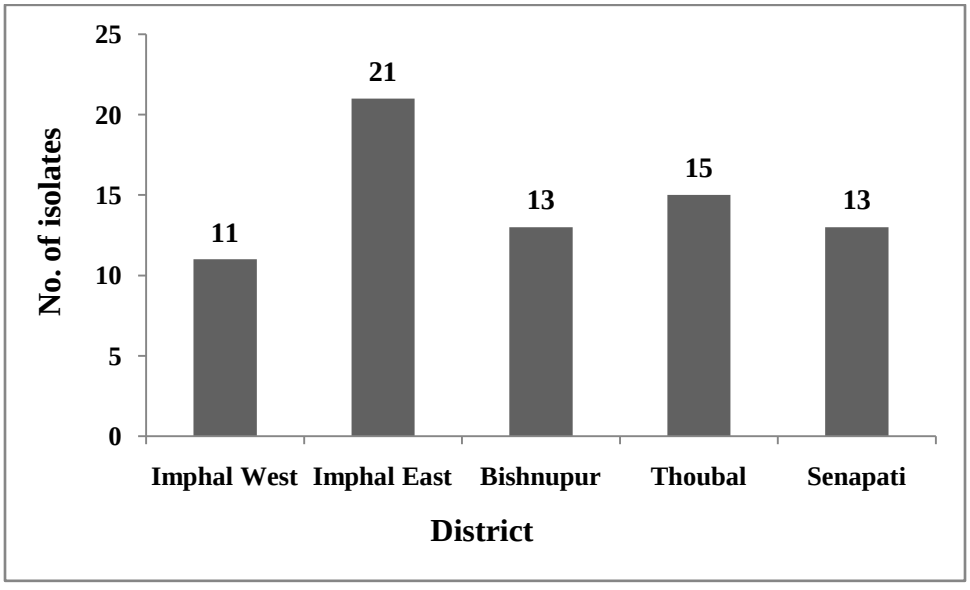


Figure 1. Number of isolates obtained from the samples**.**

4.2. Morphological, biochemical and physiological characteristics of the endophytic bacterial isolates:

The isolates grown on Yeast Extract Mannitol Agar (YEMA) formed colonies that were circular in shape, having entire margin and smooth surface texture. Both opaque as well as translucent types were found. The details of the characters are given in Table 1.

Table 1. Morphological characteristics of the isolates

Sl. No	Site	Isolates	Origin	Shape of the colony	Margin	Elevation	Color	Surface Texture	Surface Diameter	Opacity
1.	Imphal West	AL1-1	leaves	Circular	Entire	Raised	Dark pink	Smooth	3mm	opaque
		AL1-2	leaves	Circular	Entire	Raised	Off white	Smooth	3mm	opaque
		AL1-3	leaves	Circular	Entire	Raised	Off white	Smooth	3mm	opaque
		AL1-4	leaves	Circular	Entire	Flat	Off white	Smooth	2mm	Translucent
		AL1-5	leaves	Circular	Entire	Convex	Light yellow	Smooth	2mm	opaque
		AL1-6	Leaves	Irregular	Entire	Flat	yellow	Rough	3mm	Opaque
		AL1-7	Leaves	Regular	Entire	Flat	Off white	Smooth	5mm	Opaque
		AL1-8	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL1-9	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL1-10	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL1-11	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
2.	Imphal East	AL2-1	leaves	Circular	Entire	Flat	Orange	Smooth	1mm	Opaque
		AL2-2A	leaves	Circular	Entire	Flat	Yellow	Smooth	0.5mm	Opaque
		AL2-2B	leaves	Circular	Entire	Flat	Off white	Smooth	1mm	Translucent
		AL2-3	leaves	Circular	Entire	Flat	Yellow	Smooth	2mm	Opaque
		AL2-4A	leaves	Circular	Entire	Flat	Off white	Smooth	2mm	Translucent
		AL2-4B	leaves	Circular	Regular	Flat	yellow	Smooth	1mm	Opaque
		AL2-4C	leaves	Circular	Regular	Flat	Yellow	Smooth	2mm	Opaque
		AL2-4E	leaves	Circular	Undulate	Raised	Yellow	Rough	4mm	Opaque
		AL2-4F	leaves	Irregular	Undulate	Raised	Off white	Rough	7mm	Opaque
		AL2-4G	leaves	Circular	Regular	Flat	White	Rough	2mm	Translucent
		AL2-5	leaves	Irregular	Entire	Flat	Orange	Smooth	1mm	Opaque
		Al2-7A	leaves	Irregular	Entire	Flat	Orange	Smooth	1mm	Opaque
		AL2-7B	leaves	Irregular	Entire	Flat	Yellow	Smooth	2mm	Opaque
		Al2-9	leaves	Circular	Entire	Raised	orange	Smooth	2mm	Opaque
		Al2-10	leaves	Circular	Entire	Flat	White	Smooth	3mm	Opaque
		AL2-13	leaves	Circular	Entire	Flat	Light pink	Smooth	1mm	Opaque
		AL2-14B	**leaves**	**Irregular**	**Undulate**	**Flat**	**brownish**	**Rough**	**3mm**	**Opaque**
		Al2-15	leaves	Irregular	Entire	Flat	Off white	Smooth	4mm	Translucent
		AL2-16	**leaves**	**Circular**	**Entire**	**Flat**	**Pinkish**	**Smooth**	**2mm**	**Opaque**

		AL2-17	leaves	Circular	Pin head	Flat	white	Smooth	3mm	Translucent
		AL2-18	leaves	Irregular	Lobate	Flat	yellow	Smooth	2mm	Translucent
3.	Thoubal	AL3-1	leaves	Circular	Undulate	Flat	Yellow	Smooth	3mm	Opaque
		AL3-2	leaves	Circular	Entire	Convex	Yellow	Smooth	1mm	Opaque
		AL3-3	leaves	Circular	Entire	Convex	Off white	Smooth	2mm	Opaque
		AL3-4	leaves	Circular	Entire	Flat	white	Smooth	5mm	Opaque
		AL3-5	leaves	Circular	Lobate	Flat	Off white	Smooth	2mm	Opaque
		AL3-7	leaves	Irregular	Lobate	Flat	Off white	Smooth	2mm	Opaque
		AL3-8	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL3-9	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL3-10	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL3-11	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL3-12	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL3-13	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL3-14	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL3-15	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL3-16	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
4.	Bishnupur	AST4-1	Stem	Irregular	Lobate	Flat	white	Rough	6mm	Opaque
		AST4-2	Stem	Irregular	Lobate	Flat	Off white	Smooth	2mm	Opaque
		AST4-3	Stem	Irregular	Undulate	Flat	white	Rough	3mm	Opaque
		AST4-4	Stem	Irregular	Undulate	Flat	Off white	Rough	4mm	Opaque
		AST4-5	Stem	Irregular	Undulate	Flat	Off white	Rough	3mm	Opaque
		AST4-6	Stem	Irregular	Undulate	Flat	white	Rough	3mm	Opaque
		AST4-7	Stem	Irregular	Undulate	Flat	Off white	Rough	2mm	Opaque
		AST5-1	Stem	Irregular	Lobate	Flat	Off white	Smooth	2mm	Opaque
		AST5-2	**Stem**	**Irregular**	**Undulate**	**Flat**	**white**	**Rough**	**3mm**	**Opaque**
		AST5-5	Stem	Irregular	Undulate	Flat	Off white	Rough	4mm	Opaque
		AST5-6	Stem	Irregular	Undulate	Flat	Off white	Rough	3mm	Opaque
		AST5-7	Stem	Irregular	Undulate	Flat	white	Rough	3mm	Opaque
		AST5-9	Stem	Irregular	Undulate	Flat	Off white	Rough	2mm	Opaque
5.	Senapati	AL6-1	Leaves	Irregular	Rhizoidal	Flat	Off white	Smooth	4mm	Opaque
		AL6-2	Leaves	Irregular	Entire	Flat	Off white	Smooth	1mm	Opaque
		AL6-3	Leaves	Irregular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL6-4	Leaves	Irregular	Entire	Flat	white	Rough	10mm	Opaque
		AL6-5	Leaves	Irregular	Entire	Raised	Light orange	Rough	6mm	Opaque
		AL6-6	Leaves	Irregular	Entire	Flat	yellow	Rough	3mm	Opaque
		AL6-7	Leaves	Regular	Entire	Flat	Off white	Smooth	5mm	Opaque
		AL6-8	Leaves	Regular	Entire	Flat	orange	Smooth	1mm	Opaque
		AL6-9	Leaves	Regular	Entire	Flat	Greenish	Smooth	3mm	Opaque
		AL6-10	**Leaves**	**Regular**	**Entire**	**Flat**	**Pinkish**	**Smooth**	**2mm**	**Opaque**
		AL6-11	Leaves	Regular	Entire	Flat	Off white	Smooth	1mm	Opaque
		AL6-12	Leaves	Regular	Entire	Flat	Pinkish	Smooth	2mm	Opaque
		AL6-13	Leaves	Regular	Entire	Flat	Off white	Smooth	1mm	Opaque

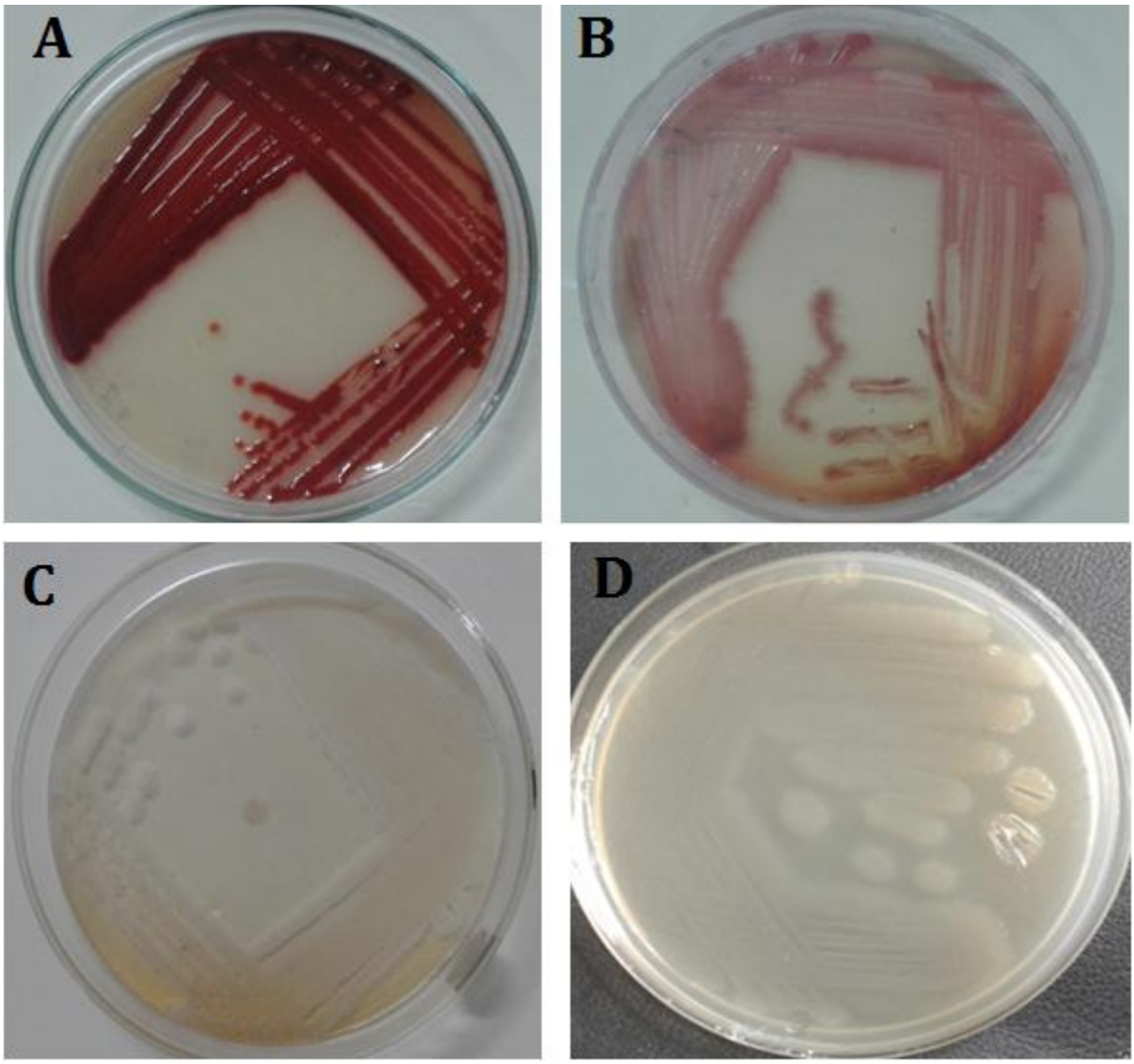
The colonies of isolate AL2-14B isolated from the leaves, appeared as irregular, undulated, flat, rough, brownish in colour, opaque and diameter of 3mm. The colonies of AL2-16, an isolate from leaves, appeared as circular, entire, flat, pinkish, smooth and opaque and diameter of 2mm. Colonies of AST5-2 appeared as irregular, undulated, flat,

off-white, rough, opaque and diameter of 3mm. Colonies of AL6-10, appeared as regular, entire, flat, dark pink in colour and diameter of 2mm (Table 1; Photoplate 1)

Table 2. Biochemical characteristics of the isolates

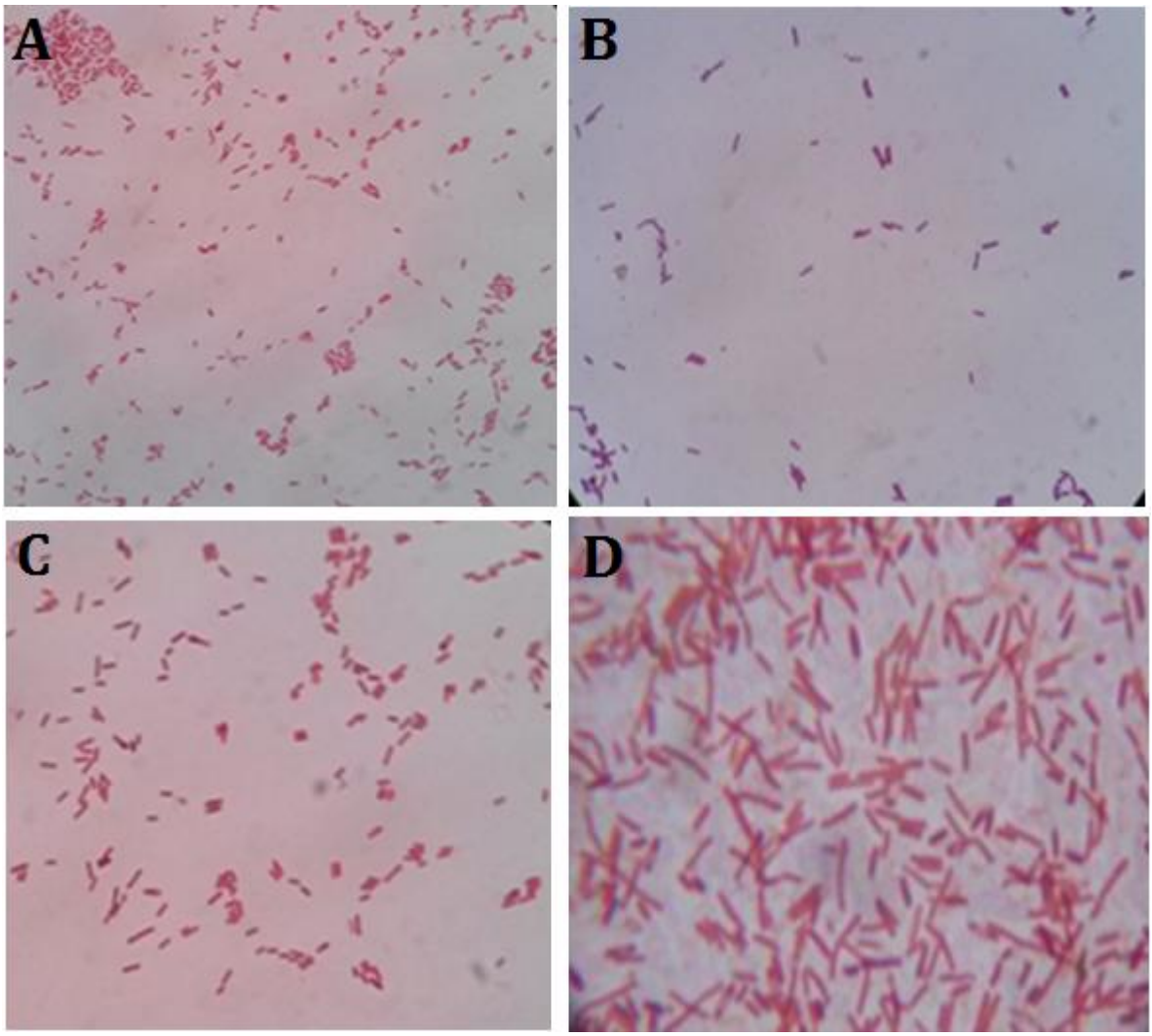
Isolates	Cell Shape	Gram Stain	Indole	MR	VP	Urease	Catalase	Oxidase	Citrate
AL1-1	Cocci	–	–	–	+	+	+	+	+
AL1-2	Cocci	–	–	–	–	+	+	+	+
AL1-3	Cocci	–	–	–	–	+	+	+	+
AL1-4	Cocci	–	–	+	–	+	+	+	+
AL1-5	Cocci	–	–	–	–	+	+	+	+
AL1-6	Cocci	–	+	–	–	+	–	+	+
AL1-7	Cocci	–	–	–	–	+	+	+	–
AL1-8	Cocci	–	–	–	–	+	+	+	–
AL1-9	Cocci	–	–	+	–	+	+	–	–
AL1-10	Cocci	–	–	+	–	+	+	+	+
AL1-11	Cocci	–	–	–	–	+	–	+	+
AL2-1	Cocci	–	–	+	+	+	+	+	+
AL2-2A	Cocci	–	–	+	+	+	+	+	+
AL2-2B	Cocci	–	–	–	–	–	–	–	–
AL2-3	Cocci	–	–	–	–	+	+	+	–
AL2-4A	Cocci	–	–	–	–	+	+	+	+
AL2-4B	Cocci	–	–	–	–	+	+	+	+
AL2-4C	Cocci	–	+	–	–	+	–	–	+
AL2-4E	Cocci	–	+	–	–	+	–	+	+
AL2-4F	Cocci	–	–	–	–	+	+	+	–
AL2-4G	Rod	–	–	–	–	+	+	+	+
AL2-5	Rod	–	–	+	–	+	+	–	+
Al2-7A	Cocci	–	–	+	–	+	+	+	+
AL2-7B	Rod	–	–	–	–	+	–	+	+
Al2-9	Cocci	–	–	+	–	–	–	–	+
Al2-10	Cocci	–	–	–	–	+	+	–	+
AL2-13	Cocci	–	–	+	–	+	+	+	–
AL2-14A	Cocci	–	–	+	–	+	+	–	–
AL2-14B	**rod**	–	**+**	**+**	**–**	**+**	**+**	**+**	+
AL2-15	Cocci	–	–	–	–	+	–	–	+
AL2-16	**rod**	**–**	**+**	**+**	**–**	**+**	**+**	**–**	+
AL2-17	Cocci	–	–	–	+	+	+	+	–
AL2-18	Cocci	–	–	+	–	+	+	+	+
AL3-1	Cocci	–	–	–	–	+	+	+	–
AL3-2	Cocci	–	–	+	–	+	+	+	+
AL3-3	Cocci	–	–	–	–	+	+	–	–
AL3-4	Rod	–	–	–	–	+	+	+	–
AL3-5	Cocci	–	–	–	+	+	+	+	–
AL3-7	Rod	–	–	+	–	–	+	–	+
AL3-8	Rod	–	–	+	–	+	+	+	+
AL3-9	Cocci	–	–	–	–	+	+	+	–
AL3-10	Cocci	–	–	+	–	+	+	+	+

AL3-11	Cocci	–	–	–	–	+	+	–	–
AL3-12	Rod	–	–	–	–	+	+	+	–
AL3-13	Cocci	–	–	–	+	+	+	+	–
AL3-14	Cocci	–	–	+	–	–	+	–	+
AL3-15	Cocci	–	–	–	+	+	+	+	–
AL3-16	Cocci	–	–	+	–	–	+	–	+
AST4-1	Bacilli	+	–	–	+	+	+	+	–
AST4-2	Bacilli	+	–	–	–	+	+	+	–
AST4-3	Bacilli	+	–	–	–	+	+	+	–
AST4-4	Bacilli	+	–	+	–	+	+	+	+
AST4-5	Bacilli	+	–	–	–	+	+	+	–
AST4-6	Bacilli	+	–	+	–	+	+	+	+
AST4-7	Bacilli	+	+	+	–	+	–	+	–
AST5-1	Bacilli	+	–	+	–	+	+	+	+
AST5-2	**Bacilli**	**+**	**+**	**+**	**–**	**+**	**–**	**+**	+
AST5-5	Bacilli	+	–	–	–	+	+	+	–
AST5-6	Bacilli	+	–	–	–	+	+	+	+
AST5-7	Bacilli	+	–	+	–	+	+	+	+
AST5-9	Bacilli	+	–	–	–	+	+	+	+
AL6-1	Cocci	–	–	+	+	+	+	+	+
AL6-2	Cocci	–	–	+	+	+	+	+	+
AL6-3	Cocci	–	–	–	–	–	–	–	+
AL6-4	Cocci	–	–	–	–	+	+	+	+
AL6-5	Rod	–	–	–	–	+	+	+	+
AL6-6	Cocci	–	–	–	–	+	+	+	+
AL6-7	Rod	–	+	–	–	+	–	–	+
AL6-8	Cocci	–	+	–	–	+	–	+	+
AL6-9	Cocci	–	–	–	–	+	+	+	–
AL6-10	**rod**	**–**	**+**	**+**	**–**	**+**	**+**	**–**	+
AL6-11	Cocci	–	–	+	–	+	+	–	-
AL6-12	Cocci	–	–	–	–	+	+	+	+
AL6-13	Rod	–	+	–	–	+	–	–	–

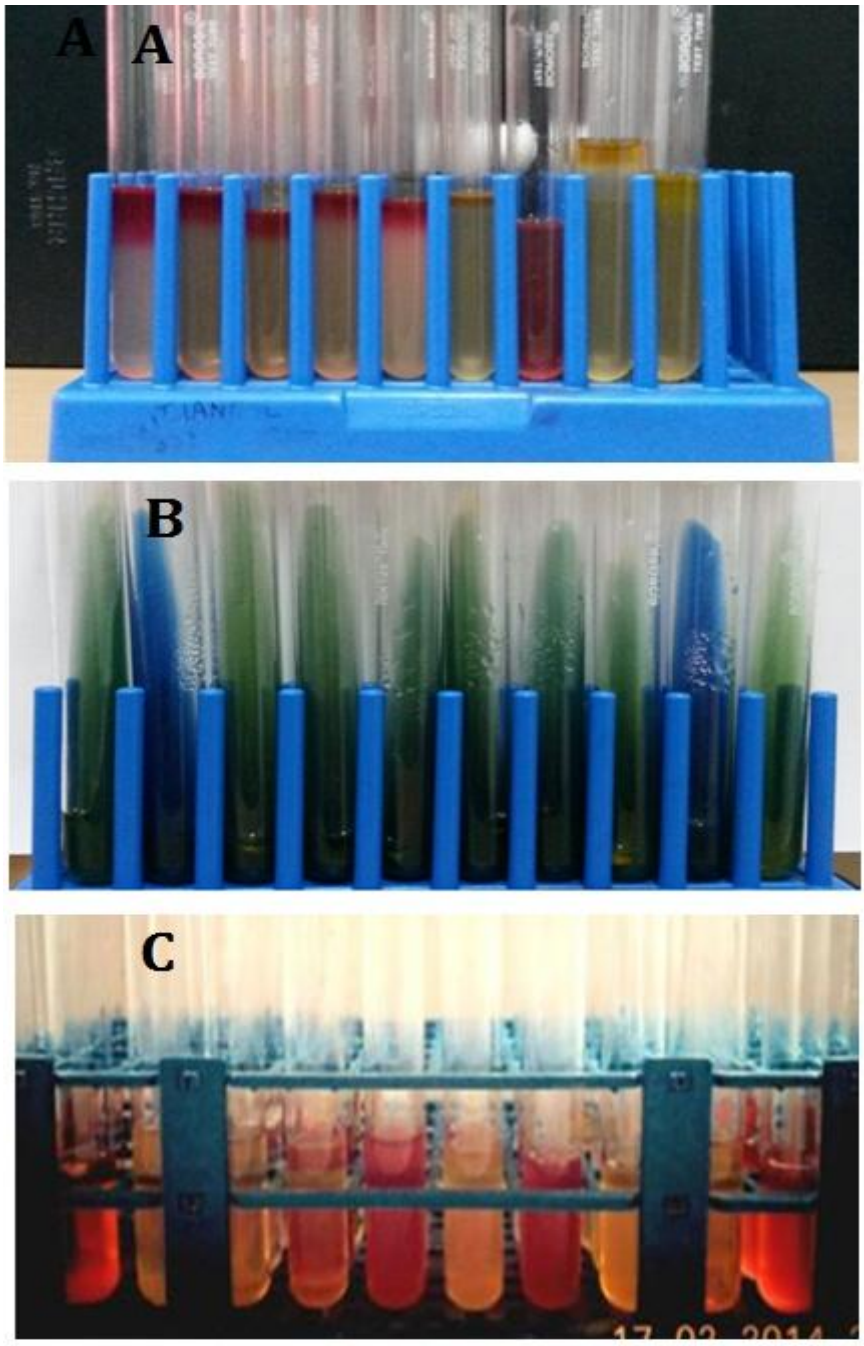


Photoplate 1: **Colony characteristics**

A. Colony characteristic of AL2-16,
B. Colony characteristic of AL6-10 ,
C. Colony characteristic of AST5-2
D. Colony characteristic of AL2-14B.



Photoplate 2. **Photomicrograph of morphological characteristics**. A) AL2-16, B) AST5-2, C) AL6-10, D) AL2-14B at 1000X magnification as observed after Gram's staining.



Photoplate 3. **Biochemical characteristics**. A) Indole test

B) Citrate test C) MR-VP test

Table 3. Physiological characteristics of endophytic bacteria

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL1-1	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL1-2	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL1-3	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL1-4	Facultative anaerobe	+++	+	+	_	_	_	_	++	++	+
AL1-5	aerobe	++	++	++	_	_	_	_	+	+++	++
AL1-6	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL1-7	Facultative anaerobe	+++	+++	++	+	_	_	_	+	+++	++
AL1-8	Facultative anaerobe	++	++	++	+	_	_	_	++	++	_
AL1-9	Facultative anaerobe	+++	++	++	+	_	_	_	+	+++	++
AL1-10	Facultative anaerobe	+++	++	+	_	_	_	_	++	++	_

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL2-1	Facultative anaerobe	+++	++	++	+	_	_	_	++	++	+
AL2-2A	aerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-2B	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-3	Facultative anaerobe	+++	+	+	_	_	_	_	++	++	_
AL2-4A	Facultative anaerobe	++	++	++	_	_	_	_	+	+++	++
AL2-4B	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-4C	Facultative anaerobe	+++	+++	++	+	_	_	_	+	++	+
AL2-4D	aerobe	++	++	++	+	_	_	_	++	+++	++
AL2-4E	Facultative anaerobe	+++	++	++	+	_	_	_	+	+++	++
AL2-4F	Facultative anaerobe	+++	++	+	_	_	_	_	++	+++	++

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL2-4G	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-5	Obligative aerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-7A	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-7B	Facultative anaerobe	+++	+	+	_	_	_	_	++	++	+
AL2-9	Facultative anaerobe	++	++	++	_	_	_	_	+	+++	++
AL2-10	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-13	Facultative anaerobe	+++	+++	++	+	_	_	_	+	+++	++
AL2-14A	Facultative anaerobe	++	++	++	+	_	_	_	++	++	_
AL2-14B	**Facultative anaerobe**	**+++**	**++**	**++**	**+**	**_**	**_**	**_**	**+**	**+++**	**++**
AL2-16	**Facultative anaerobe**	**+++**	**++**	**+**	**_**	**_**	**_**	**_**	**++**	**++**	**_**

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL2-17	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL2-18	Obligative aerobe	+++	++	++	+	_	_	_	++	+++	++
AL3-1	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL3-2	Facultative anaerobe	+++	+	+	_	_	_	_	++	++	+
AL3-3	Facultative anaerobe	++	++	++	_	_	_	_	+	+++	++
AL3-4	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL3-5	Facultative anaerobe	+++	+++	++	+	_	_	_	+	+++	++
AL3-7	Facultative anaerobe	++	++	++	+	_	_	_	++	++	_
AL3-8	Facultative anaerobe	+++	++	++	+	_	_	_	+	+++	++
AL3-9	Facultative anaerobe	+++	++	+	_	_	_	_	++	++	_

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL3-10	Facultative anaerobe	+++	++	++	+	_	_	_	++	++	+
AL3-11	Facultative anaerobe	+++	++	++	+	_	_	_	++	++	+
AL3-12	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL3-13	Facultative anaerobe	+++	+	+	_	_	_	_	++	+++	++
AL3-14	Facultative anaerobe	++	++	+	_	_	_	_	+	+++	++
AL3-15	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL3-16	Facultative anaerobe	+++	+++	++	+	_	_	_	+	++	+
AST4-1	Facultative anaerobe	++	++	++	+	_	_	_	++	++	_
AST4-2	Facultative anaerobe	+++	++	++	+	_	_	_	+	+++	++
AST4-3	aerobe	+++	++	+	_	_	_	_	++	++	_

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AST5-1	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AST5-2	**Facultative anaerobe**	**+++**	**++**	**++**	**+**	**_**	**_**	**_**	**++**	**+++**	**++**
AST5-5	Facultative anaerobe	+++	++	++	+	_	_	_	++	++	+
AST5-6	Facultative anaerobe	+++	+	+	_	_	_	_	++	++	+
AST5-7	Facultative anaerobe	++	++	++	_	_	_	_	+	+++	++
AST5-9	Facultative anaerobe	+++	++	++	+	_	_	_	++	++	+
AL6-1	Facultative anaerobe	+++	+++	++	+	_	_	_	+	+++	++
AL6-2	Obligative aerobe	++	++	++	+	_	_	_	++	++	_
AL6-3	Facultative anaerobe	+++	++	++	+	_	_	_	+	++	+
AL6-4	Facultative anaerobe	+++	++	+	_	_	_	_	++	+++	+

Table 3. Contd.

Isolates	Oxygen requirement	Growth at different NaCl concentration(%)					Growth at different p^H				
		0.5	2	5	10	20	4	5	6	7	8
AL6-5	Obligative aerobe	+++	++	+	_	_	_	_	++	+++	++
AL6-6	Facultative anaerobe	++	++	+	_	_	_	_	++	+++	++
AL6-6A	aerobe	+++	++	++	+	_	_	_	++	+++	++
AL6-7	Facultative anaerobe	+++	+	+	_	_	_	_	++	+++	+
AL6-8	Facultative anaerobe	++	++	++	_	_	_	_	++	+++	++
AL6-9	Facultative anaerobe	+++	++	++	+	_	_	_	++	+++	++
AL6-10	**Facultative anaerobe**	**+++**	**+++**	**++**	**+**	**_**	**_**	**_**	**++**	**+++**	**++**
AL6-11	Facultative anaerobe	++	++	++	+	_	_	_	+	++	_
AL6-12	Facultative anaerobe	++	++	++	+	_	_	_	++	+++	++
AL6-13	Facultative anaerobe	+++	++	+	_	_	_	_	++	++	_

Out of 73 isolates, 83% (61) isolates were facultative anaerobes, 6.8% (5) isolates were aerobes and only 5.4% (4) were obligate aerobes. All the isolates were able to grow well at 0.5% NaCl concentration, and even survive up to a range of 2-5% NaCl concentration. None of the isolate was able to grow at 20% NaCl concentration. All the isolates grew well at pH 6 and 7, however no isolate was able to grow at pH 4 and 5 (Table 3).

AL2-14B was Gram negative rod, indole, catalase, urease, citrate and oxidase positive and methyl red and Voges-Proskauer negative. It was able to ferment glucose but found negative for other carbohydrates such as fructose, mannitol, sucrose, cellulose and maltose. It was facultative anaerobe, able to grow well at a range of 0.5-5% NaCl concentration and pH of 6 and 7 (Table 2; Table 3; Photoplate 1; Photoplate 2).

AL2-16 was Gram negative, rod in shape, indole, catalase, urease, citrate and oxidase positive and methyl red and Voges-Proskauer negative. It was able to ferment glucose, fructose, mannitol, sucrose, and maltose but found negative for cellulose. It was facultative anaerobes, able to grow well at a range of 0.5-5% NaCl concentration and pH of 6 and 7 (Table 2; Table 3; Photoplate 1; Photoplate 2).

AST5-2 was Gram positive in nature, indole, oxidase, citrate, methyl red, urease positive; catalase and Voges-Proskauer negative. It was able to ferment glucose, fructose, sucrose, but found negative for maltose, lignin and mannitol. It was facultative anaerobes, able to grow well at a range of 0.5-5% NaCl concentration and pH of 6 and 7 (Table 2; Table 3; Photoplate 1; Photoplate 2).

AL6-10 was Gram negative, rod in nature; urease, citrate, catalase and oxidase positive whereas indole, methyl red and Voges-Proskauer negative. It was able to ferment

glucose, fructose, sucrose, but found negative for maltose, lignin and mannitol. It was facultative anaerobes, able to grow well at a range of 0.5-5% NaCl concentration and pH of 6 and 7 (Table 2; Table 3; Photoplate 1; Photoplate 2).

4.3. Preliminary screening for plant growth bioassays:

All the collected pure cultures were checked for Plant Growth Promoting (PGP) attributes such as Indole Acetic Acid production (IAA), Phosphate solubilization, Siderophore production, Nitrogen fixation and Ammonia production. The results are presented in Table 4.

Table 4. Plant growth promotion assay

Isolate	IAA Production (µg/ml)	Siderophore Production	PO_4 solubilization	N_2 Fixation	Ammonification
AL1-1	12.008	-	+	+	++
AL1-2	11.7	-	+	+	++
AL1-3	8.9	-	-	+	++
AL1-4	9.2	-	+	–	++
AL1-5	7.51	-	++	+	++
AL1-6	6.20	-	-	+	++
AL1-7	7.18	-	-	+	++
AL1-8	4.79	-	+	+	++
AL1-9	10.01	-	+	+	++
AL1-10	12.88	-	-	-	++
AL1-11	11.76	-	+	-	+
AL2-1	5.22	-	++	+	+
AL2-2A	6.25	-	+	+	+
AL2-2B	9.01	-	-	+	++
AL2-3	8.34	-	-	–	+
AL2-4A	4.98	+	+	–	+
AL2-4B	5.67	-	+	+	++
AL2-4C	8.72	-	-	+	+
AL2-4E	12.126	+	-	+	++
AL2-4F	5.33	-	-	+	+
AL2-4G	7.11	-	-	+	+
AL2-5	8.33	-	-	+	+
Al2-7A	9.01	-	-	+	++
AL2-7B	5.475	-	+	–	++
Al2-9	8.2	-	-	–	+
Al2-10	7.65	-	-	+	+
AL2-13	12.502	-	-	+	+

AL2-14A	7.35	-	+	+	++
AL2-14B	**23.01**	**+++**	**+++**	**+++**	**+++**
AL2-15	6.45	-	+	+	++
AL2-16	**31.361**	**+++**	**+++**	**+++**	**+++**
AL2-17	7.12	-	+	+	+
AL2-18	5.6	-	-	+	+
AL3-1	6.4	+	–	–	++
AL3-2	5.36	-	+	+	+
AL3-3	8.01	-	-	+	+
AL3-4	7.11	-	-	+	+
AL3-5	6.54	-	-	+	++
AL3-7	11.78	-	-	+	++
AL3-8	5.76	-	-	+	++
AL3-9	10.11	-	-	+	++
AL3-10	9.56	-	-	+	++
AL3-11	4.4	-	-	+	++
AL3-12	3.2	+	+	+	+
AL3-13	5.09	+	+	+	+
AL3-14	3.64	-	-	+	+
AL3-15	9.34	-	+	+	++
AL3-16	7.81	-	-	+	++
AST4-1	7.12	-	+	+	+
AST4-2	10.09	-	+	+	+
AST4-3	4.54	-	-	+	++
AST4-4	11.11	-	-	+	++
AST4-5	6.72	+	-	+	+
AST4-6	3.88	-	-	+	+
AST4-7	5.27	+	-	-	++
AST5-1	6.34	-	-	+	++
AST5-2	**19.32**	**-**	**+++**	**++**	**+++**
AST5-5	8.56	-	-	+	++
AST5-6	10.64	-	-	+	+
AST5-7	7.12	-	-	+	+
AST5-9	10.66	-	-	+	++
AL6-1	3.572	-	–	–	++
AL6-2	11.362	-	-	+	+
AL6-3	7.01	-	-	+	+
AL6-4	5.34	-	-	+	+
AL6-5	7.21	-	-	+	+
AL6-6	5.01	-	-	+	+
AL6-7	6.87	-	-	–	+
AL6-8	8.72	-	-	+	++
AL6-9	5.34	-		+	++
AL6-10	**21.112**	**+++**	**+++**	**+++**	**+++**
AL6-11	10.784	-	-	+	+
AL6-12	8.91	-	-	++	+

AL6-13	3.01	-	-	+++	++

All the endophytes were found to release IAA in presence of 0.2% L-tryptophan. The 4 isolates namely, AL2-14B, AL2-16, AL6-10 and AST5-2 produced 23.01, 31.361, 19.32, 21.112 µg/ml of IAA respectively, which was found to be higher than any other isolates. These isolates have shown good halozone size for siderophore production in CAS agar medium and phosphate production in Pikovskaya's agar medium. And also these isolates were able to grow on nitrogen free media which was indicative of their ability to fix atmospheric nitrogen. The N_2-fixing ability was further quantified by Acetylene Reduction Assay (ARA) for selected isolates (described later).

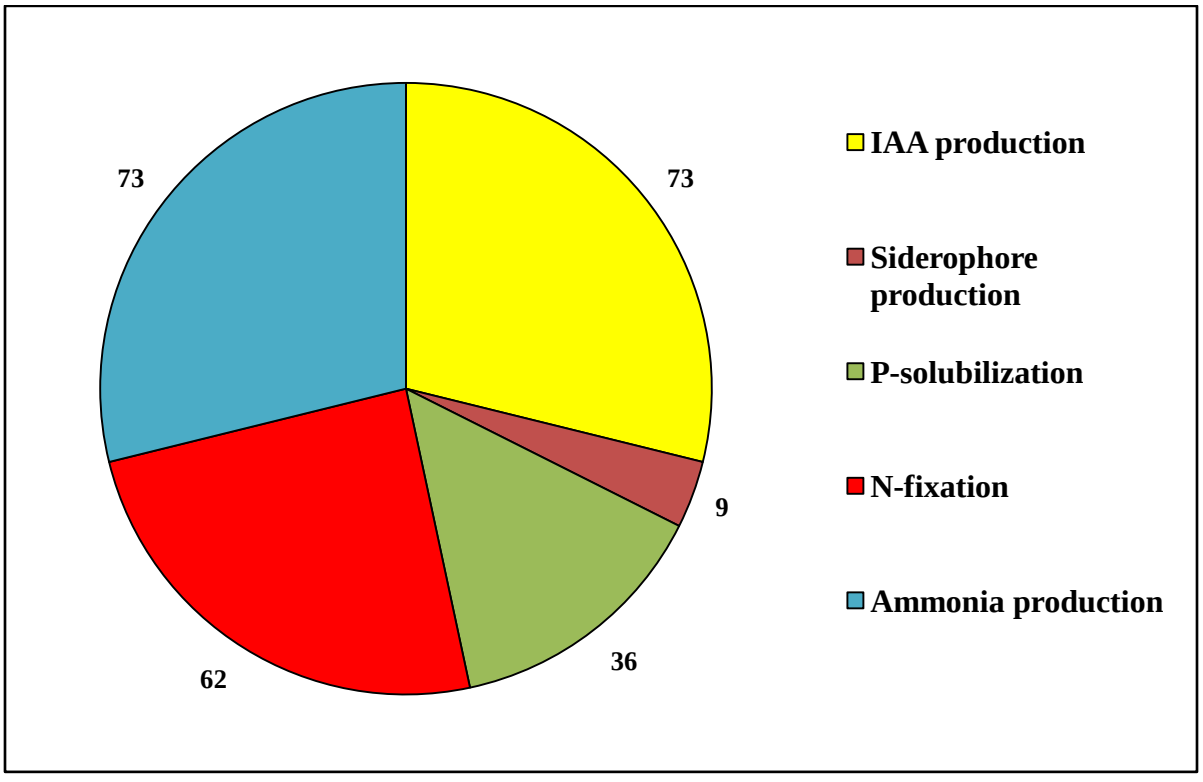


Figure 2. Pie chart represents the ability of plant growth promoting attributes.

In initial screening, all the endophytic isolates from *A. aspera* were found to released IAA and has the ability of ammonification (100%). 84.93% of endophytes were able to fix atmospheric nitrogen, while 49.31% of endophytes solubilized tricalcium phosphate. Only 12.32% of endophytes showed halozone on CAS agar medium which was indicative of their siderophore production ability (Figure 2).

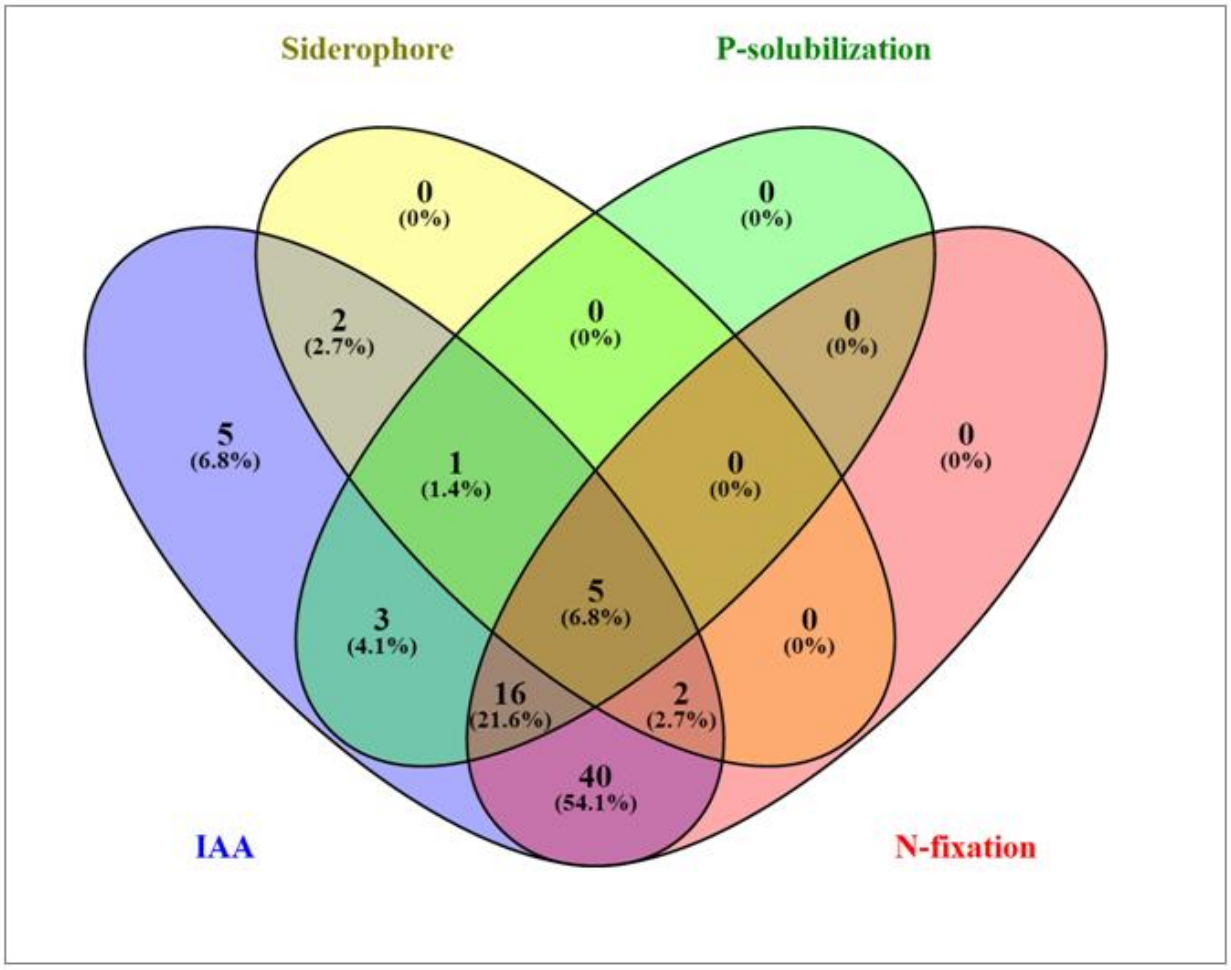
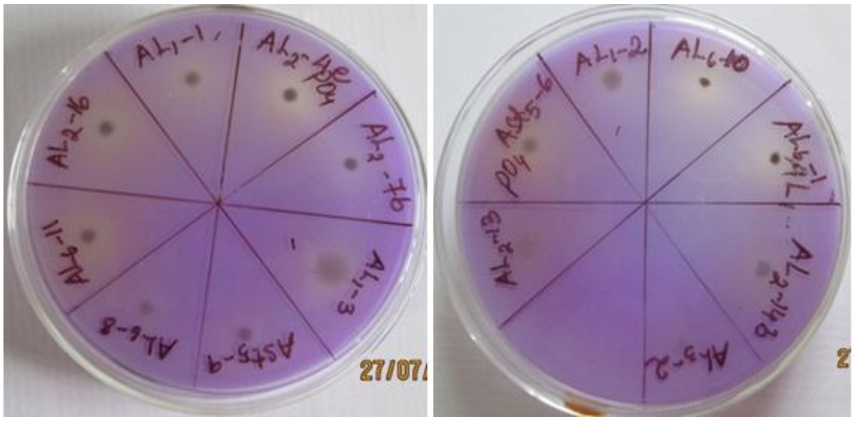


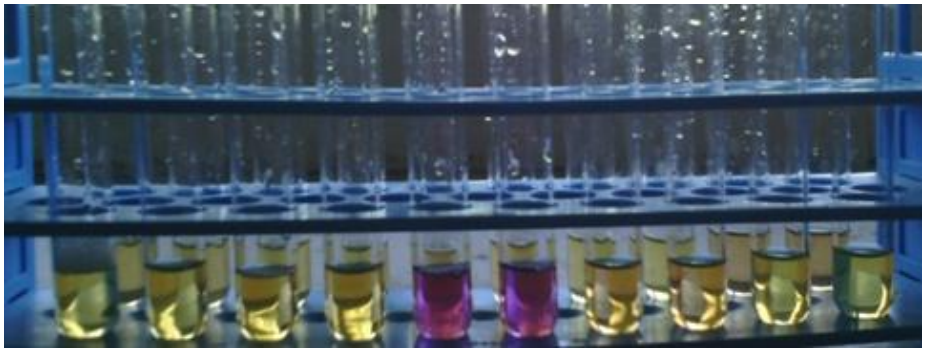
Figure 3. Venn diagram depicting the overlap of multiple PGP traits among isolates.

From the figure 3, it may be summarized that 54.1% of the isolates have the ability to produced IAA and also fixed atmospheric nitrogen, 21.6% of the isolates

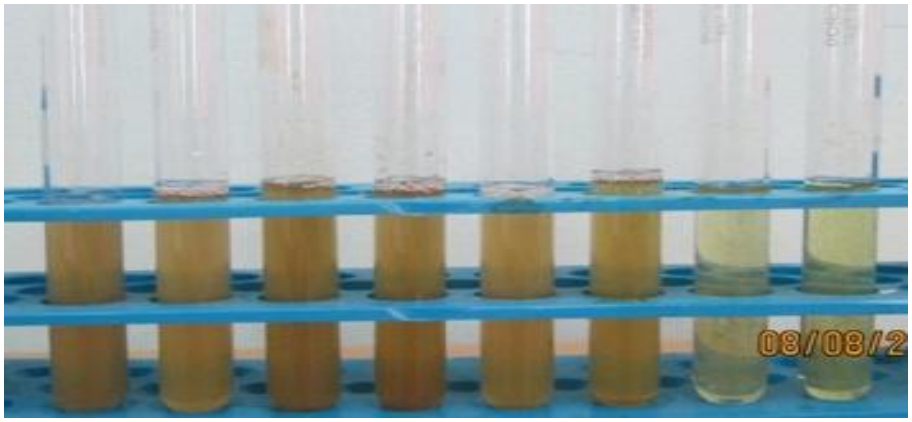
produced IAA along with phosphate solubilization and nitrogen fixation. 4.1% of isolates had IAA along with phosphate solubilization activity. 2.7% isolates produced siderophore along with IAA production. 6.8% of isolates have IAA and siderophore production along with phosphate solubilization Only 1 isolate produced IAA, siderophore and showed phosphate solubilization.



Photoplate 4. Zone formation on Pikovskaya's agar plate



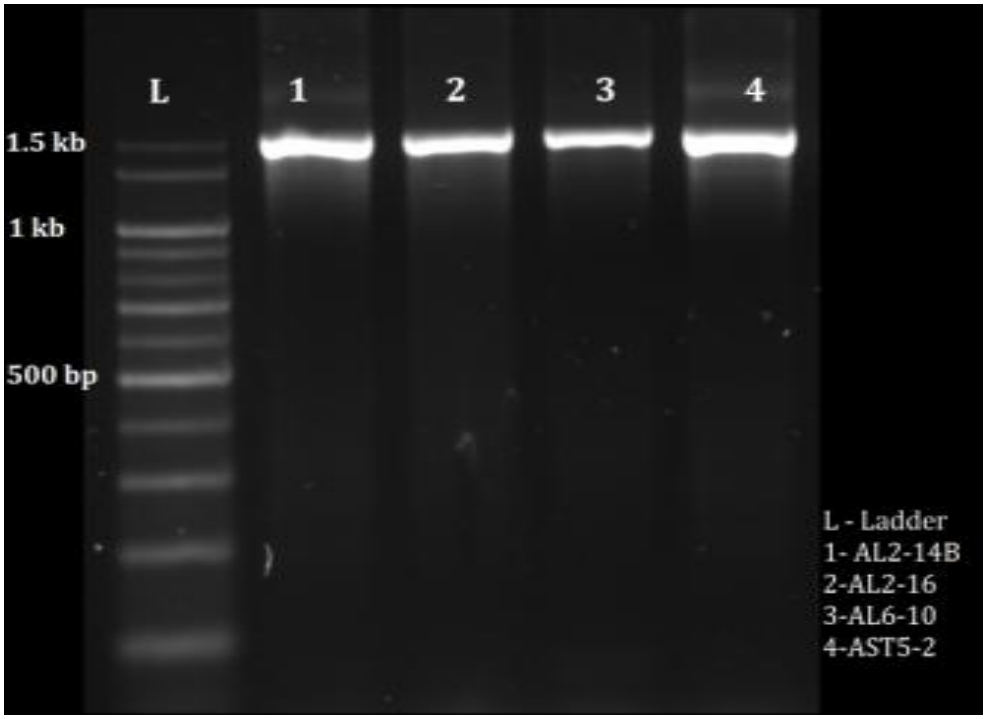
Photoplate 5. Siderophore Production in CAS broth



Photoplate 6. Ammonia production in peptone broth

4.4. Molecular identification of the isolate

On the basis of initial screening of PGP attributes, four isolated were selected for further studies i.e. AL2-14B, AL2-16, AL6-10 and AST5-2. Identification of the bacterial isolates was carried out with HiPurA Bacterial DNA isolation Mini Kit. The primers 27F (5'-CAGAGTTTGATCCTGGCT-3') and 1492R (5'-AGGAGGTGATCCAGCCGCA-3') were used for amplification of 16S rRNA gene. A specific bands were formed at 1500 bp which confirmed the bacterial 16S conserved region (Photoplate 7).



Photoplate 7. Gel image of 16S rRNA amplification of four isolates in1% Agarose gel.

The 16S rRNA was sequenced. The respective sequences (in NCBI GenBank formate) and phylogenetic tree of four isolates are given below

The 16S rRNA sequence of AL2-14B is given below.

Pseudomonas sp. strain aeruginosa 16S ribosomal RNA gene, partial sequence

GenBank: KY087983.1

FASTA Graphics

```
LOCUS       KY087983               1200 bp    DNA     linear   BCT 13-NOV-2016
DEFINITION  Pseudomonas sp. strain aeruginosa 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   KY087983
VERSION     KY087983.1
KEYWORDS    .
SOURCE      Pseudomonas sp.
  ORGANISM  Pseudomonas sp.
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (bases 1 to 1200)
  AUTHORS   Khaidem,A. and Pandey,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-NOV-2016) Department of Microbiology, Assam
            University, Silchar, Durgakona, Silchar, Assam 788011, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..1200
                     /organism="Pseudomonas sp."
                     /mol_type="genomic DNA"
                     /strain="aeruginosa"
                     /isolation_source="plant"
                     /host="Plant"
                     /db_xref="taxon:306"
     rRNA            <1..>1200
                     /product="16S ribosomal RNA"
ORIGIN
        1 gcttataaca caatttgcaa agtcggagcg gatgaaggga gcttgctcct ggattcagcg
       61 gcggacgggt gagtaatgcc taggaatctg cctggtagtg ggggataacg tccggaaacg
      121 ggcgctaata ccgcatacgt cctgagggag aaagtggggg atcttcggac ctcacgctat
      181 cagatgagcc taggtcggat tagctagttg gtggggtaaa ggcctaccaa ggcgacgatc
      241 cgtaactggt ctgagaggat gatcagtcac actggaactg agacacggtc cagactccta
      301 cgggaggcag cagtggggaa tattggacaa tgggcgaaag cctgatccag ccatgccgcg
      361 tgtgtgaaga aggtcttcgg attgtaaagc actttaagtt gggaggaagg gcagtaagtt
      421 aataccttgc tgttttgacg ttaccaacag aataagcacc ggctaacttc gtgccagcag
      481 ccgcggtaat acgaagggtg caagcgttaa tcggaattac tgggcgtaaa gcgcgcgtag
      541 gtggttcagc aagttggatg tgaaatcccc gggctcaacc tgggaactgc atccaaaact
      601 actgagctag agtacggtag agggtggtgg aatttcctgt gtagcggtga aatgcgtaga
      661 tataggaagg aacaccagtg gcgaaggcga ccacctggac tgatactgac actgaggtgc
      721 gaaagcgtgg ggagcaaaca ggattagata ccctggtagt ccacgccgta aacgatgtcg
      781 actagccgtt gggatccttg agatcttagt ggcgcagcta acgcgataag tcgaccgcct
      841 ggggagtacg ggccgcaggg ttaaaactca atgaattgtc aggggcccgc acaagcggtg
      901 gagcatgtgg tttaatttca aacaacgcgc agaaccttac ctgggcttga catgctgaga
      961 actttccaga gatgtattgg tgccttcggg aactcagaca caggtgctgc atgggctgtc
     1021 gtcagctctg tcgtgagatg ttgggttaag tcccgttacc acccaacctt gtccttagta
     1081 cgactctcgg tggtcactct aggaaactgc cgtgacaacc cggaggaagg tgggatgacg
     1141 ttcagtgtta tgggccttac ggcaaggcat accacgtgct gcatgggtcg gtacataggg
```

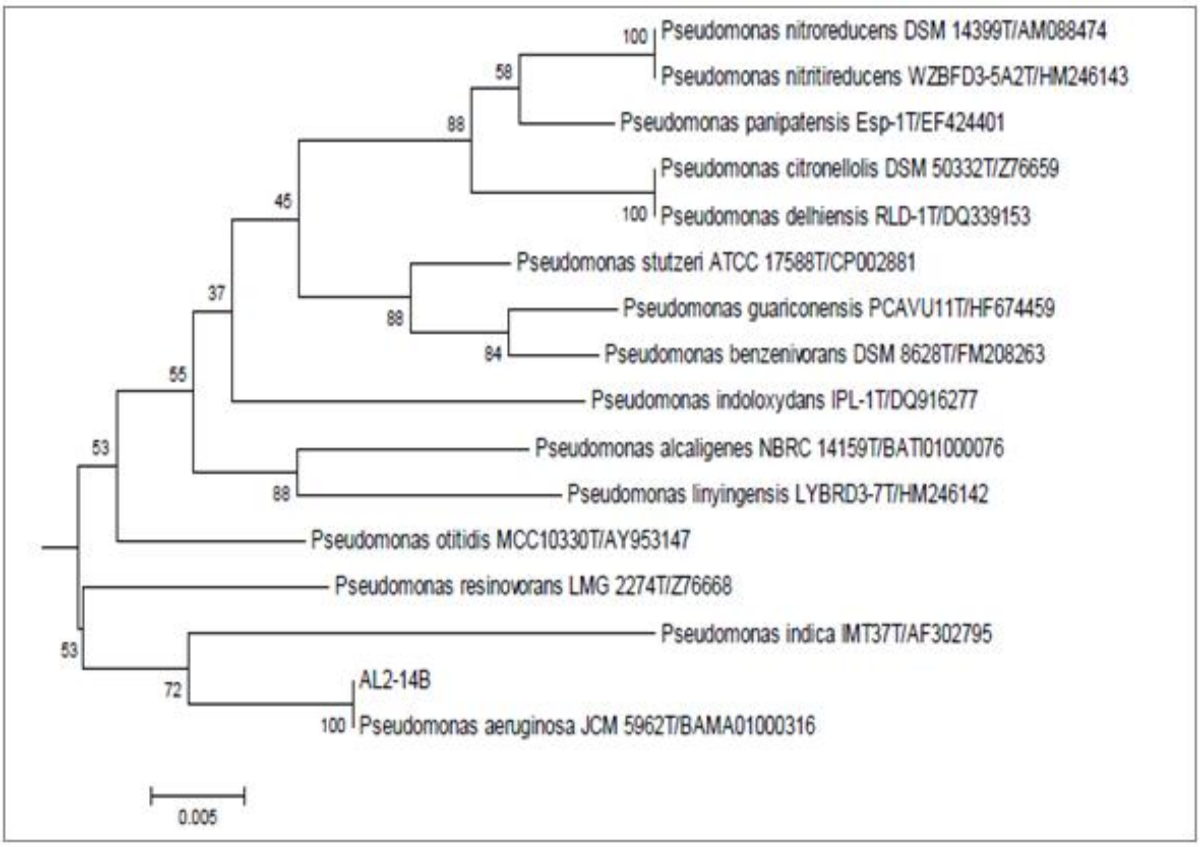


Figure 4. Phylogenetic analysis of 16S rRNA sequences of the bacterial isolate AL2-14B isolated from *A. aspera* L. The analysis was conducted with MEGA6 using neighbor-joining method.

The results of the BLAST search of the 16S rRNA gene sequences indicated AL2-14B isolate as closely related to *Pseudomonas aeruginosa*. A phylogenetic tree was constructed on the basis of 16S rRNA sequence homology. AL2-14B clustered with *P. aeruginosa* JCM 5962T/ BAMA01000316 and these two were closely related to *P. indica* IMT37T/AF302795. All type strains clustered in 3 major and 2 minor clades. Based on phylogenetic analysis, AL2-14B was identified as *P. aeruginosa*, maximum similarity was observed with isolate *P. aeruginosa* JCM 5962T/ BAMA01000316 (Figure 4). Strain AL2-14B clustered with *P. indica* and *P. aeruginosa.*

The 16S rRNA sequence of AL2-16 is given below

Serratia sp. strain marcescens 16S ribosomal RNA gene, partial sequence

GenBank: KY087982.1

FASTA Graphics

```
LOCUS       KY087982                957 bp    DNA     linear   BCT 13-NOV-2016
DEFINITION  Serratia sp. strain marcescens 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   KY087982
VERSION     KY087982.1
KEYWORDS    .
SOURCE      Serratia sp.
  ORGANISM  Serratia sp.
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Yersiniaceae; Serratia.
REFERENCE   1  (bases 1 to 957)
  AUTHORS   Khaidem,A. and Pandey,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-NOV-2016) Department of Microbiology, Assam
            University, Silchar, Durgakona, Silchar, Assam 788011, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..957
                     /organism="Serratia sp."
                     /mol_type="genomic DNA"
                     /strain="marcescens"
                     /isolation_source="plant"
                     /host="Plant"
                     /db_xref="taxon:616"
     rRNA            <1..>957
                     /product="16S ribosomal RNA"
ORIGIN
        1 cggcgggggc accgcttaaa acatgcaagt cgagcggtag cacaagggga gcttgctccc
       61 tgggtgacga gcggcggacg ggtgagtaat gtctgggaaa ctgcctgatg gaggggggata
      121 actactggaa acggtagcta ataccgcata acgtcgcaag accaaagagg gggaccttcg
      181 ggcctcttgc catcagatgt gcccagatgg gattagctag taggtggggt aatggctcac
      241 ctaggcgacg atccctagct ggtctgagag gatgaccagc cacactggaa ctgagacacg
      301 gtccagactc ctacgggagg cagcagtggg gaatattgca caatgggcgc aagcctgatg
      361 cagccatgcc gcgtgtgtga agaaggcctt cgggttgtaa agcactttca gcgaggagga
      421 aggtggtgaa cttaatacgt tcatcaattg acgttactcg cagaagaagc accggctaac
      481 tccgtgccag cagccgcggt aatacggagg gtgcaagcgt taatcggaat tactgggcgt
      541 aaagcgcacg caggcggttt gttaagtcag atgtgaaatc cccgggctca acctgggaac
      601 tgcatttgaa actggcaagc tagagtctcg tagaggggg tagaattcca ggtgtagcgg
      661 tgaaatgcgt agagatctgg aggaataccg gtggcgaagg cggccccctg gacgaagact
      721 gacgctcagg tgcgaaaagc gtggggagca aacaggatta gataccctgg tagtccacgc
      781 tgtaaacgat gtcgatttgg aggttgtgcc cttgaggcgt ggcttccgga gctaacgcgt
      841 taaatcgacc gcctgggagt acggccgcaa ggttaaaact caatgaattg acgggggcgc
      901 gcacaagcgg tggagcatgt ggtttaattc gatgcaccgc gaaaaccttа cctactc
```

The 16S rRNA sequence of AL6-10 is given below

Serratia sp. strain marcescens 16S ribosomal RNA gene, partial sequence

GenBank: KY087984.1

FASTA Graphics

```
LOCUS       KY087984               1200 bp    DNA     linear   BCT 13-NOV-2016
DEFINITION  Serratia sp. strain marcescens 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   KY087984
VERSION     KY087984.1
KEYWORDS    .
SOURCE      Serratia sp.
  ORGANISM  Serratia sp.
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Yersiniaceae; Serratia.
REFERENCE   1  (bases 1 to 1200)
  AUTHORS   Khaidem,A. and pandey,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-NOV-2016) Department of Microbiology, Assam
            University, Silchar, Durgakona, Silchar, Assam 788011, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..1200
                     /organism="Serratia sp."
                     /mol_type="genomic DNA"
                     /strain="marcescens"
                     /isolation_source="plant"
                     /host="Plant"
                     /db_xref="taxon:616"
     rRNA            <1..>1200
                     /product="16S ribosomal RNA"
ORIGIN
        1 gaaggcatga agggtgcgcg cttataacat gcaagtcgag cggtagcaca aggggagctt
       61 gctccctggg tgacgagcgg cggacgggtg agtaatgtct gggaaactgc ctgatggagg
      121 gggataacta ctggaaacgg tagctaatac cgcataacgt cgcaagacca aagaggggga
      181 ccttcgggcc tcttgccatc agatgtgccc agatgggatt agctagtagg tggggtaatg
      241 gctcacctag gcgacgatcc ctagctggtc tgagaggatg accagccaca ctggaactga
      301 gacacggtcc agactcctac gggaggcagc agtggggaat attgcacaat gggcgcaagc
      361 ctgatgcagc catgccgcgt gtgtgaagaa ggccttcggg ttgtaaagca ctttcagcga
      421 ggaggaaggt ggtgaactta atacgctcat caattgacgt tactcgcaga agaagcaccg
      481 gctaactccg tgccagcagc cgcggtaata cggagggtgc aagcgttaat cggaattact
      541 gggcgtaaag cgcacgcagg cggtttgtta agtcagatgt gaaatccccg ggctcaacct
      601 gggaactgca tttgaaactg gcaagctaga gtctcgtaga gggggggtaga attccaggtg
      661 tagcggtgaa atgcgtagag atctggagga ataccggtgg cgaaggcggc cccctggacg
      721 aagactgacg ctcaggtgcg aaagcgtggg gagcaaacag gattagatac cctggtagtc
      781 cacgctgtaa acgatgtcga tttggaggtt gtgcccttga gcgtggcttc cggagctaac
      841 gcgttaaatc gaccgcctgg ggagtacggc cgcaaggtta aaactcaaat gaattgacgg
      901 gggcccgcac aagcggtgga gcatgtggtt taattcgatg caacgcgaag aaccttacct
      961 actcttgaca tccagagaac tttccagaga tggattggtg ccttcgggaa ctctgagaca
     1021 ggtgctgcat ggctgtcgtca gctcgtgtgt gaaatgttgg ggttagtccc gcacgagcgc
     1081 accctatctt tgttgccgcg gttcggccgg taactcatgg aaactgcagt gatatatgtg
     1141 aggtacgtgg gatgacgtca gtcatcatgg tcccttacaa gtatgcttac acggtctgac
```

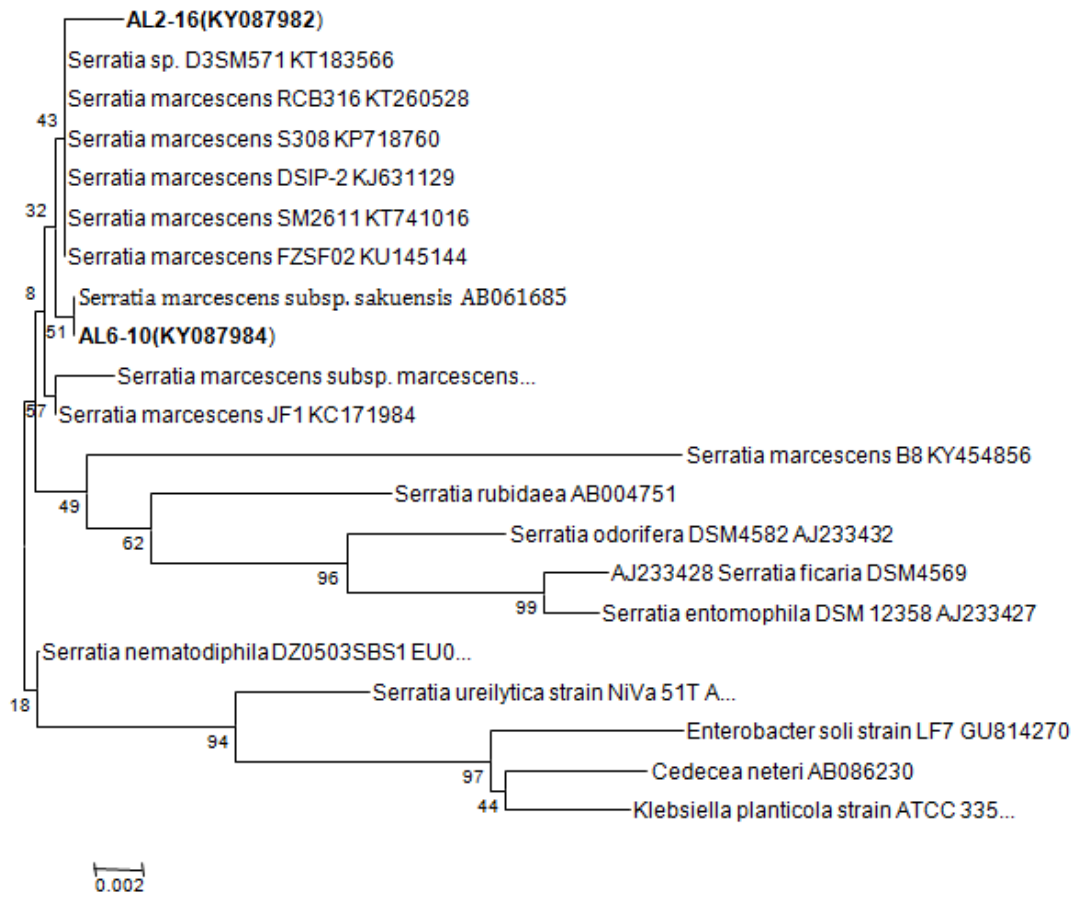


Figure 5. Phylogenetic analysis of 16S rRNA sequences of the bacterial isolate AL2-16 and AL6-10 isolated from *A. aspera* L. The analysis was conducted with MEGA6 using neighbor-joining method.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.12083594 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were

eliminated from the dataset (Complete deletion option). There were a total of 815 positions in the final dataset. Phylogenetic analyses were conducted in MEGA6 (Tamura *et al*., 2011).

The results of the BLAST search of the 16S rRNA gene sequences indicated AL2-16 isolate as closely related to *Serratia marcescens*. Based on the phylogenetic tree constructed with the 16S rRNA similarity (%), it was identified as *Serratia marcescens,* maximum similarity was observed with isolate *Serratia marcescens* subsp. *Sakuensis* AB061685. Strain AL6-10 was also found to be closely clustered with *Serratia marcescens* subsp. *Sakuensis* and *Serratia marcescens* subsp.*marcescens* (Figure 5).

The 16S rRNA sequence of AST5-2 is given below

Bacillus sp. strain methylotrophicus 16S ribosomal RNA gene, partial sequence

GenBank: KY087985.1

FASTA Graphics

Go to:

```
LOCUS       KY087985                882 bp    DNA     linear   BCT 13-NOV-2016
DEFINITION  Bacillus sp. strain methylotrophicus 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   KY087985
VERSION     KY087985.1
KEYWORDS    .
SOURCE      Bacillus sp.
  ORGANISM  Bacillus sp.
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 882)
  AUTHORS   Khaidem,A. and Pandey,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-NOV-2016) Department of Microbiology, Assam
            University, Silchar, Durgakona, Silchar, Assam 788011, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..882
                     /organism="Bacillus sp."
                     /mol_type="genomic DNA"
                     /strain="methylotrophicus"
                     /isolation_source="plant"
                     /host="Plant"
                     /db_xref="taxon:1409"
     rRNA            <1..>882
                     /product="16S ribosomal RNA"
ORIGIN
        1 ctaatgaaga cagttgtcaa cgtgcggacg cggacaaatg ggagcttgct ccctgatgtt
       61 agcggcggac gggtgagtaa cacgtgggta acctgcctgt aagactggga taactccggg
      121 aaaccggggc taataccgga tggttgtttg aaccgcatgg ttcagacata aaaggtggct
      181 tcggctacca cttacagatg gacccgcggc gcattagcta gttggtgagg taacggctca
      241 ccaaggcgac gatgcgtagc cgacctgaga gggtgatcgg ccacactggg actgagacac
      301 ggcccagact cctacgggag gcagcagtag ggaatcttcc gcaatggacg aaagtctgac
      361 ggagcaacgc cgcgtgagtg atgaaggttt tcggatcgta aagctctgtt gttagggaag
      421 aacaagtgcc gttcaaatag ggcggcacct tgacggtacc taaccagaaa gccacggcta
      481 actacgtgcc agcagccgcg gtaatacgta ggtggcaagc gttgtccgga attattgggc
      541 gtaaagggct cgcaggcggt ttcttaagtc tgatgtgaaa gccccgggct caaccgggga
      601 gggtcattgg aaactgggga acttgagtgc agaagaggag agtggaattc cacgtgtagc
      661 ggtgaaatgc gtagagatgt ggaggaacac cagtggcgaa cgcgactctc tggtctgtaa
      721 ctgacgctga gagcgaaagc gtggggagcg aacaggatta tataccctgg tagtccactc
      781 cgtaacgatg agtgctaagt gttagggggt ttccccccct tatgctgcag ctaaacgcat
      841 taagcactcc gcctgggggа gtacgttcca tagactgaaa ta
```

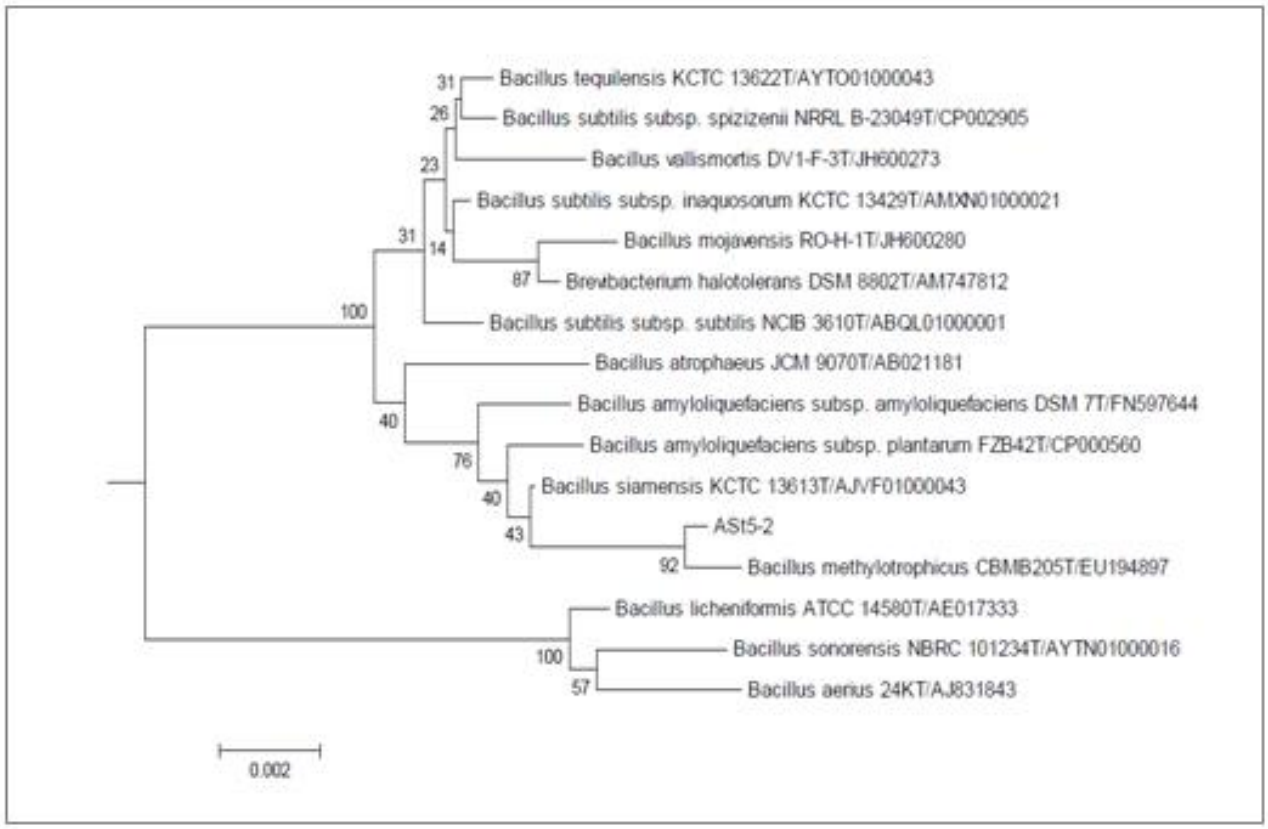


Figure 6. Phylogenetic analysis of 16S rRNA sequences of the bacterial isolate AST5-2 isolated from *A. aspera* L. The analysis was conducted with MEGA6 using neighbor-joining method.

The results of the BLAST search of the 16S rRNA gene sequences indicated AST5-2 isolate as closely related to *Bacillus substilis* JX286682 with only 96% similarity. However the phylogenetic analysis with the 16S rRNA similarity placed with *Bacillus methylotrophicus.* CMBM205T/EU194897 (Figure 6). AST5-2 was therefore identified as *B. methylotrophicus* however possibility of new species may not be rule out. Most of the *Bacillus* sp. were placed together in one mega clustered including AST5-2, *B. substilis*, *B. siamensis* being close to each other while *B. substilis* distantly placed in the same mega clustered. Three other species of Bacillus formed a separate small clustered.

The result for BLAST similarity sequence of 16s rRNA of AL2-14B, AL2-16, AL6-10 and AST5-2 were summarized in Table 5.

Table 5. Molecular characterization of the isolates

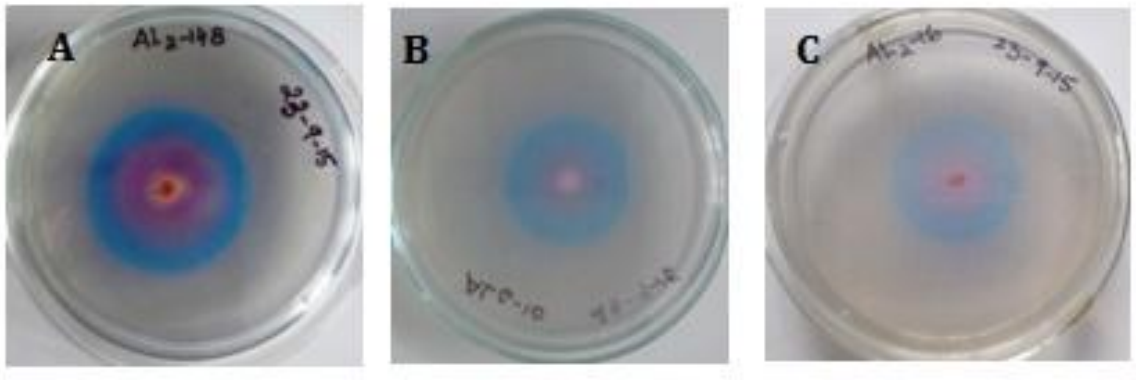
Isolates	Length[a]	Most closely related organism		
		Species (strains)	Accession no.	% Gene identity
AL2-14B	1200	*Pseudomonas aeruginosa* JF510526.1	KY087983.1	99%
AL2-16	957	*Serratia marcescens* KF938667.1	KY087982.1	98%
AL6-10	1200	*Serratia marcescens* KX852703.1	KY087984.1	97%
AST5-2	882	*Bacillus substilis.* JX286682.1	KY087985.1	96%

[a] Length: length of 16S rRNA gene sequenced.

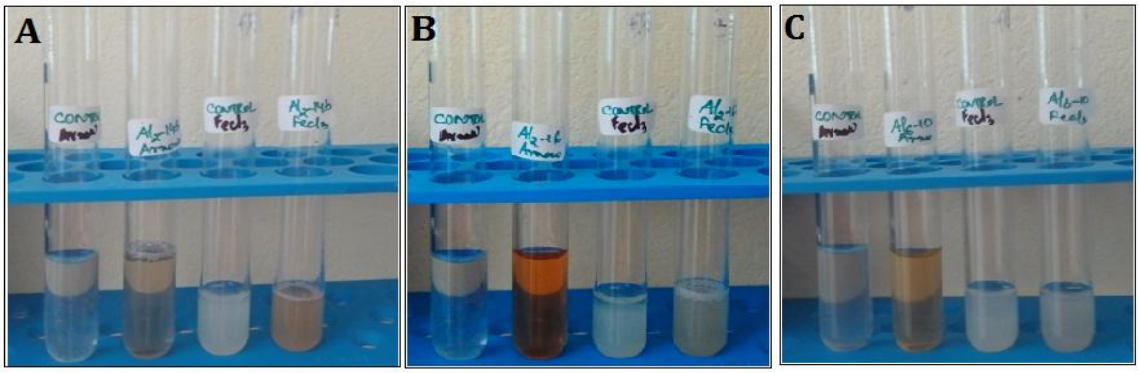
4.5. *In vitro* tests for plant growth promoting (PGP) traits by endophytes:

4.5.1. Siderophore production assay:

Out of the selected four isolates, three isolates *Pseudomonas aeruginosa* AL2-14B, *Serratia marcensens* AL2-16, *Serratia marcensens* AL6-10 were able to produce siderophore by forming orange halo zone on CAS agar medium after 24 hours of incubation. However, *Bacillus methylotrophicus* AST5-2 showed negative result (Photoplate 8).



Photoplate 8. **Siderophore production** A) Pink halozone shown by *P. aeruginosa* AL14B, B) Pink halozone shown by *S. marcescens* AL6-10, C) Pink halozone shown by *S. marcescens* AL2-16.



Photoplate 9.**Types of Siderophore** A) Catecholate and hydroxamate production by *P. aeruginosa* AL2-14B, B) Catecholate production by *S. marcescens* AL2-16, C) Catecholate production by *S. marcescens* AL6-10

Estimation of siderophore production in iron free succinate medium, was confirmed by the instant decolourization of CAS reagent from blue to orange. Siderophore production by isolates *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16, *S. marcescens* AL6-10 was quantified in succinate medium.

The siderophore production by *P. aeruginosa* AL2-14B was initiated after 24 hours of incubation during the growth phase. Synthesis of siderophore continued till the

end of stationary phase and was reduced with initiation of death phase. Siderophore production was found maximum (71.806%) at 72 hours with growth was maximum (Figure 7).

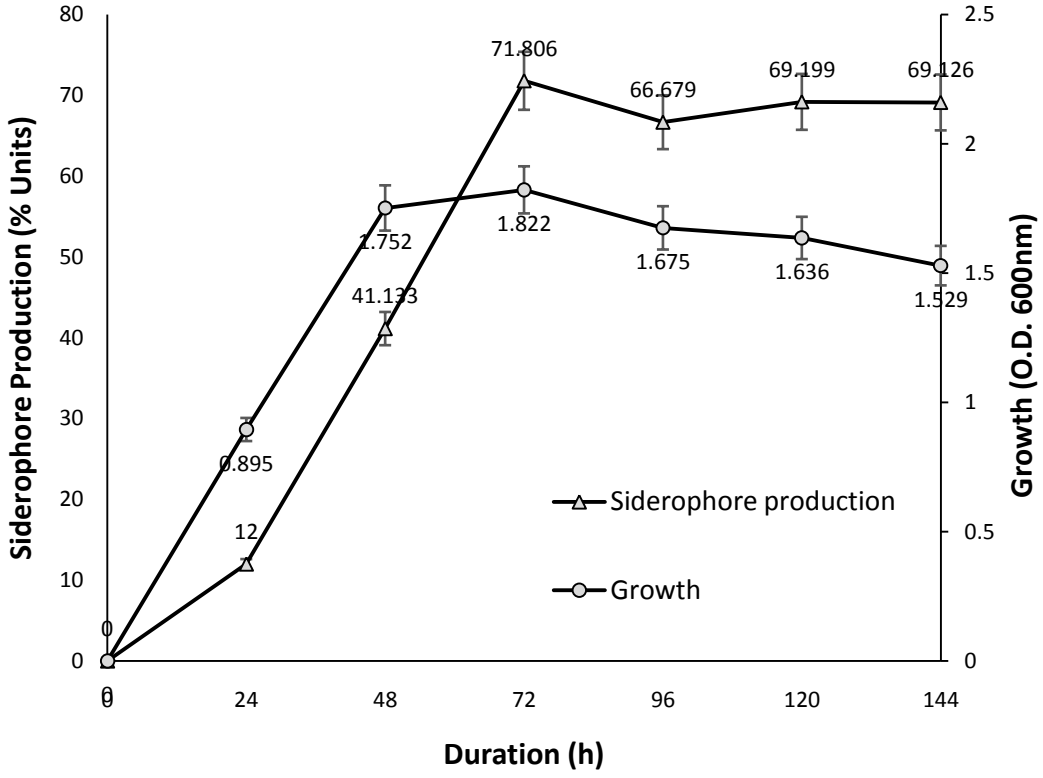


Figure 7. Quantitative estimation of siderophore release by *P. aeruginosa* AL2-14B against growth of bacteria.

S. marcescens AL2-16 produced maximum siderophore at 48 hours of incubation period with 74.931% siderophore production. It was evident that the siderophore production was high at late log phase, and amount of siderophore release was in accordance with the growth profile of isolates (Figure 8).

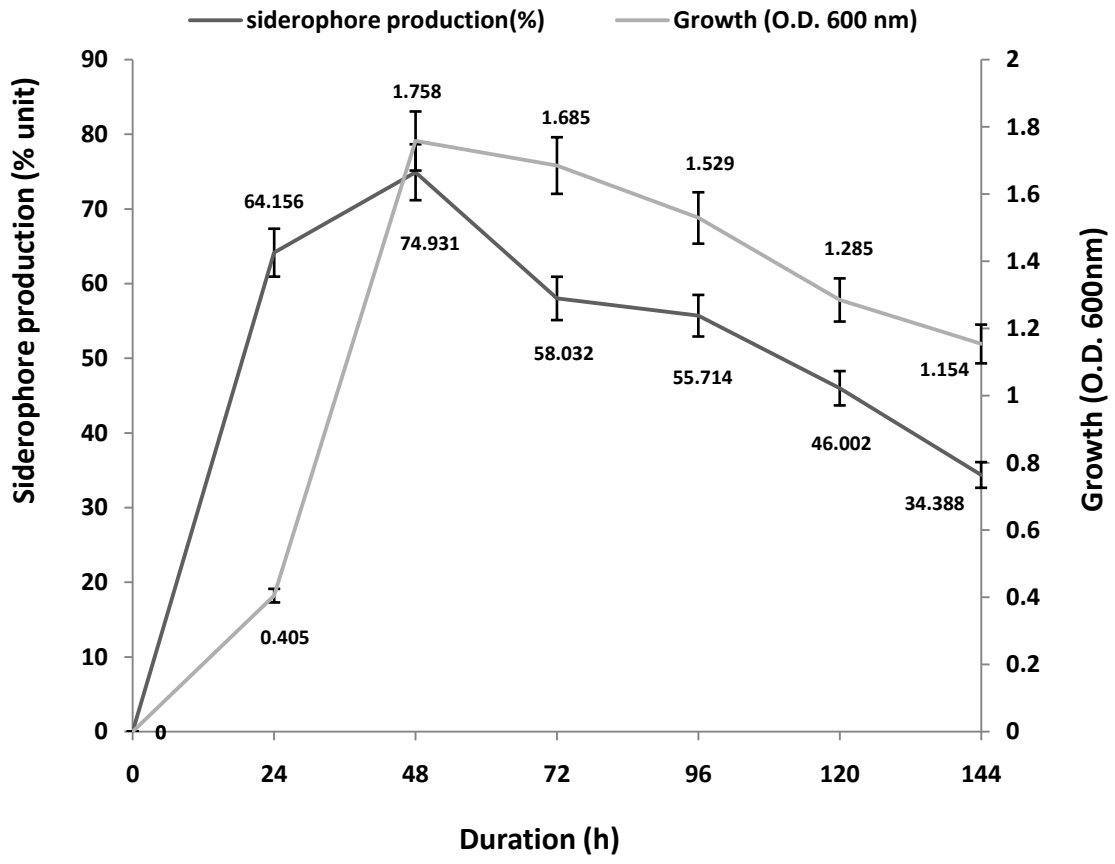


Figure 8. Quantitative estimation of siderophore release by *S. marcescens* AL2-16 against growth of bacteria.

S. marcensens AL6-10 also released maximum siderophore at 48 hours of incubation with 80.72% sierophore production. The growth of the isolate and its production of siderophore simultaneously increased till 48 hours and gradually decreased at same time (Figure 9).

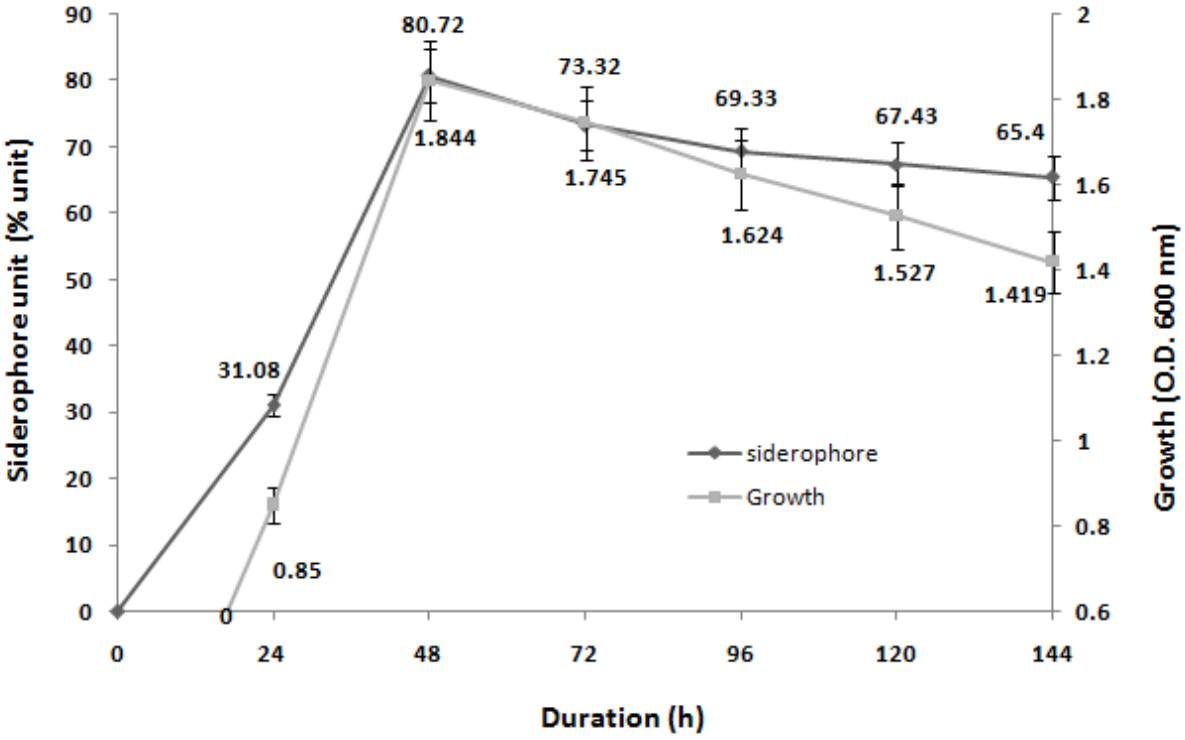


Figure 9. Quantitative estimation of siderophore release by *S. marcescens* AL6-10 against growth of bacteria.

Siderophore type determination:

When cell free supernatant of *P. aeruginosa* AL2-14B was subjected to Arnow' assay, it gave light pink which confirmed the presence of catecholate type of siderophore. And also it gave light orange colour in $FeCl_3$ test, which confirmed the presence of hydroxamate type of siderophore. Both *Serratia marcescens* AL2-16 and AL6-10 gave pink colour in Arnow's assay which indicated the presence of catecholate type of siderophore (Photoplate 9).

4.5.1.1 Effect of growth medium on siderophore release:

The effect of medium on siderophore production was studied in four different media i.e. Succinic medium (SM), Luria bertani broth (LB), Nutrient broth (NB) and

Yeast Extract Mannitol broth (YEMB). SM was found to be the best medium with maximum siderophore release for all the three isolates (*P. aeruginosa* AL2-14B-71.806%, *S. marcescens* AL2-16-74.931%, *S. marcescens* AL6-10-80.720%). Based on these results, SM was selected as the base medium for subsequent experiments (Figure 10, Figure 11, Figure 12).

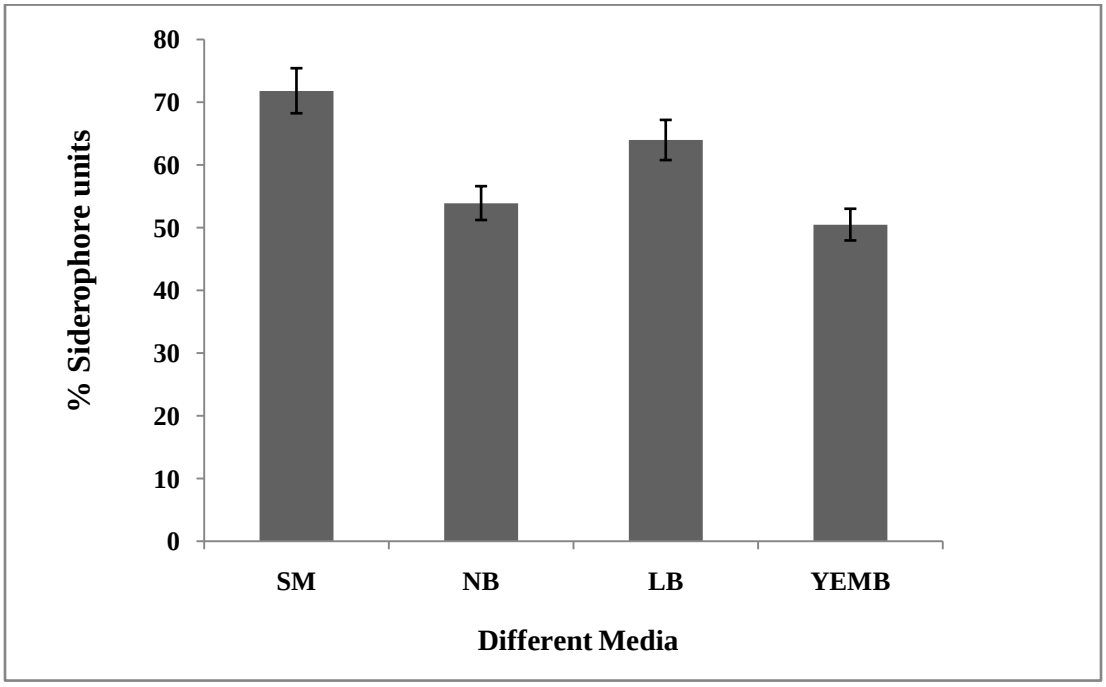


Figure 10. Effect of media composition on Siderophore production by *P. aeruginosa* AL2-14B.

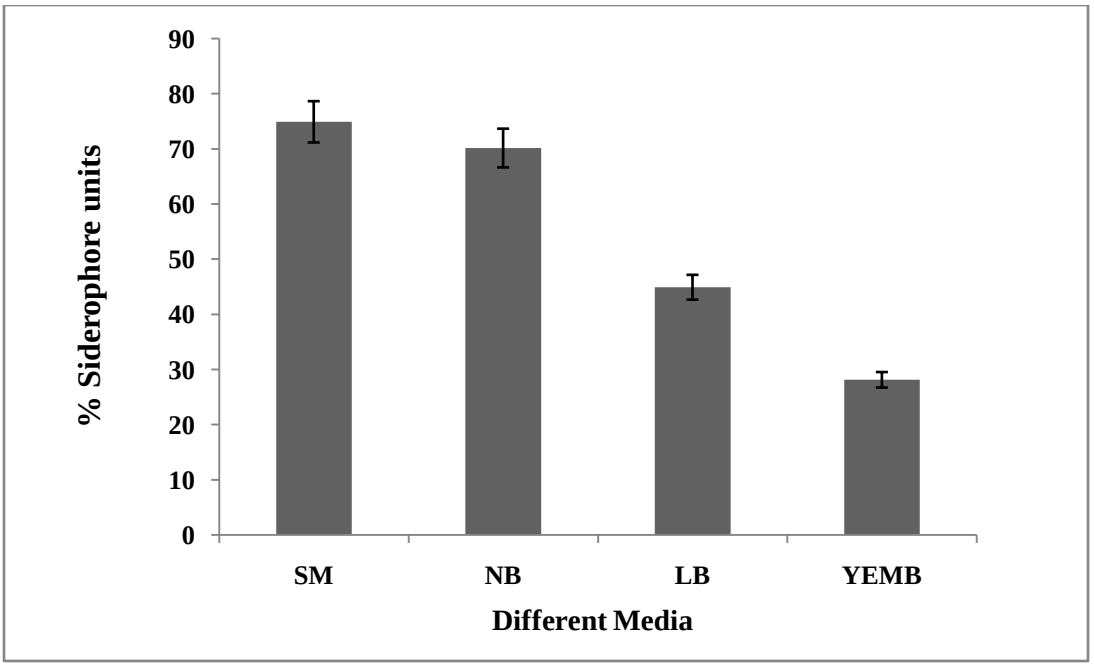


Figure 11. Effect of media composition on Siderophore production by *S. marcescens* AL2-16

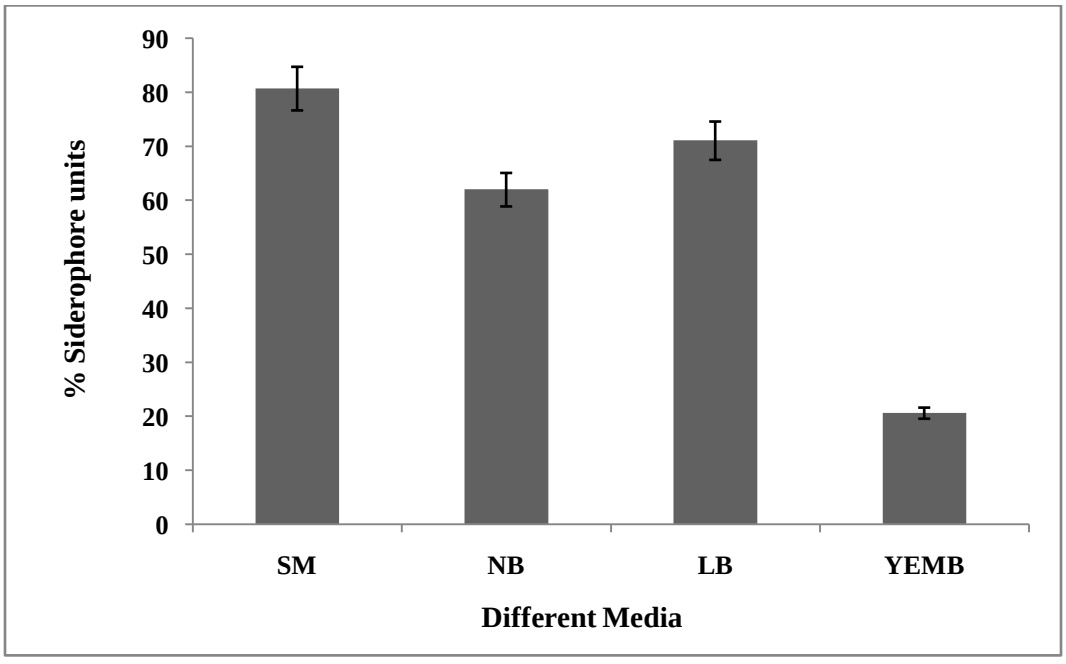


Figure 12. Influence of media composition on Siderophore production by *S. marcescens* AL6-10.

4.5.1.2 Effect of Carbon Sources, Nitrogen Sources and Organic acids on Siderophore production

Isolates were grown in Succinic medium supplemented with different carbon sources - glucose, sucrose, mannitol, fructose and maltose one at a time. Fructose was proved to be the best carbon sources resulting in appreciable amount of siderophore production in all the three isolates. Fructose followed by sucrose and mannitol were proved to be the best carbon sources resulting in appreciable amount of siderophore production i.e. 87.975%, 84.568%, 72.534% by AL2-14B (Figure 13). Similarly, AL2-16 gave good amount of siderophore production (fructose-77.223%, glucose-73.584%, sucrose-65.363 %) in different carbon source (Figure 14). AL6-10 also resulted in good amount of siderophore unit in different carbon sources (fructose-87.735%, glucose-85.309%, maltose-52.547%) (Figure 15).

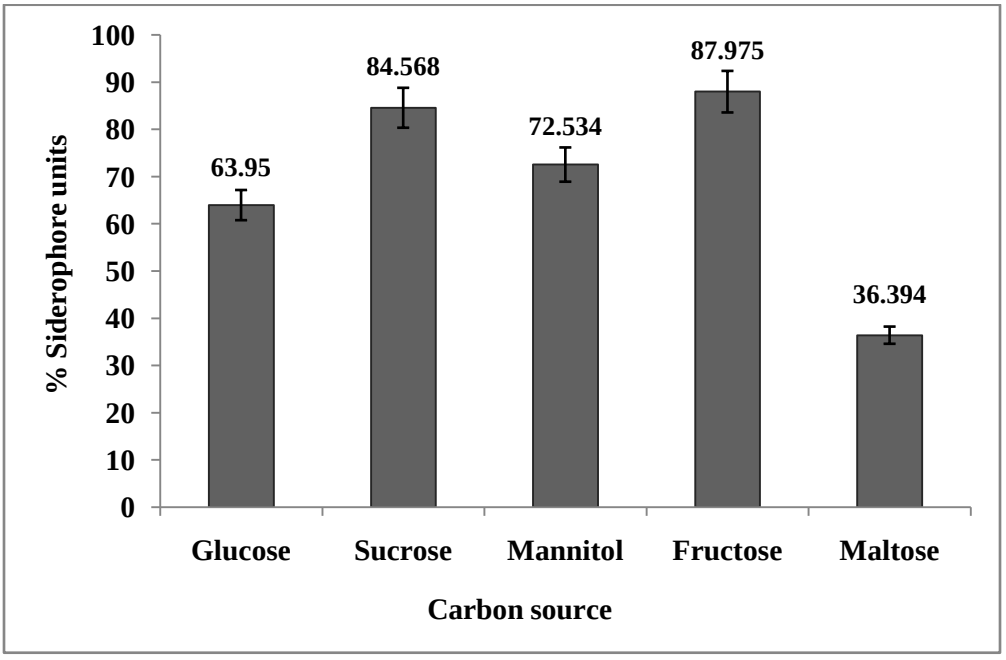


Figure 13. Effect of different carbon source on siderophore production by *P. aeruginosa* AL2-14B.

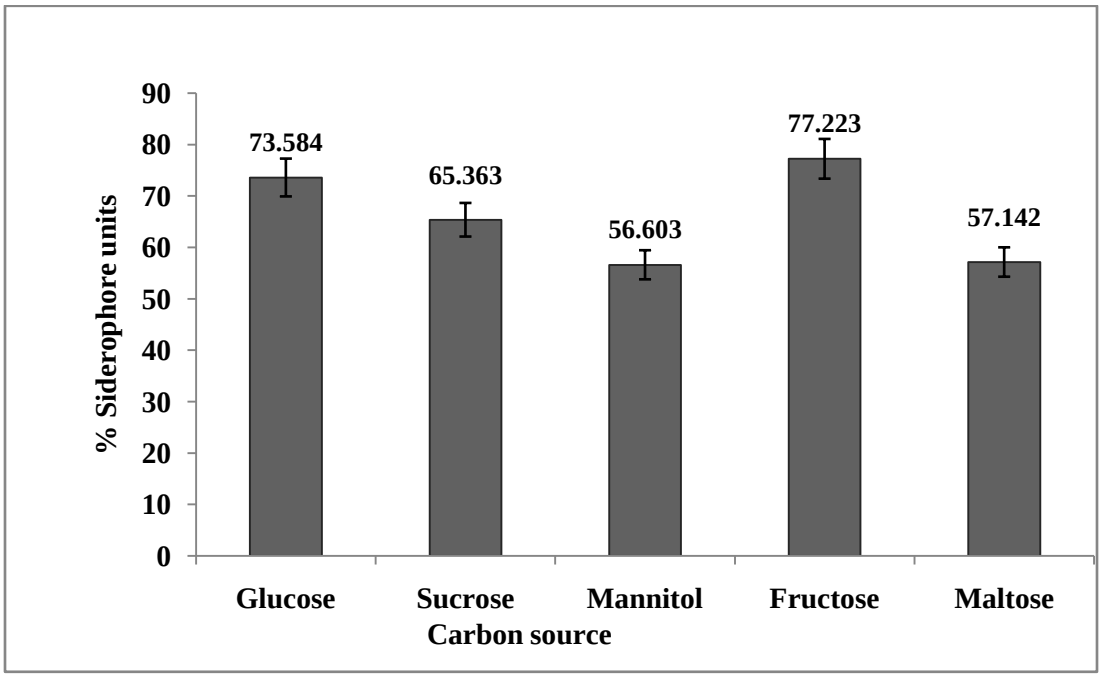


Figure 14. Effect of different carbon source on siderophore production by *S. marcescens* AL2-16.

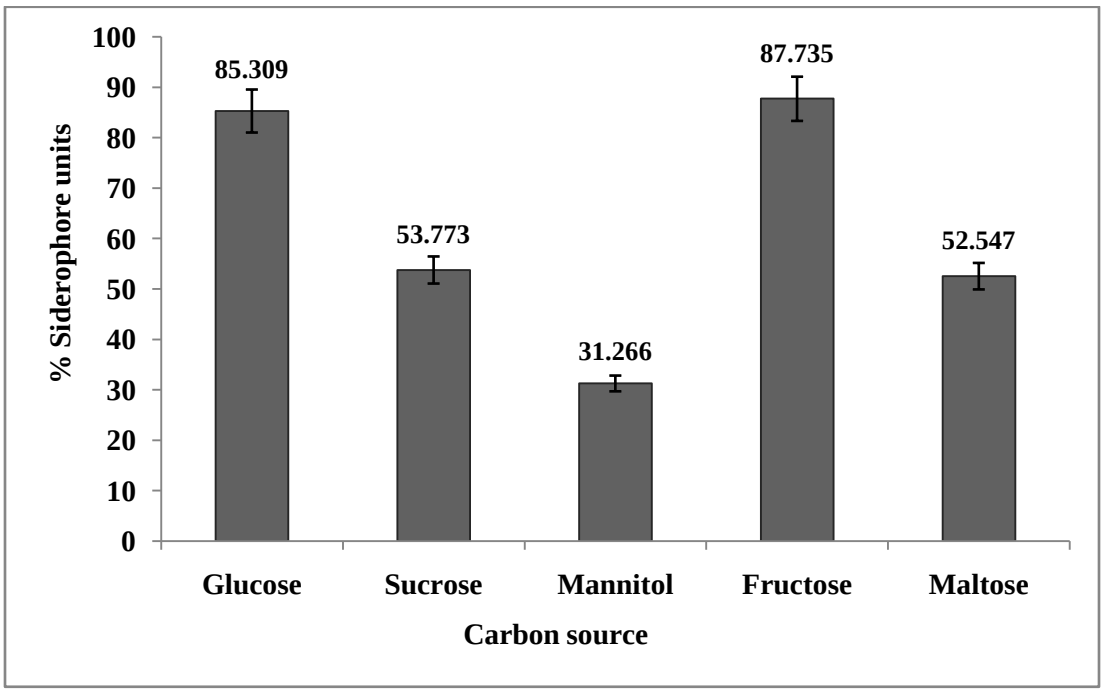


Figure 15. Effect of different carbon source on siderophore production by *S. marcescens* AL6-10.

Growth and siderophore biosynthesis were studied after replacement of the nitrogen source ammonium sulfate in succinate medium by urea and sodium nitrate. Amongst nitrogen sources, urea proved to be the best utilizable nitrogen source for maximum siderophore peoduction by *P. aeruginosa* AL2-14B (Figure 16) whereas succinic acid was best nitrogen source for both *S. marcescens* AL2-16 (Figure 17) and *S. marcescens* AL6-10 (Figure 18).

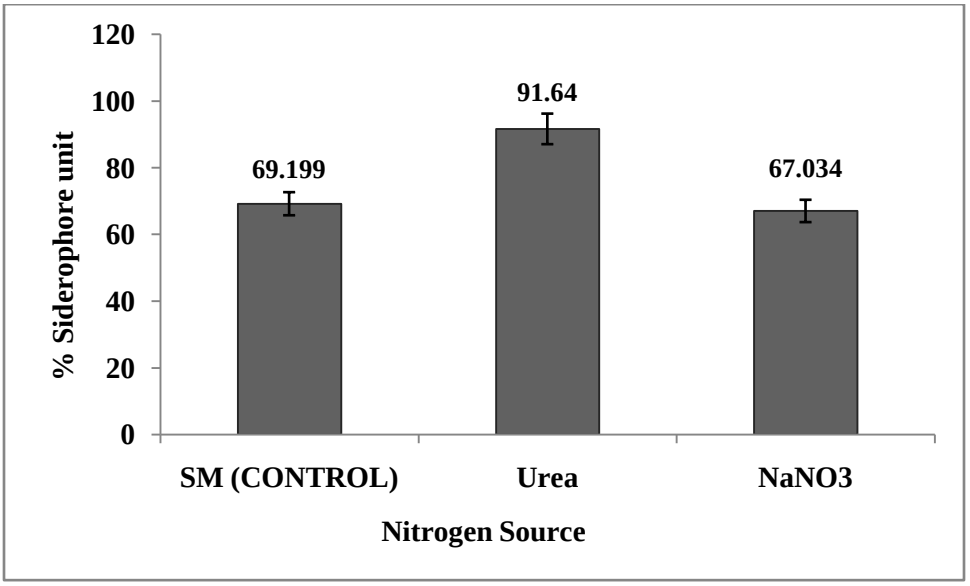


Figure 16. Effect of nitrogen sources on siderophore production by *P. aeruginosa* AL2-14B.

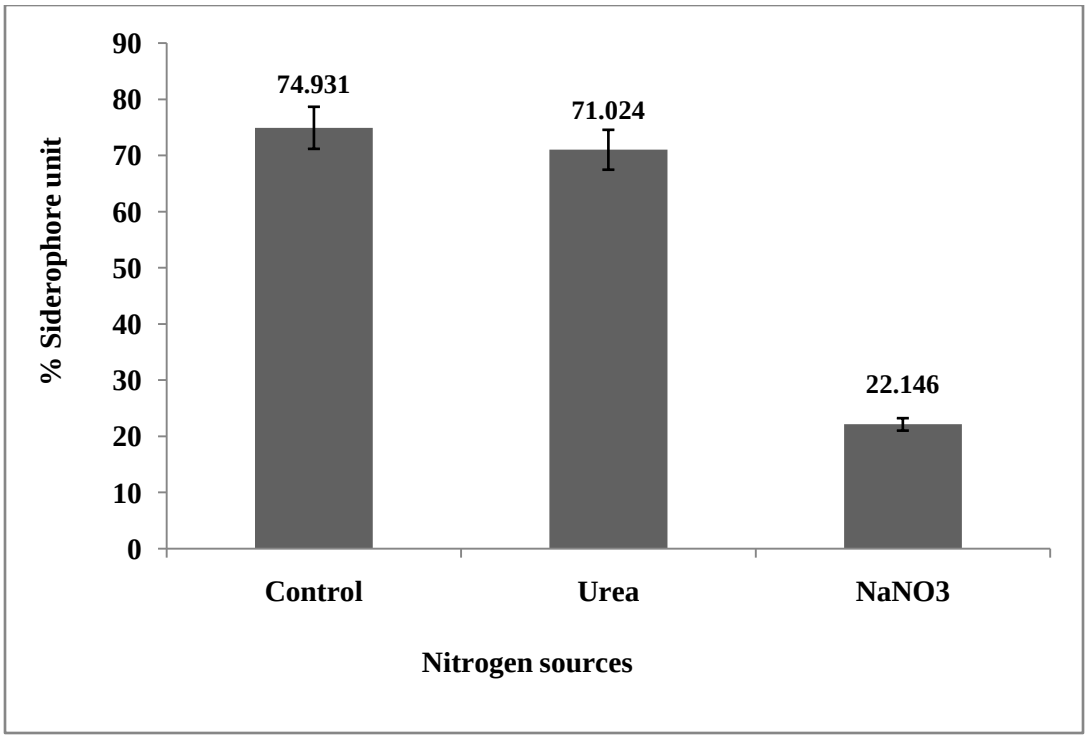


Figure 17. Effect of nitrogen sources on siderophore production by *S. marcescens* AL2-16.

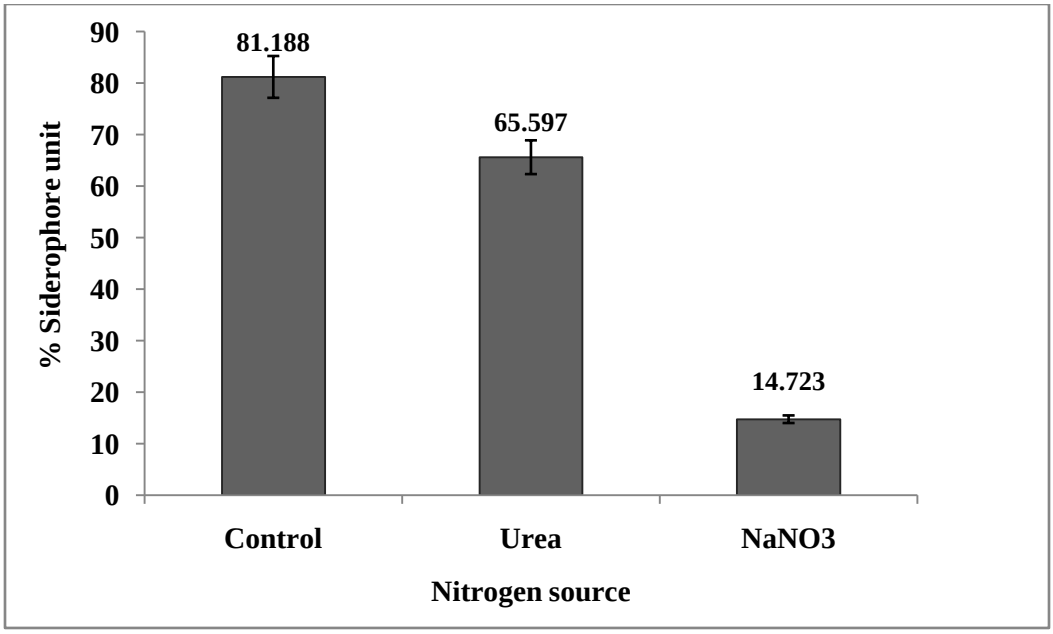


Figure 18. Effect of nitrogen sources on siderophore production by *S. marcescens* AL6-10.

Further, amendment of SM with different organic acids resulted in wide variation in siderophore production. Malate was proved to be the best organic acid source resulting in appreciable amount of siderophore production (81.683%) by *P. aeruginosa* AL2-14B, followed by citrate and oxalate (Figure 19). In case of *S. marcensens* AL2-16, oxalate showed highest siderophore production, followed by citrate and malate (Figure 20). Whereas, in AL6-10, control (SM) served to be the best production of siderophore among other orgaic acids (malate, oxalate and citrate) (Figure 21).

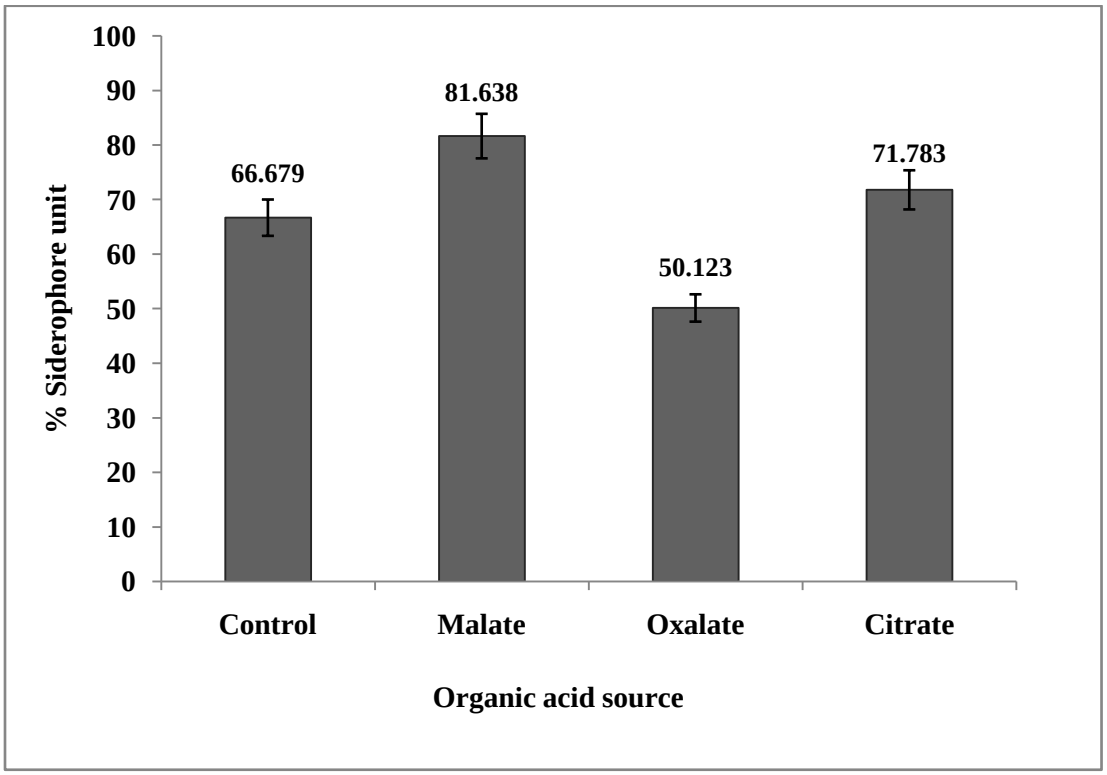


Figure 19. Effect of different organic acids sources on siderophore production by *P. aeruginosa* Al2-14B

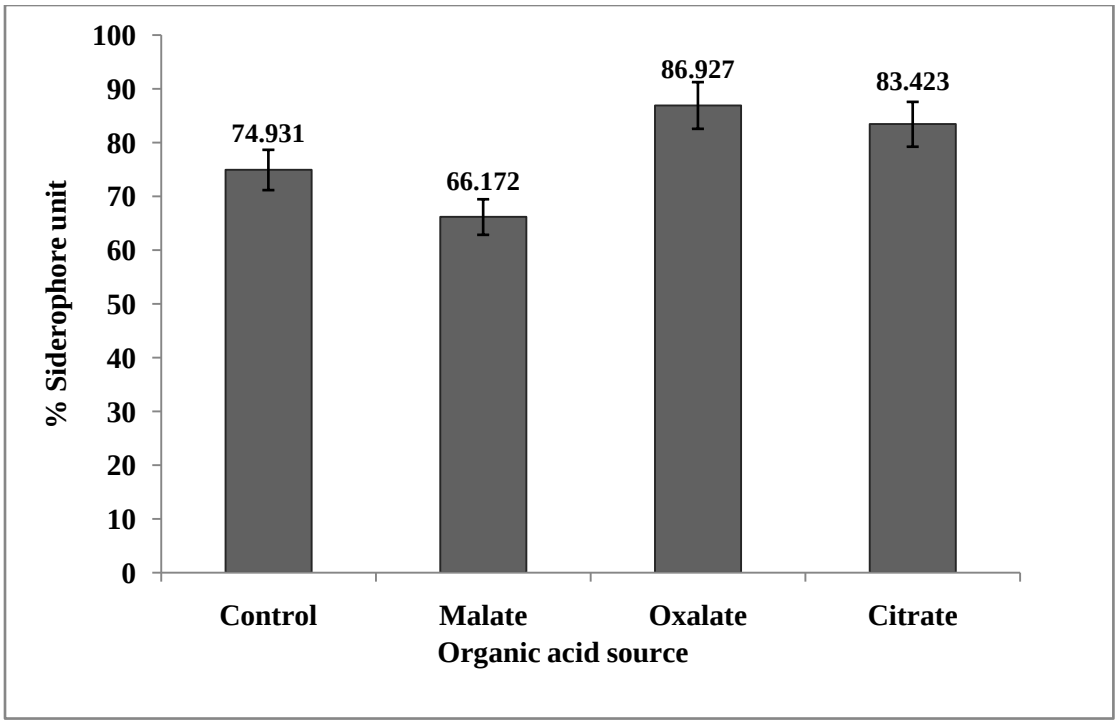


Figure 20. Effect of different organic acids sources on siderophore production by *S. marcescens* AL2-16.

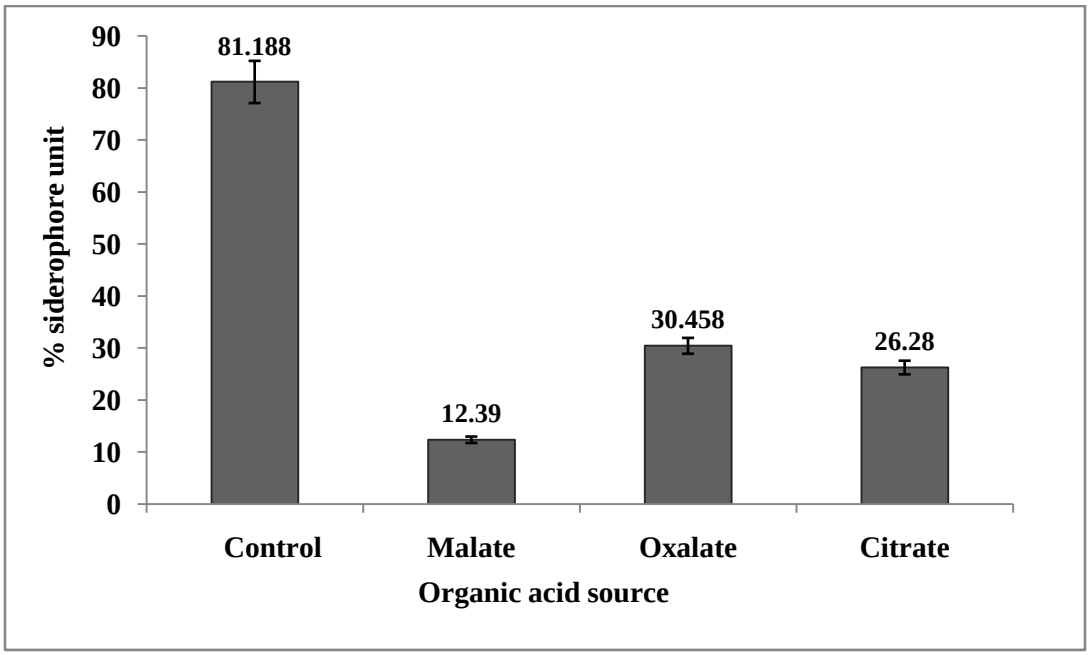


Figure 21. Effect of different organic acids sources on siderophore production by *S. marcescens* AL6-10.

4.5.1.3. Effect of iron on siderophore release:

Siderophore production was considerably affected by the presence of iron in medium. Initial increase in iron concentration induced siderophore production. Increase in iron concentration resulted in successive decrease of siderophore production. The level of iron in succinate medium (SM) at which siderophore synthesis was determined by growing test culture in SM supplemented with iron as ferric chloride within the range of 0-30µM and growth along siderophore production was measured. Maximum siderophore production was observed at the concentration of 1µM of iron by *P. aeruginosa* AL2-14B (Figure 22) and *S. marcescens* AL2-16 (Figure 23) with the siderophore production of 70.007% and 83.483% respectively. However *S. marcescens* AL6-10 showed its maximum production of siderophore (81.188%) at 0 µM of iron concentration (Figure 24).

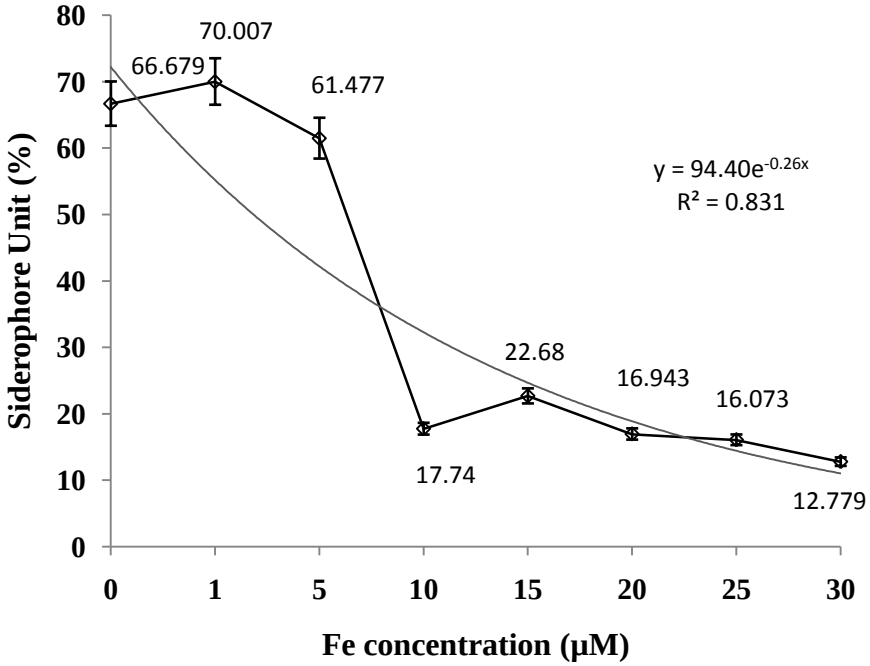


Figure 22. Effect of iron concentration on siderophore production by *P. aeruginosa* AL2-14B

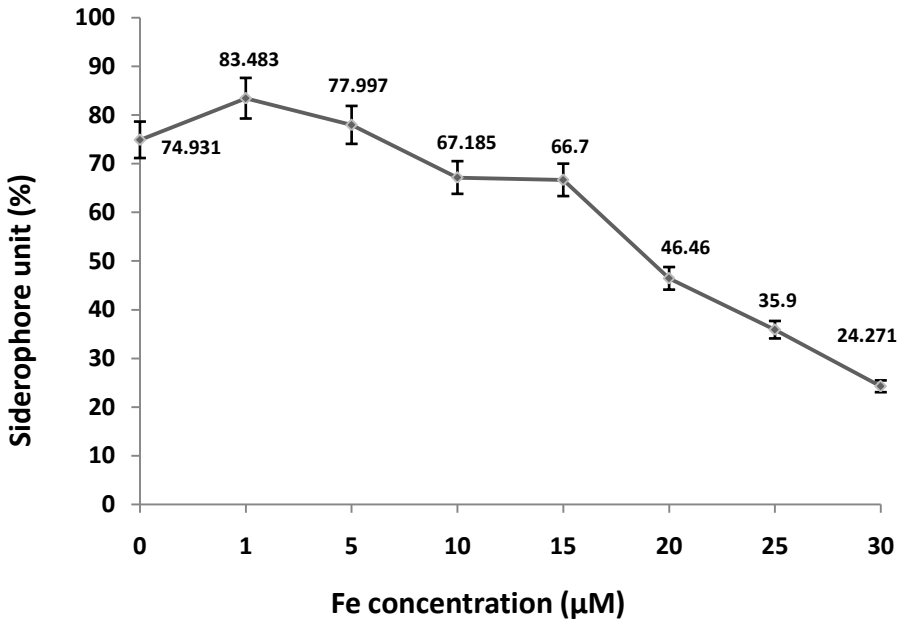


Figure 23. Effect of iron concentration on siderophore production by *S. marcescens* AL2-16.

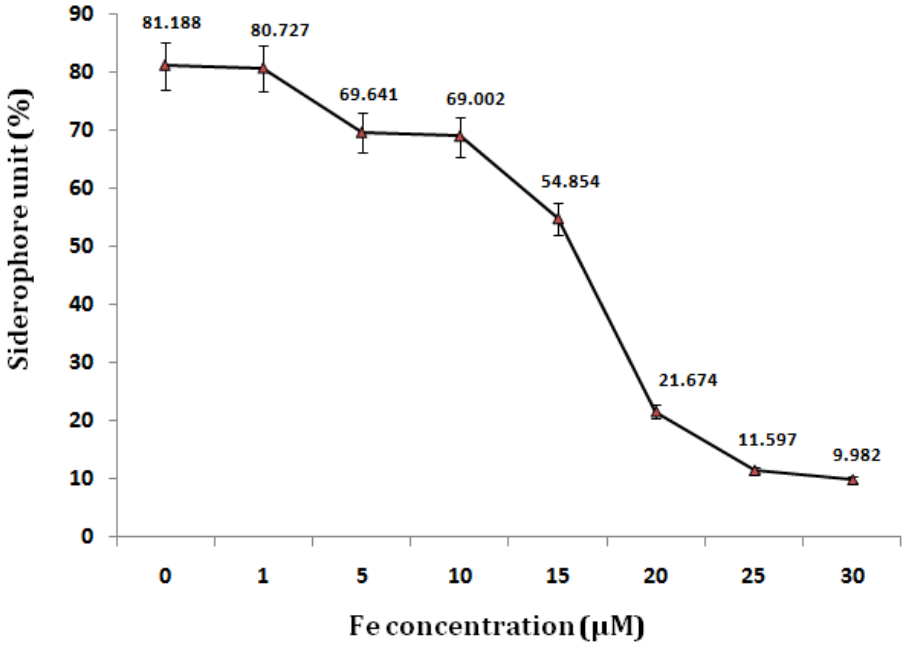


Figure 24. Effect of iron concentration on siderophore production by *S. marcescens* AL6-10.

4.5.2. IAA production assay:

Production of plant growth regulator (Indole Acetic Acid) by PGPB plays a direct role in stimulation of plant growth. IAA production by selected isolates, *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2 were studied by growing the isolates in YEM broth supplemented with different concentration of L-Tryptophan (0%, 0.2%, 0.4%, 0.6%, 0.8 and 1.0%). The effect of incubation period on IAA production was studied by withdrawing the samples from the production media at every 24 h upto 168 h. IAA production was observed to be in correlation to growth, and maximum IAA production was observed at 96 h (114.79 µg/ml) by *P. aeruginosa* AL2-14B (Figure 26) and *S. marcescens* AL6-10 (107.56 µg/ml) (Figure 30). In case of *S. marcescens* AL2-16, IAA production was found maximum at 144 h with the production of 133.2 µg/ml (Figure 28), however *B. methylotrophicus* AST5-2 showed its maximum production of IAA (102.26 µg/ml) at 48 h of incubation (Figure 31). The IAA production were in the range of 6.64 µg/ml – 114.79 µg/ml for *P. aeruginosa* AL2-14B, 0.8µg/ml – 133.2µg/ml for *S. marcescens* AL2-16, 1.54 µg/ml-107.56 µg/ml for *S. marcescens* AL6-10 and 1.56 µg/mL-102.26 µg/ml in case of *B. methylotrophicus* AST5-2. 1.0 % concentration of L-Tryptophan was found to be optimum for IAA production by all the isolates except AST5-2 where it was found to be maximum at 0.6% L-Tryptophan. IAA production decreased at higher concentrations of tryptophan.

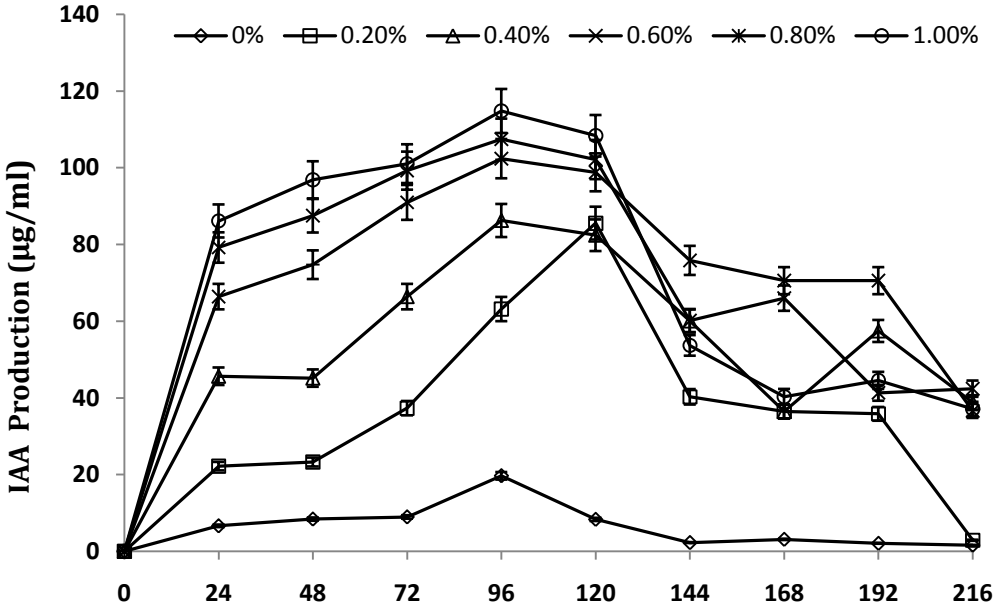


Figure 25. Quantitative estimation of IAA produced by *P. aeruginosa* AL2-14B at different L-tryptophan concentrations.

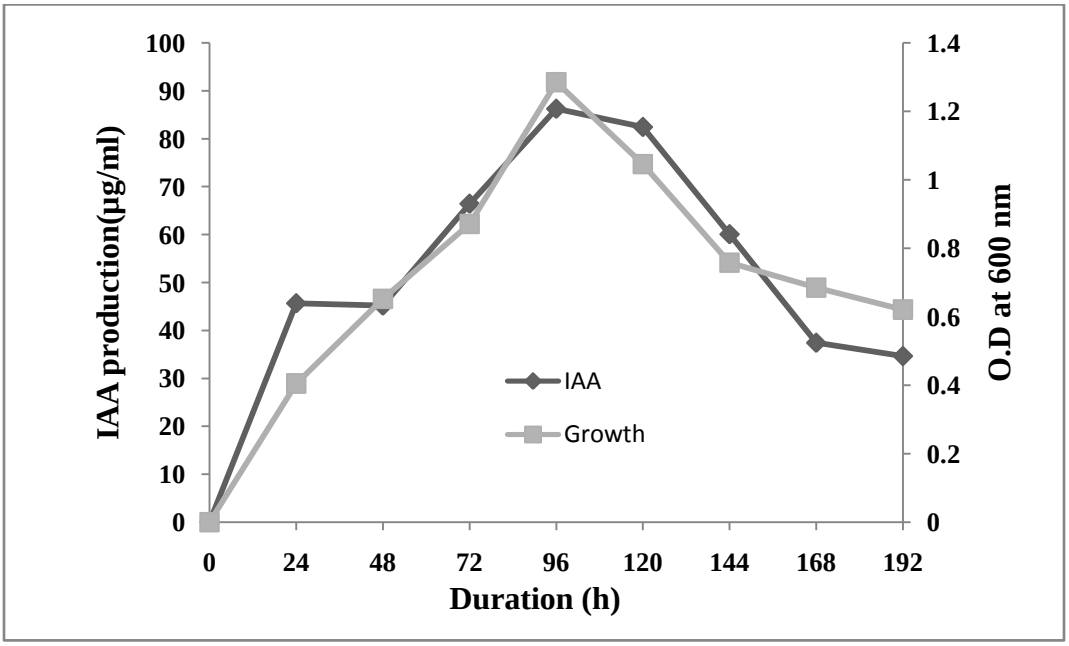


Figure 26. IAA production profile of *P. aeruginosa* AL2-14B.

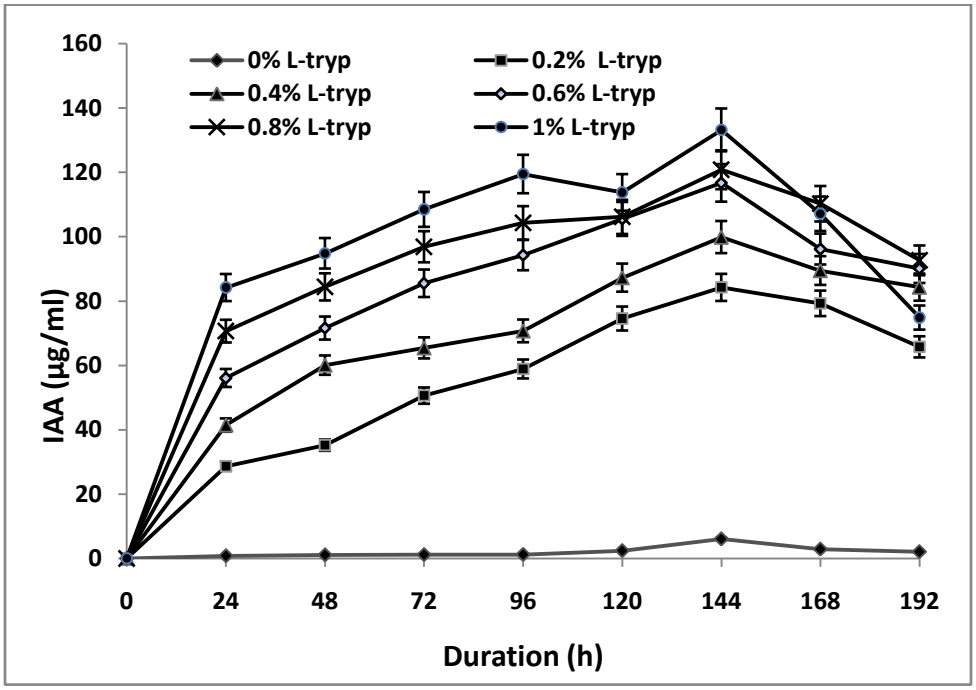


Figure 27. Quantitative estimation of IAA produced by *S. marcescens* AL2-16 at different L-tryptophan concentrations.

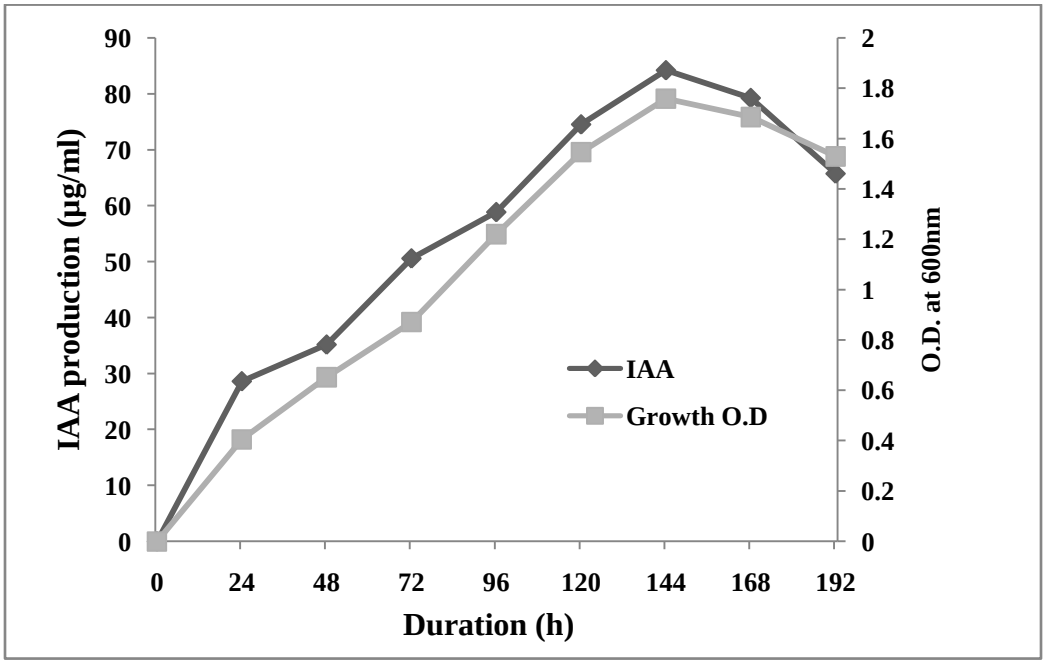


Figure 28. IAA production profile of *S. marcescens* AL2-16.

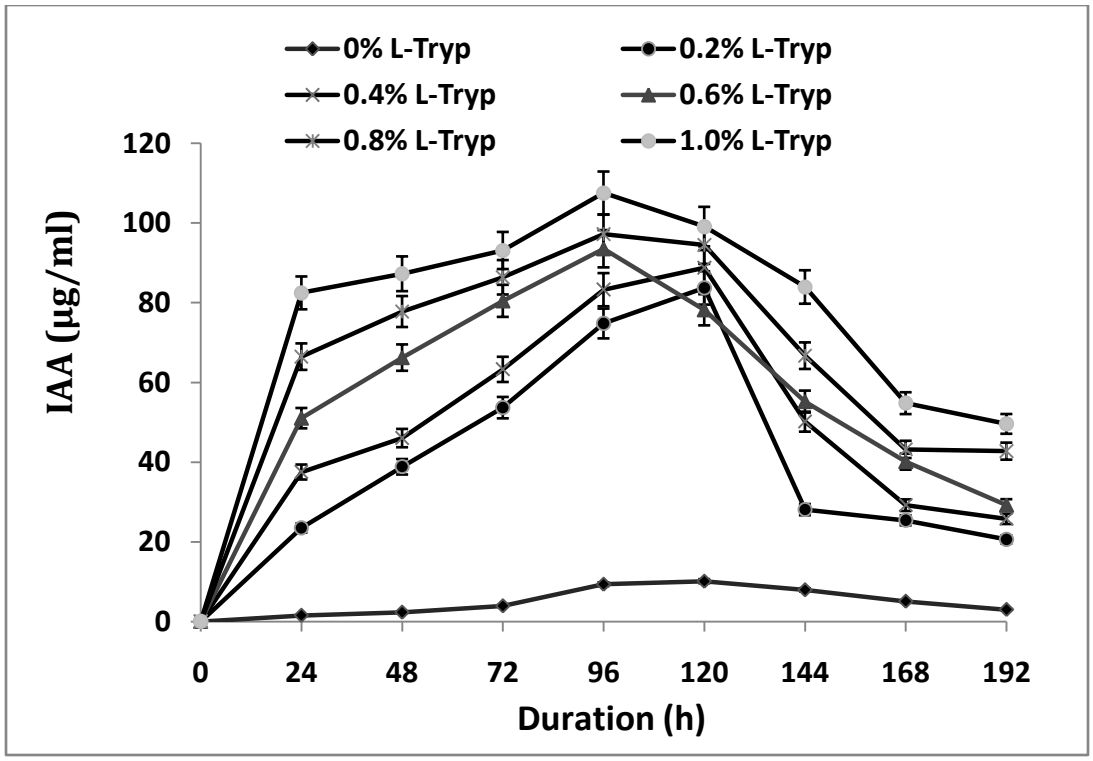


Figure 29. Quantitative estimation of IAA produced by *S. marcescens* AL6-10 at different L-tryptophan concentrations.

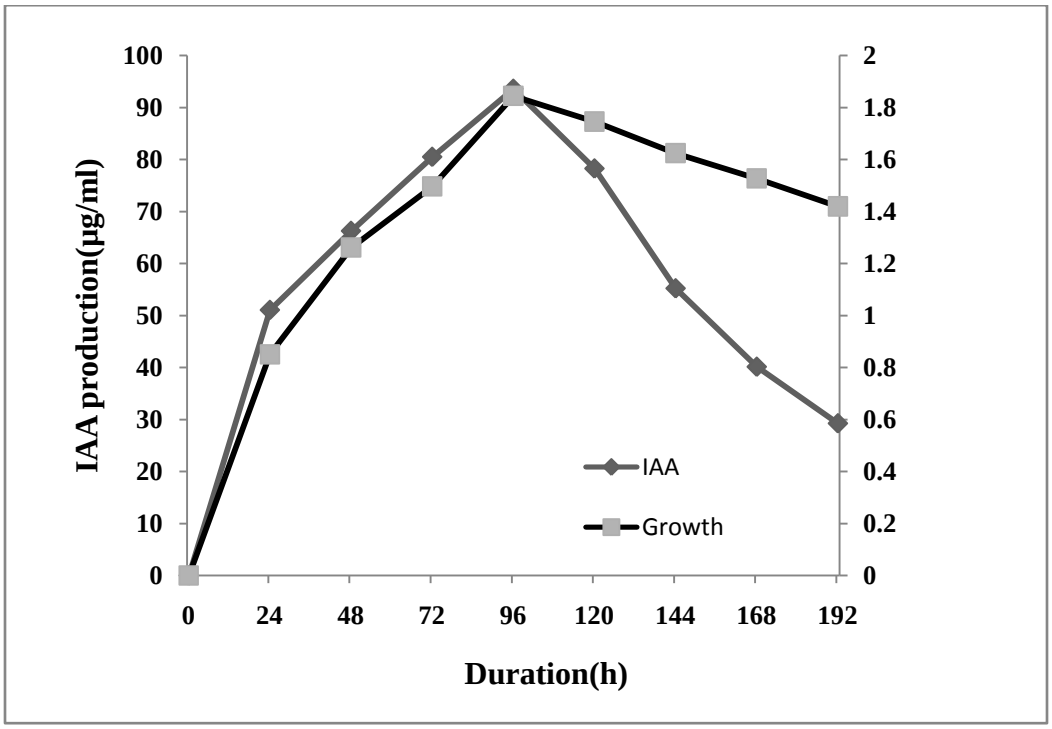


Figure 30. IAA production profile of *S. marcensens* AL6-10.

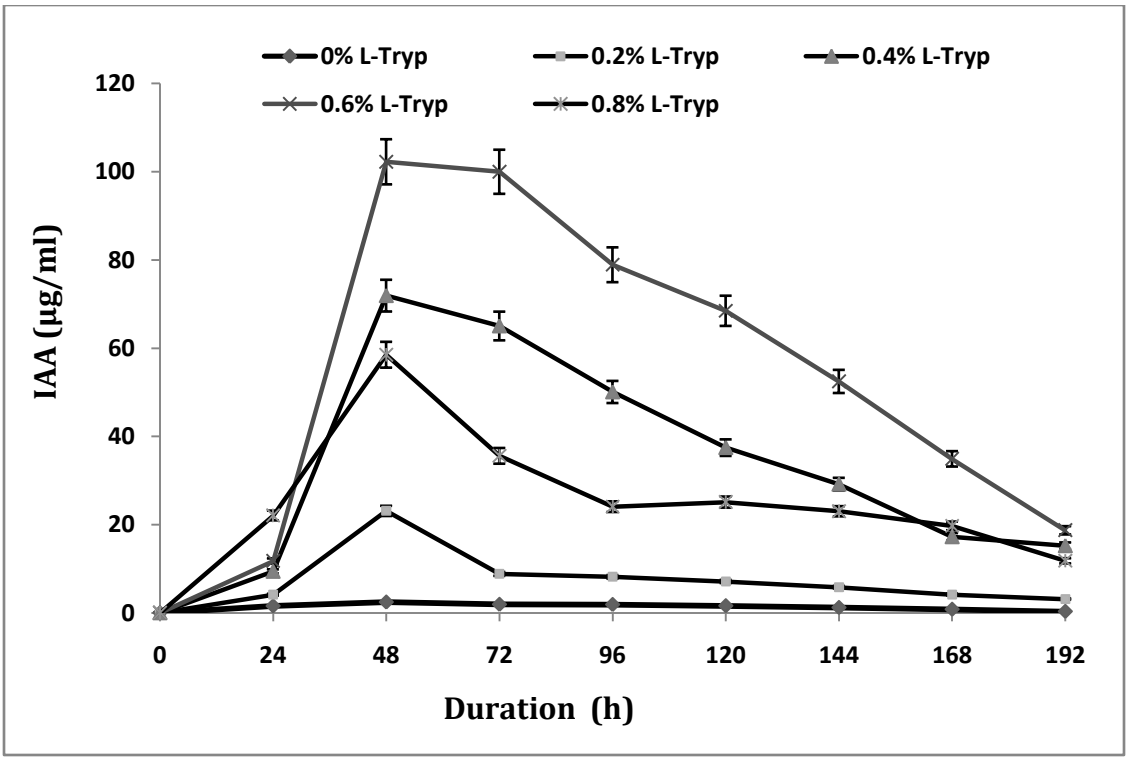


Figure 31. Quantitative estimation of IAA produced by *B. methylotrophicus* AST5-2 at different L-tryptophan concentrations.

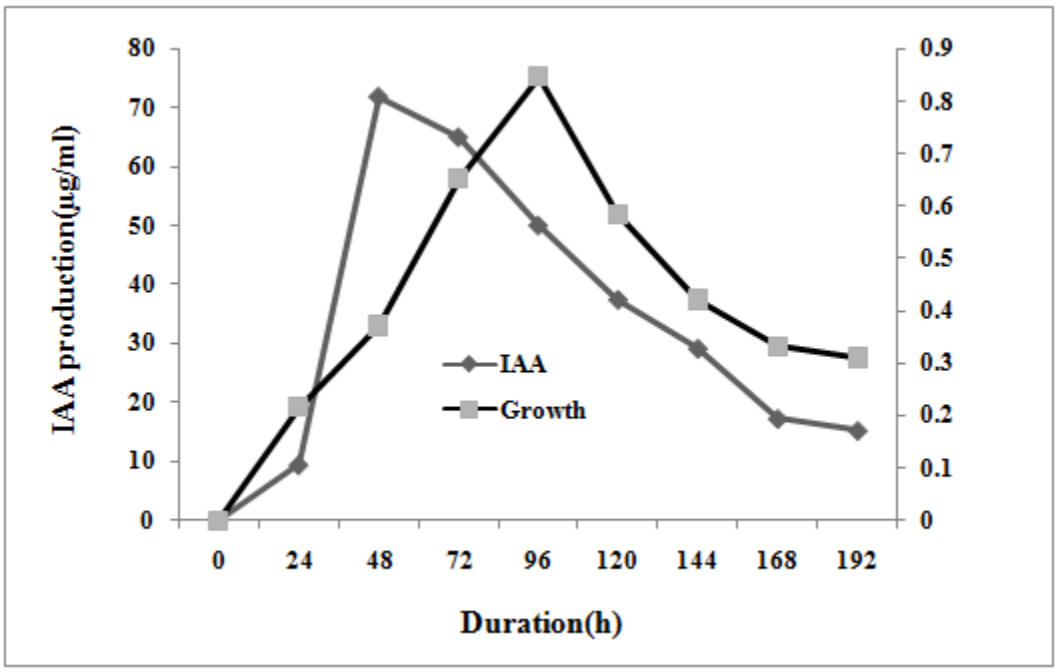
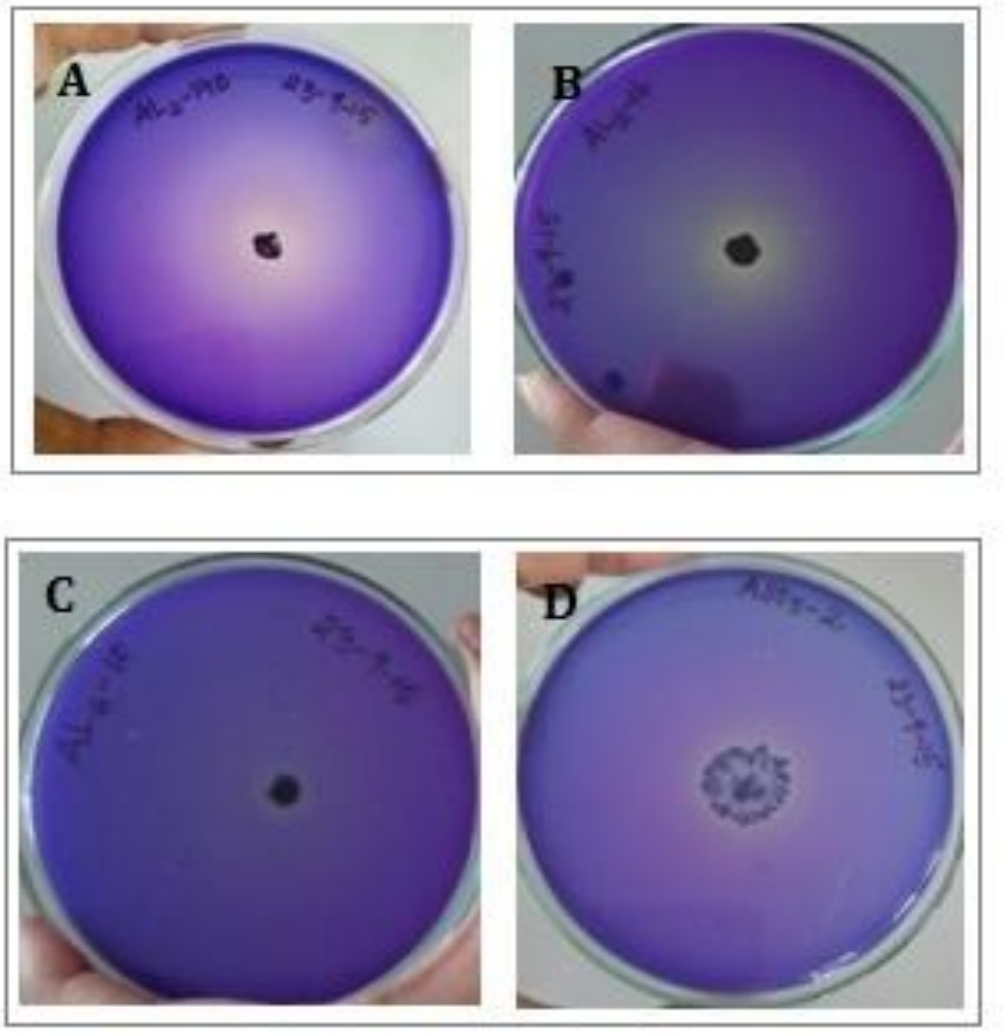


Figure 32. IAA production profile of *B.methylotrophicus* AST5-2

4.5.3. Phosphate solubilisation:

The selected isolates solubilized tri calcium phosphate in Pikovskaya's agar, forming a clear halo around the colony after 48 h of incubation. The Phosphate Solubilization Index (PSI) of *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2 were found to be 7 mm, 4.6mm, 2.3 mm and 2.7 mm respectively (Photoplate 10).



Photoplate 10: **Phosphate solubilization** on Pikovskaya agar medium
A) *P. aeruginosa* AL2- 14B; B) *S. marcescens* AL2-16; C) *S. marcescens* AL6-10
D) *B. methylotrophicus* AST5-2

Quantitative analysis for phosphate solubilization was carried in Pikovskaya broth and the p^H of the liquid broth was also recorded simultaneously. The P-solubilization initiated after 24 hours of incubation, reached its maximum at 144 hours (383 µg/ml) and keep on decreased by *P. aeruginosa* AL2-14B. The range of soluble P-released by *P. aeruginosa* AL2-14B was found between 200- 383 µg/ml, where the p^H decreased from 7 to 3.9 (Figure 33).

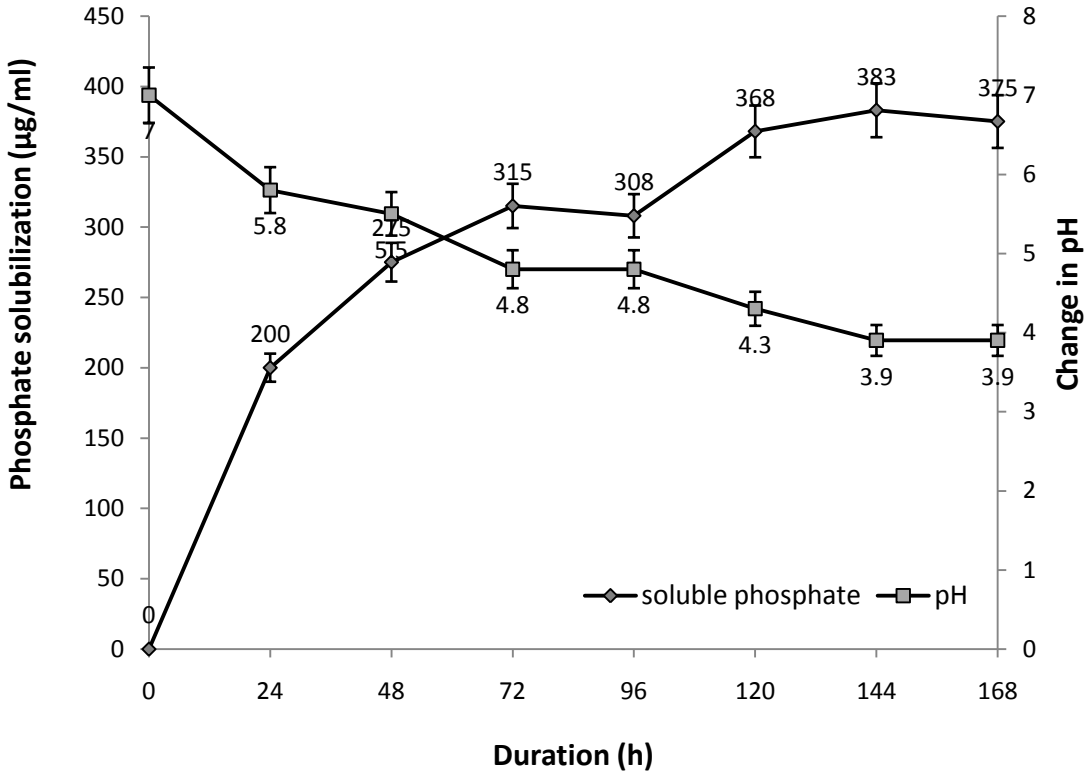


Figure 33. Phosphate solubilization by *P. aeruginosa* AL2-14B after different time intervals. Soluble free phosphate concentration is given against primary Y axis, while variation of pH in the culture medium is given at secondary Y axis. Standard deviation showed as bars.

S. marcescens AL2-16 showed its maximum released of free P at 72 hours of incubation (259 µg/ml) at p^H 4.9. The soluble P was found to be in range of 96 to 259 µg/ml, however, the p^H of the broth drop to 4.9 from the initial value of p^H 7.

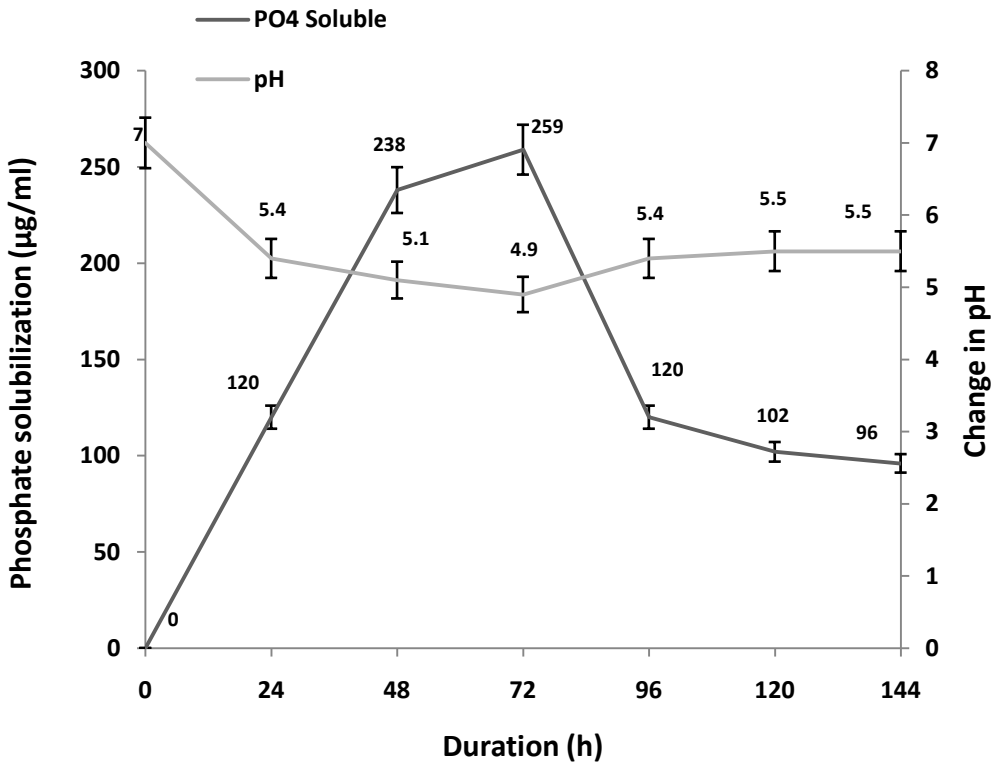


Figure 34. Phosphate solubilization by *S. marcescens* AL2-16 after different time intervals. Soluble free phosphate concentration is given against primary Y axis, while variation of pH in the culture medium is given at secondary Y axis. Standard deviation showed as bars.

Similarly, *S. marcescens* AL6-10 facilitated maximum released of soluble phosphate at 72 hours of incubation (353 µg/ml) at p^H 4.3. The range was found from 106 to 353 µg/ml. Notably, lowering of p^H of the culture broth was found to be correlated with better P solubilization in *S. marcescens* AL6-10 (Figure 35).

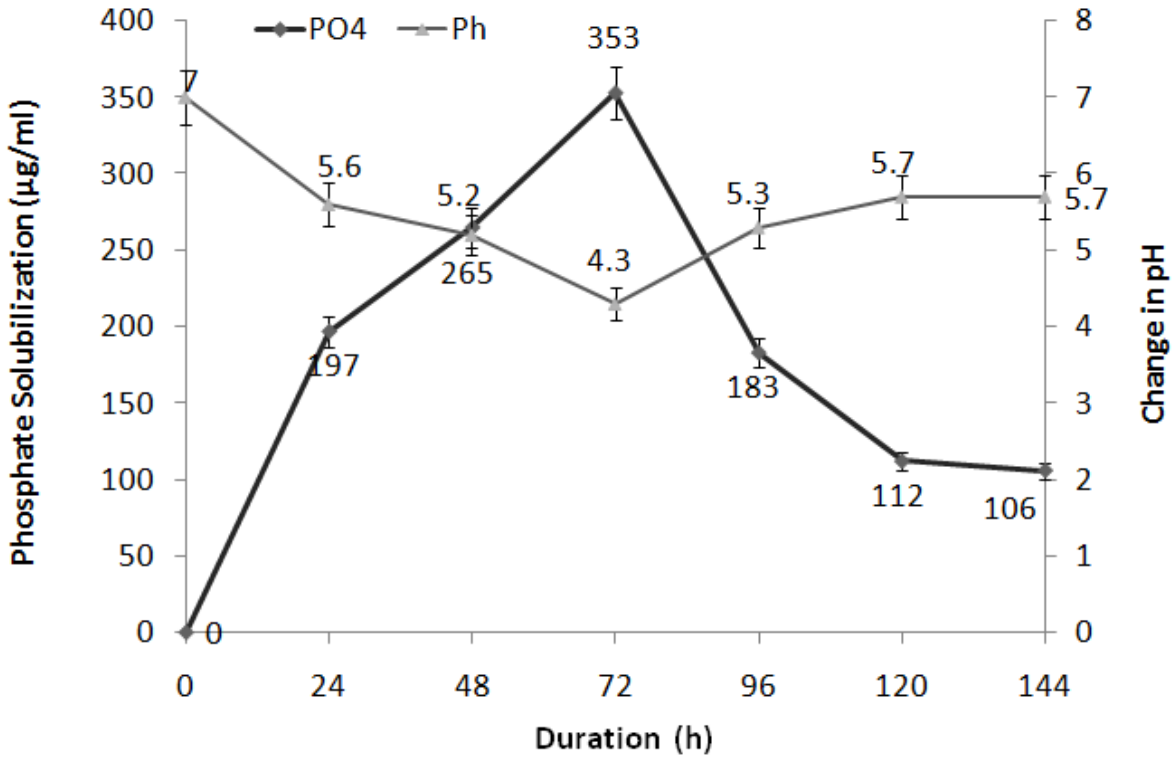


Figure 35. Phosphate solubilization by *S. marcescens* AL6-10 after different time intervals. Soluble free phosphate concentration is given against primary Y axis, while variation of pH in the culture medium is given at secondary Y axis. Standard deviation showed as bars.

The isolate *B. methylotrophicus* AST5-2 released 149 µg/ml of soluble phosphate after 48 hours of incubation. The maximum P-solubilization was found at p^H of 5.2. The range of P solubilized was from 80 to 149 µg/ml, with the decrased of pH from initial the p^H of 7 (Figure 36).

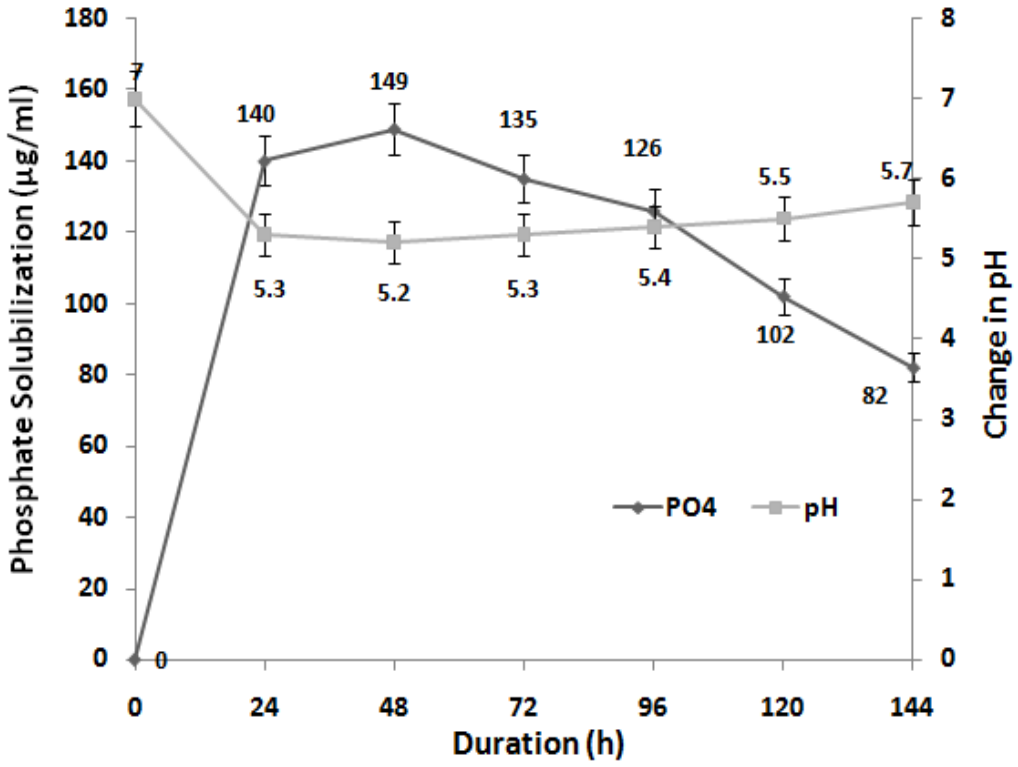
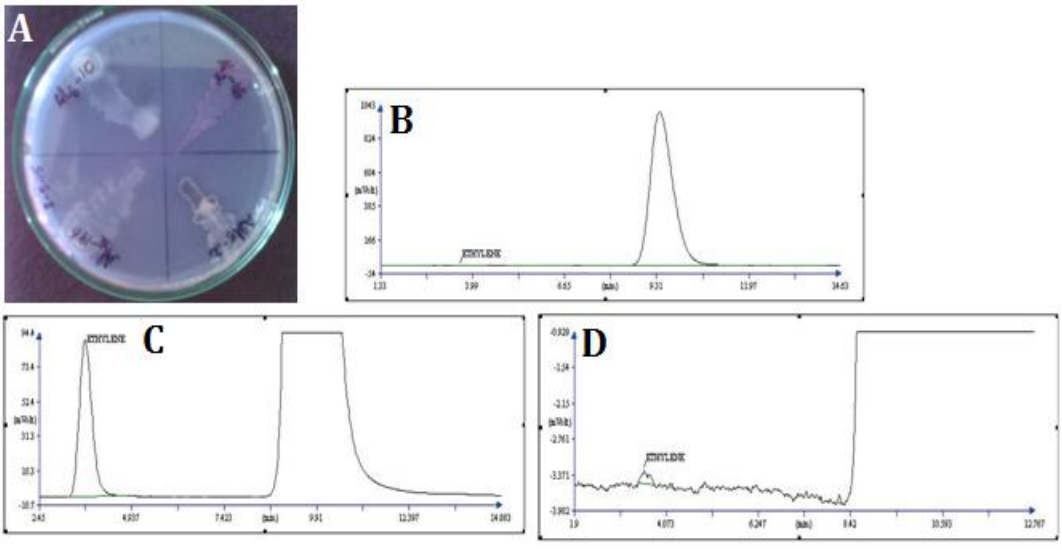


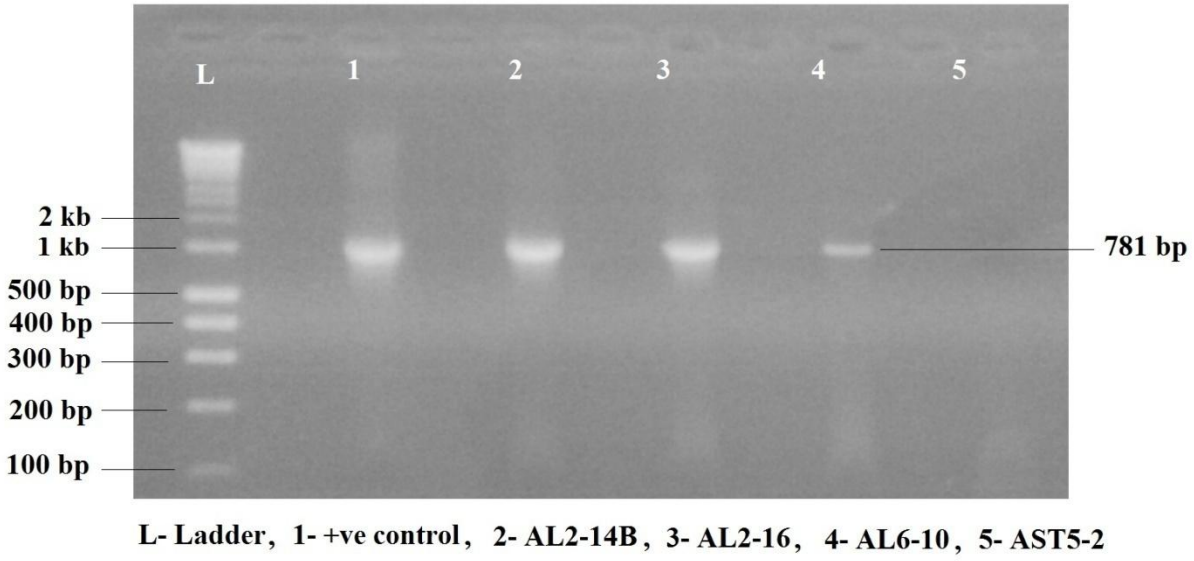
Figure 36. Phosphate solubilization by *B. methylotrophicus* AST5-2 after different time intervals. Soluble free phosphate concentration is given against primary Y axis, while variation of pH in the culture medium is given at secondary Y axis. Standard deviation showed as bars.

4.5.4. Nitogen fixation

All the four selected isolates were able to grow on plates containing nitrogen-free medium. This was further confirmed by Acetylene reduction assay by using Gas chromatography-Flame Ionization Detector (GC-FID) and *nifH* gene amplification. Nitrogenase activity was quantified and it was found to be 1.8617, 2.523 and 32.968 nmol ethylene μg^{-1} $protein^{-1}$ hr^{-1} by *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10 respectively which indicated varied nitrogen fixation efficiency of different isolates. However ethylene production was not detected in case of *B. methylotrophicus* AST5-2. Amplification of *nifH* gene was performed using the pair of *nifH* specific universal primers to confirm diazotrophy of bacterial isolates on the molecular level. However 3 out of 4 isolates showed amplification for *nifH* gene. The desired amplicon of 781 bp corresponding to *nifH* gene was obtained by *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16 and *S. marcescens* AL6-10. The results are given in Photoplate 11, Photoplate 12.



Photoplate 11. A) Growth of the isolates in nitrogen free medium.
Acetylene reduction assay for Nitrogenase activity by
B) AL2-14B; C) AL2-16; D) AL6-10.



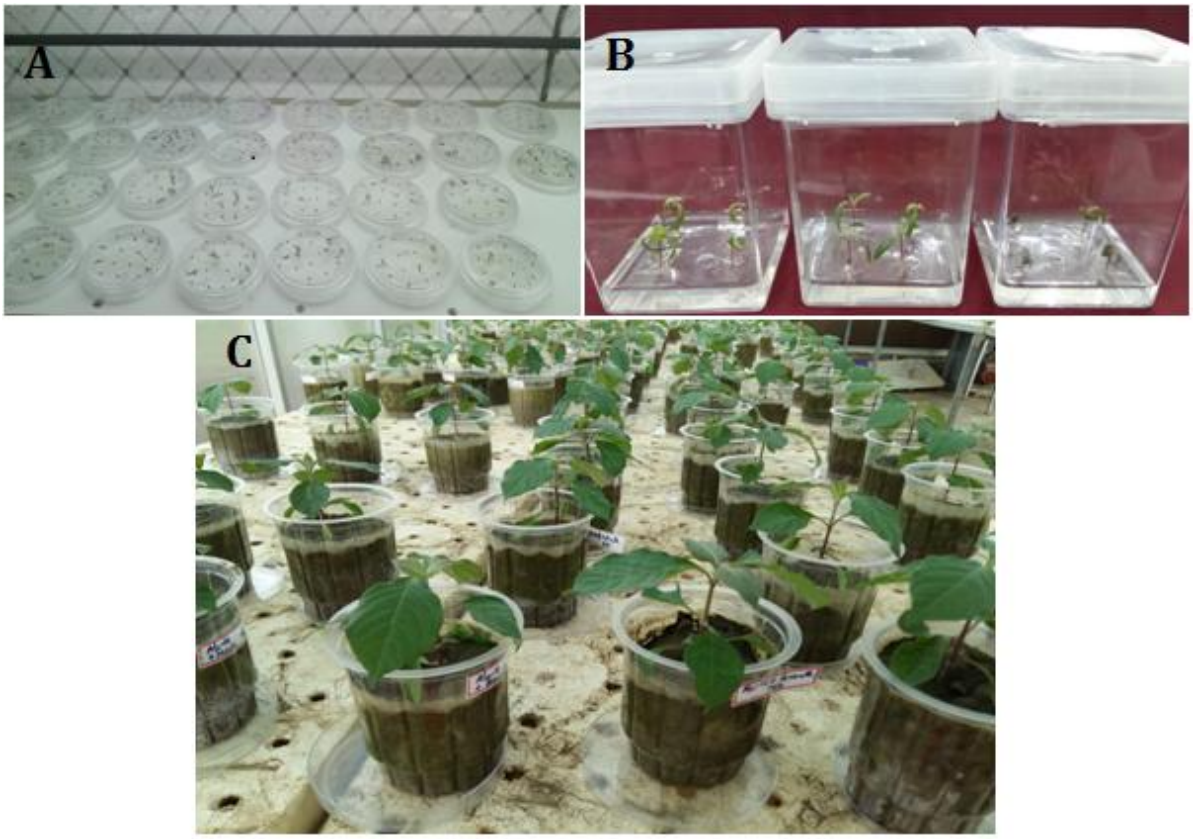
Photoplate 12. Gel image of *nifH* gene amplification of four isolates.

4.6. Colonization study of isolates from experimentally inoculated plants and its effect on plant growth in Pot trial experiment:

Seeds germination started on 9th day by the emergence of radical and plumule. Seeds germination was obtained maximum (29%) in half strength MS medium as compared to full strength MS medium which germinated 22%. Seed germination status depends on embryo potential. This potential depends on seed structure especially embryo structure. Seeds without husk help in easy germination compared with husk coated seeds. The germinated seeds were transferred to MS medium to get proper nutrient for growth of the plant. After development of extensive root system and had 6 leaflets, the plantlets were gradually acclimatized to natural environment and finally planted in sterile soil under greenhouse conditions (26 ± 2^{0}C and 70% RH) (Table 6. Photoplate 13).

Table 6. Percentage of seed germination

Condition of seed	½ MS+light	½ MS+dark	MS+light	MS+dark
Seed+husk	10	10	15	11
Seed-husk	**29**	16	22	16



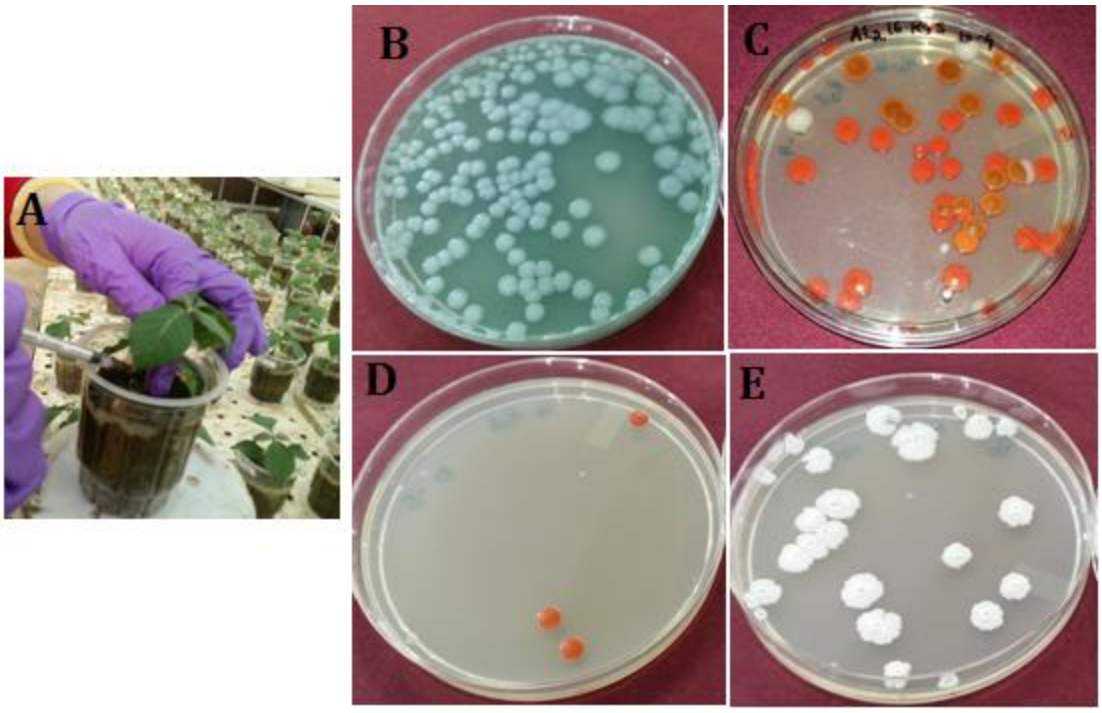
Photoplate 13. *In vitro* propagation of *A. aspera* L. in germ free condition
A. Germination of *A. aspera* seeds on ½ MS
B. Transfer of germinated seeds on MS
C. Transfer of plantlets on soil for acclimatization

The four potential isolates were re-isolated from infected seedlings of *A. aspera* L. In addition, the morphological and physiological characteristics of the endophytic

bacterium recovered after experimental inoculations were indistinguishable from the colony morphologies of the inoculated organisms. This was further confirmed by 16S sequence similarity of inoculated and recovered bacteria. 10 µL of 1.O.D_{600} was inoculated into the homologous plant hosts and grown in the greenhouse. The population of *P. aeruginosa* AL2-14B, *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2 increased from 70×10^6 to 32.3×10^{10}, 16×10^6 to11.2×10^8, 43×10^6 to 52.7×10^8, 10×10^6 to 51×10^7 CFU/g (fresh weight) between 3rd and 5th days after inoculation (DAI) respectively. Further, All these isolates were not detected in leaves till 3DAI, however after 5 DAI, they were recovered from leaves as well as from stem of bacteria-treated *A. aspera* plants. The representative control trials yielded no indigenous bacteria. The results are presented in Table 7.

Table 7. **Population of endophytic bacteria from *A. aspera* grown in greenhouse condition**

Bacteria	**Initial population of bacteria as inoculated**		**Final population of bacteria CFU/g (fresh weight)**			
			3 DAI		**5 DAI**	
	Stem	Leaf	Stem	Leaf	Stem	Leaf
AL2-14B	12.9×10^5	Nil	70×10^6	Nil	21×10^6	11.3×10^4
AL2-16	12.4×10^5	Nil	16×10^6	Nil	93×10^3	18.9×10^4
AL6-10	56.8×10^5	Nil	43×10^6	Nil	11×10^5	41×10^3
AST5-2	20.3×10^5	Nil	10×10^6	Nil	7×10^4	44×10^3



Photoplate 14. Population density study from experimental plants inoculated with isolates

A. Inoculation of the endophytic bacteria into *A. aspera* plantlets.
B. Endophytic *P. aeruginosa* AL2-14B re isolated from the infected plant
C. Endophytic *S. marcescens* AL2-16 re isolated from the infected plant
D. Endophytic *S. marcescens* AL6-10 re isolated from the infected plant
E. Endophytic *B. methylotrophicus* AST5-2 re isolated from the infected plant

The experimented pot trials for the study of growth of *A. aspera* L inoculated individually with the four potential isolates at different pots were harvested for a total of 10 traits (Shoot length, Root length, No. of leaves, Fresh leaves weight, Dry leaves weight, Fresh shoot weight, Dry shoot weight, Fresh root weight, Dry root weight, Area of leaves), after 150 days of incubation.

The study revealed that plants inoculated with *P. aeruginosa* AL2-14B showed shoot length, root length, number of leaves, fresh leaves weight, dry leaves weight, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and area of leaves of 35.50±0.93, 22.86±1.63, 20.2±0.83, 0.50±0.12, 0.12±0.03, 3.38±0.64, 0.88±0.43, 1.02±0.53, 0.32±0.17 and 58.28±5.95 respectively with the control having 20.50±2.5, 10.20±2.28, 9.60±0.89, 0.26 ±0.12, 0.05±0.023, 0.84±0.14, 0.15±0.02, 0.21±0.35, 0.04±0.005 and 24.77±2.11 respectively. The endophytic bacteria treatment increased all growth parameters of *A. aspera* L. It significantly (P≤0.05) increased shoot length by 72.83%, fresh shoot weight by 302%, dry shoot weight by 486%, fresh root weight by 385.71%, dry root weight by 700%, area of leaves by 135.28% by *P. areuginosa* AL2-14B. The results are presented in Table 8, Photoplate 15.

Table 8. Effect of *P. aeruginosa* Al2-14B on the growth characteristics of the *A. aspera* L.

Parameters	Control	Inoculated with AL2-14B
Shoot length (cm)	20.54±2.5(a)	35.50±0.93(b)
Root length (cm)	10.20±2.28(a)	22.86±1.63(a)
No. of leaves	9.60±0.89(a)	20.2±0.83(a)
Fresh leaves weight (g)	0.26±0.12(a)	0.50±0.12(a)
Dry leaves weight (g)	0.05±0.023(a)	0.12±0.03(a)
Fresh shoot weight (g)	0.84±0.14(a)	3.38±0.64(b)
Dry shoot weight (g)	0.15±0.02(a)	0.88±0.43(b)
Fresh root weight (g)	0.21±0.35(a)	1.02±0.53(b)
Dry root weight (g)	0.04±0.005(a)	0.32±0.17(b)
Area of leaves	24.77±2.11(a)	58.28±5.95(b)

Each value is the mean of 5 plants. Values with the same letter within a row are not significant at P≤0.05.

Achyranthes aspera plants treated with *S. marcescens* AL2-16 significantly (P≤0.05) increased shoot length by 95%, fresh shoot weight by 602%, fresh root weight by 438%, dry root weight by 675% and area of leaves by 127%. The untreated control plant recorded shoot length, root length, number of leaves, fresh leaves weight, dry leaves weight, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and area of leaves to be 20.54±2.5, 10.20±2.28, 9.60±0.89, 0.26±0.12, 0.05±0.023, 0.84±0.14, 0.15±0.02, 0.21±0.35, 0.04±0.005 and 24.77±2.11 respectively after 150 days of plantation with the treated plant having 40.16±0.59, 21.31±0.72, 20.00±1.00, 0.63±0.13, 0.14±0.018, 5.90±0.82, 1.76±0.25, 1.13±0.25, 0.31±0.163 and 56.28±4.95 respectively. The results are presented in Table 9, Photoplate 15.

Table 9. **Effect of *S. marcescens* AL2-16 on the growth characteristics of the *A. aspera* L.**

Parameters	Control	Inoculated with AL2-16
Shoot length (cm)	20.54±2.5(a)	40.16±0.59(b)
Root length (cm)	10.20±2.28(a)	21.31±0.72(a)
No. of leaves	9.60±0.89(a)	20.00±1.00(a)
Fresh leaves weight (g)	0.26±0.12(a)	0.63±0.13(a)
Dry leaves weight (g)	0.05±0.023(a)	0.14±0.018(a)
Fresh shoot weight (g)	0.84±0.14(a)	5.90±0.82(b)
Dry shoot weight (g)	0.15±0.02(a)	1.76±0.25(b)
Fresh root weight (g)	0.21±0.35(a)	1.13±0.25(b)
Dry root weight (g)	0.04±0.005(a)	0.31±0.163(b)
Area of leaves	24.77±2.11(a)	56.28±4.95(b)

Each value is the mean of 5 plants. Values with the same letter within a row are not significant at P≤0.05.

Similarly, *Achyranthes aspera* plants treated with *S. marcescens* AL6-10 significantly (P≤0.05) increased shoot length by 47.90%, fresh shoot weight by 197%, dry shoot weight 393%, fresh root weight by 342%, dry root weight by 690%, area of leaves by 123%. Plants inoculated with *S. marcescens* AL6-10 showed shoot length, root length, number of leaves, fresh leaves weight, dry leaves weight, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and area of leaves of 30.38±1.01, 30.26±1.35, 10.8±1.788, 0.50±0.025, 0.10±0.22, 2.50±0.45, 0.74±0.371, 0.93±0.152, 0.316±0.122 and 55.28±5.15 respectively with the control plant having 20.54±2.5, 10.20±2.28, 9.60±0.89, 0.26±0.12, 0.05±0.023, 0.84±0.14, 0.15±0.02, 0.21±0.35, 0.04±0.005 and 24.77±2.11 respectively. The results are presented in Table 10, Photoplate 15.

Table 10. Effect of *S. marcescens* AL6-10 on the growth characteristics of the *A. aspera* L.

Parameters	Control	Inoculated with AL6-10
Shoot length (cm)	20.54±2.5(a)	30.38±1.01(b)
Root length (cm)	10.20±2.28(a)	30.26±1.35(a)
No. of leaves	9.60±0.89(a)	10.8±1.788(a)
Fresh leaves weight (g)	0.26±0.12(a)	0.50±0.025(a)
Dry leaves weight (g)	0.05±0.023(a)	0.10±0.22(a)
Fresh shoot weight (g)	0.84±0.14(a)	2.50±0.45(b)
Dry shoot weight (g)	0.15±0.02(a)	0.74±0.371(b)
Fresh root weight (g)	0.21±0.35(a)	0.93±0.152(b)
Dry root weight (g)	0.04±0.005(a)	0.316±0.122(b)
Area of leaves	24.77±2.11(a)	55.28±5.15(b)

Each value is the mean of 5 plants. Values with the same letter within a row are not significant at P≤0.05.

Photoplate 15. Plant growth promotion of *A. aspera* L by endophytic bacteria

4.7. Availability and uptake of NPK (Nitrogen, Phosphorus, Potassium):

The NPK availability was estimated in the rhizospheric soil of *A. aspera* L. at the first day and after 30th day of the treatments. It showed that the NPK concentration in soil at the first day was 56.00 mg/kg, 37.81 mg/kg and 80.5 mg/kg, respectively, which was found to decreased in inoculated with endophytes. The NPK content in soil inoculated with AL2-14B decreased up to 36 mg/kg, 33.20 mg/kg and 34.60 mg/kg respectively. Similarly there were decreased in NPK content inoculated with AL2-16, AL6-10 and AST5-2.

NPK content was also analysed in leaf tissues. In the case of control plant (without inoculation), the NPK content in leaves was found to be 29400 mg/kg, 2300.85

mg/kg and 48550 mg/kg respectively. Concentration of NPK was higher as recorded in the leaves of *A. aspera* L. inoculated with endophytes. The NPK concentration in leaves with AL2-14 was recorded at 32480 mg/kg, 2677.54 mg/kg and 57850 mg/kg respectively. Similarly there were increased in NPK content in leaves inoculated with AL2-16, AL6-10 and AST5-2.

4.8. Determination of *in vitro* Antioxidant activity of *Achyranthes aspera* L. by endophytes:

4.8.1. Quantitative analysis of total phenol content:

Phenolics compounds are the key phytochemical with high free radical scavenging activity. Phenolics compounds also possess anti mutagenic and anti tumor activities. In the present work, the phenolic content was detected in both the control and treated plants of *A. aspera* L. The amount of total phenol content in the treated plant by *S. marcescens* AL6-10 was found to be 23.58 µg/mg extract which was higher than the control plant (20.49 µg/mg). However other three isolates treated plants gave lesser amount of phenol content than the control plant. The results are represented in Table 11.

Table 11. Effect of isolates on total phenol content *A. aspera* L.

Sample name	Total Phenol Content (µg/mg extract)
Control	20.49
P. aeruginosa AL2-14B	19.85
S. marcescens AL2-16	18.03
S. marcescens AL6-10	23.58
B. methylotrophicus AST5-2	19.21

4.8.2. Scavenging effect on DPPH free radical:

DPPH assay evaluates the ability of antioxidants to scavenge free radicals. Hydrogen-donating ability is an index of the primary antioxidants. *A. aspera* L. plant

treated with the isolates showed higher DPPH radical scavenging activity compared to control plant. The free radical scavenging activity of the extract was concentration dependent. The values of DPPH activity of treated plant with *B. methylotrophicus* AST5-2 ranged from 14.27 to 76.46 while the values of DPPH activity of control plants were in the range of 5.24 to 29.1. IC50 was observed at a concentration of 2.743 mg/ml for DPPH free radical scavenging activity of *B. methylotrophicus* AST5-2 with the control plant having IC50 of 8.11mg/ml. The value of DPPH activity of plants treated with AL2-14B and AL6-10 were found to have similar result. Whereas DPPH free radical activity of plant treated with AL2-16 ranged from 11.91 to 34.24. The results are presented in Figure 37 and Figure 38.

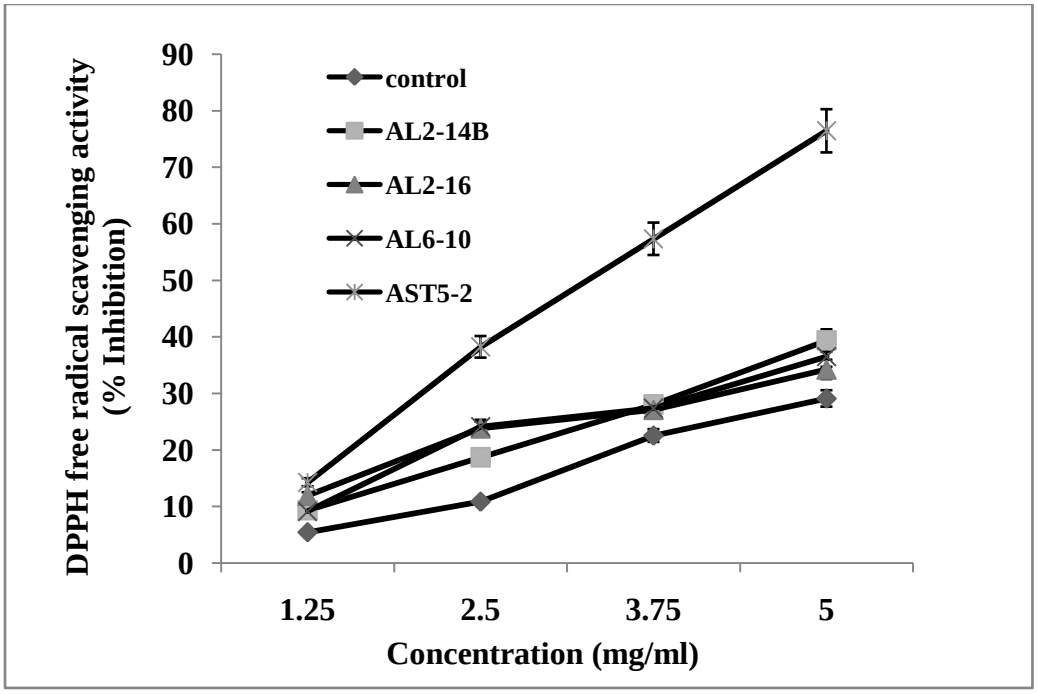


Figure 37. DPPH free radical scavenging activity of samples. Results are expressed as Mean ± SD of three determinations.

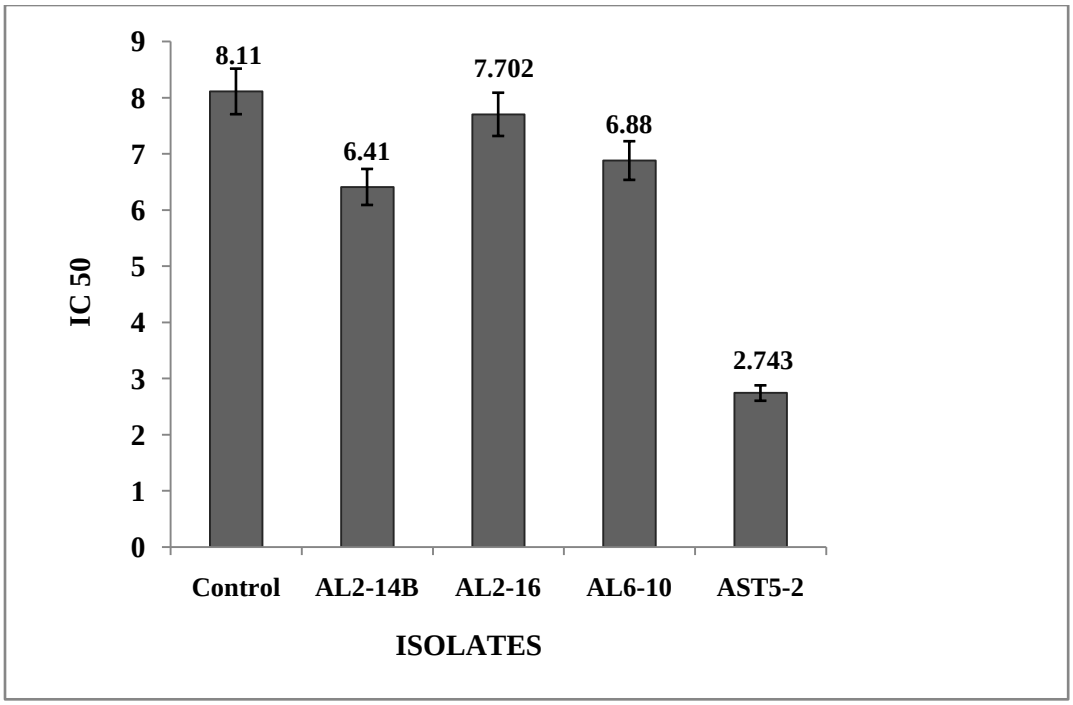


Figure 38. Concentration of samples that scavenge 50% free radicals of DPPH, expressed as IC_{50}(mg/ml). Results are expressed as Mean ± SD of three determinations.

4.8.3.. β-Carotene-linoleic acid assay:

β-Carotene-linoleic acid assay of extract obtained from *P. aeruginosa* AL2-14B inoculated plant was found to be slightly increased than the control plant. It ranged from 15.77± 2.52 to 78.85 ± 4.52 with the control having 15.35±2.12 to76.76 ± 4.05. Whereas the extract obtained from *S. marcescens* AL2-16, *S. marcescens* AL6-10 and *B. methylotrophicus* AST5-2 inoculated plants were found to be lower than the control plant. The results are given in Figure 39.

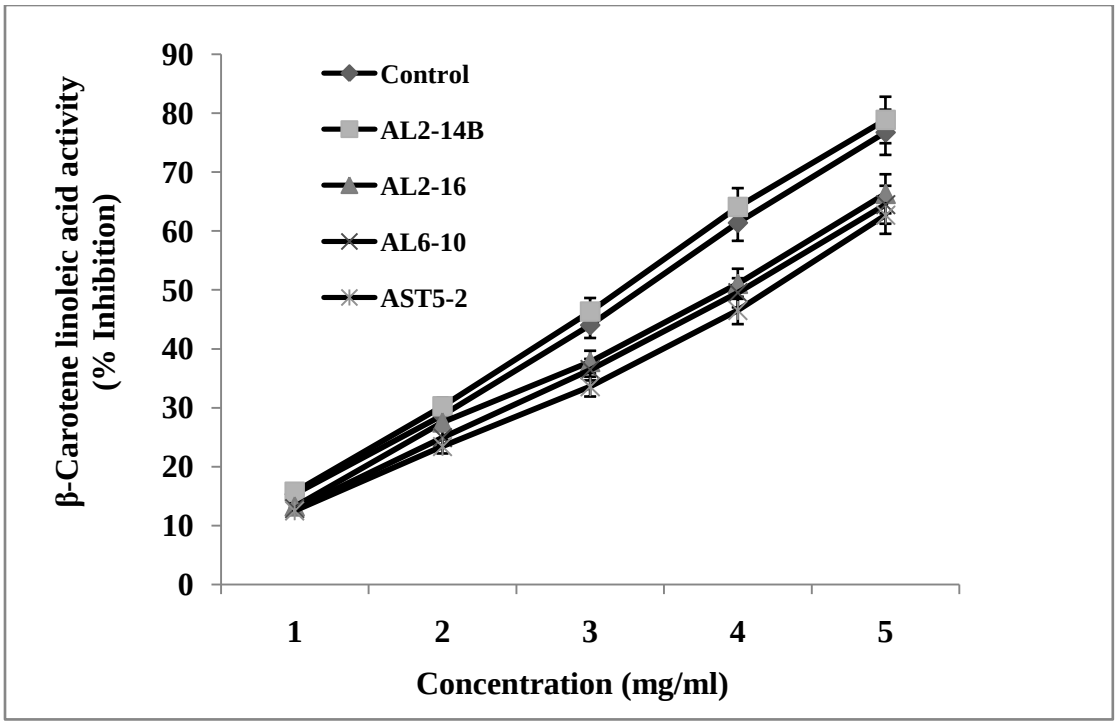


Figure 39. Antioxidant activity of samples using β-carotene bleaching assay. Results expressed as Mean ± SD of three determinations.

4.8.4. Determination of reducing power:

Reducing power serve as a good indicator of antioxidant potential of the plant. Compounds possessing reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation, thus acting as both, primary and secondary antioxidants. The reducing power of the plant extract increased with respective increase in concentration. The reducing power of *A. aspera* L. leaves inoculated with *P. aeruginosa* AL2-14B and *B. methylotrophicus* AST5-2 were found to be higher than that of control plant. Whereas the reducing power of the extract inoculated with *S. marcescens* AL2-16 and *S. marcescens* AL6-10 were found to be slightly lower than the control plant.

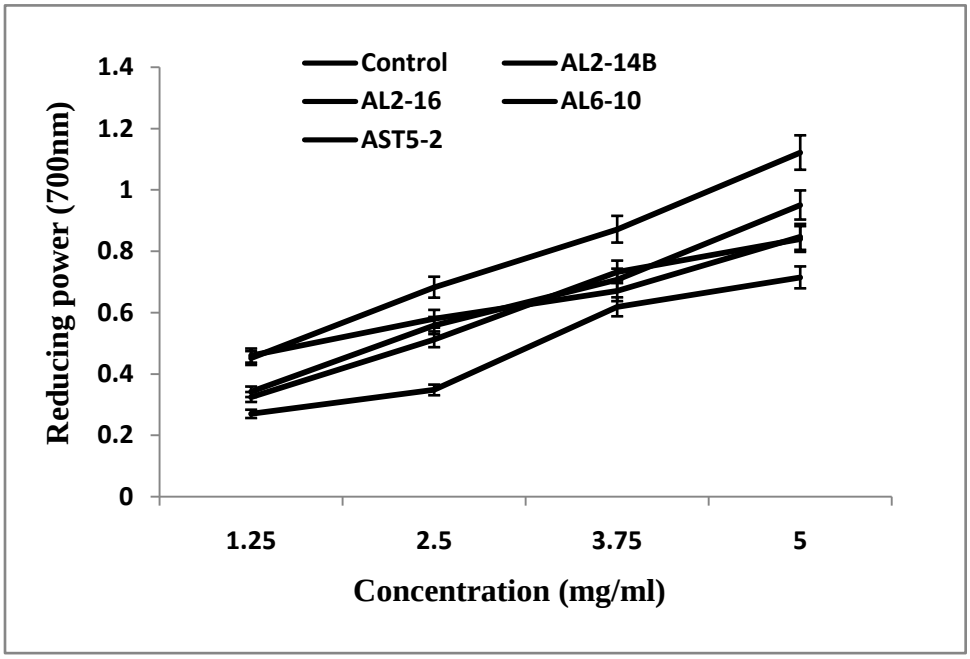


Figure 40. Reducing power of samples at 700nm. Results are expressed as Mean ± SD of three determinations**.**