A SEMINAR PAPER

ON

Plant Growth Promotion Activities of Biofilm-Producing Bacteria

Course Title: Seminar

Course Code: ENS 598

Summer, 2018

SUBMITTED TO

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PLANT GROWTH PROMOTION ACTIVITIES OF BIOFILM-PRODUCING BACTERIA

BY

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ABSTRACT

Plant growth promoting bacteria play a important role in influencing plant growth through a range of beneficialfunction.Biofilm development by PGPR is considered as a survival strategy over the planktonic mode of growth under stress and natural condition.Plant growth promoting bacteria with efficient colonization ability and exhibiting multiple PGP traits are to perform better. Bacteria which has ability to form biofilm , that has also multiferous PGP activities such as production of indole acetic acid ,siderophore ,phosphate solubilization ,hydrogen cyanide ,ammonia production and biocontrol activity .These bacteria can tolerant to salinity and heavy metals.Different bacterial strains play active role in plant growth promotion activities.*Pseudomonasentomophila* FAP1 strains produced IAA (2009.25±1.32 µg ml−1.Other isolates were also showed varying level of the production of IAA is PS2(149.21 ± 1.16 µg ml−1) ,Ps3 (169.31±1.26 µg ml−1) ,PS4(159.21±1.16 µg ml−1), PS5 (189.41±1.26 µg ml−1), PS6(139.91±1.66 µg ml−1) and PS7(179.81±1.06 µg ml−1) .(Ansari et. al.;2018).IAA also reduced the lateral elongation of root and balance root/shoot ratio.*Bacillus sp. ,Aeronomus* , strain of these bacteria has effect on root/shoot ratio.Psedomonas bacterial strain also plays an impotant role in phosphate solubilization , siderophore production and N_2-Fixation.

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CHAPTER I

INTRODUCTION

Biofilm are external layer of bacteria that is self replicating.The main component of the biofilm is mainly cellulose(exopolysaccarides),protein(the highly aggregative, unbranched, amyloid-like cell surface known as curli) and nucleic acid.Biofilms arestructuded communitiesof microbial cells enclosed in a self-producedetracellular polymeric substances (EPS) adherent to differentabiotic (e.g., plastic, rubber, cement,glass, stainless steel, rocks, hull of the ships.) andbiotic (e..g., plant surface, animal epithelial cells, gallstones etc.)solid surfaces (Costerton et al ., 1999; Dolan and Costerton,2002).

Biofilm may influence resource competition or production of inhibitory compounds and increase resistant to abiotic stresses (Angus et. al., 2013).Naturally biofilm constituets a protected growth modality allowing bacteria to survive in hostailenvironment.The different characteristic such as EPS production .swarming and swimming motility,cell surface hydrophobicity and alginate productionin *pseudomonas sp.* and other bacteria (Angus et. al.,2013).Bacteria can protect its self by producing biofilm.By this mechanism bacteria can survive in adverse condition like high ph ,higher salinity ,low temperature,higher temperature, alkanity etc.

In present condition of the agriculture is so much threat for environment because of heavy use of pesticide ,fertilizer,higher exploitation of soil.Thats affect environment in many ways.At this moment biofilm has great chances for agriculture by preparing biofertilizer with biofilm producing bacteria.Biofilm producing bacteria can induce IAA production.siderophoreproduction,Ammoniaproduction,phosphatesolubilisation activity lastly and most important nitrogen fixation.All these characteristics can promote the plant growth and keep environment safe.

Plant growth –promoting rhizobacteria are bacteria influenced by plant root exudates that have the ability to improve plant growth over the short term (Masciarelli et al.,2014) and crop production over the long term (Morales et. al.,2011).Rhizobia develop structurted biofilms which is the specific information about the four major genera :Mesorhizobium,Sinorhizobium ,Bradyrhizobium and rhizobium have been summarized .(Rinaudi et. al.,2010). Particularly for the S. meliloti Rm1021 strain ,attachment to polystyrene and growth as a biofilm depend on the environmental condition.(Rinaudi et.al.,2006)and biotic and abiotic surface colonization is affected by succinoglycanproduction (Fujishige et.al., 2006).S. meliloti strain Rm8530,Which has a mucoid phenotype ,forms a highly structured architectural biofilm,in contrast to the unstructured one-fromed by non-EPS 2 producing strain Rm1021 (Rinaudi et. al.,2009) The presence of a functional copy of the expR regulate gene is necessary for autoaggregation LMW EPS 2, either alone or in combination with the HMW fraction, may function as a polymeric extracellular matrix that agglutinates bacterial cells. (Sorroche et. al.,2010)

In consequence, wild-type S. meliloti reference strains carrying nonfunctional expR loci fail to autoaggregate and develop a relatively small biomass attached to plastic surface and,therefore ,a function EPS2 synthetic pathway and its proper regulation as essential for cell-cell interactions and surface attachment of S. meliloti. In addition ,we found a positive correlation between bacterial autoaggregation and biofilm in native S. meliloti strains isolated from root nodules of alfalfa.(Sorroche et. al., 2012).

The biofilm may influence resource competition or production inhibitory compounds and increase resistance to abiotic stresses.(Rao et. al., 2005).Beneficial plant growth promoting rhizobacteria (PGPR) associated with plant root surfaces are known to contribute towards increase in plant yield.(Ryu et. al.,2004)The mechanisms of plant growth promotion by various PGPR has been well documented by both direct (production of plant hormones, nitrogen fixation, phosphate solubilization and sequestering iron) and indirect (antibiotics and lytic enzymes,induced resistance ,HCN production and competition) mechanism

Objectives:

1.To know about biofilm producing bacteria

2.To understand the plant growth promotion activities of biofilm producing bacteria

CHAPTER II

MATERIALS AND METHODS

The topic of this seminar paper was selected with the consultation ofMajor Professor. This paper is exclusively a review paper. So, all of the information has been collected from the secondary sources such as various relevant books, journals, proceedings, reports, publications etc. for preparing this manuscript. Topic related findings have been reviewed with the help of the library facilities of Bangabandhu Sheikh MujiburRahman Agricultural University. Some information has also been collected by searching internet web sites. Valuable suggestion and information were collected from course instructors, major professor and other resource personnel. After collecting information, these were compiled and used for preparing this seminar manuscript.

CHAPTER III

REVIEW OF LITERATURE

Plant Growth promotion:

Plant growth promotion,Which can regulated by the production of hormones and other compounds related to the auxin (IAA) is a class of plant hormones important in the promotion of lateral root formation .Increased lateral root formation leads to an enhanced ability to take up nutrients for the plant(Van,et.al.,2007).Plant growth promoting bacteria occupy the rhizosphere of many plant species and have beneficial effect on the host plant.They may influence the plant in a direct or indirect manner.A direct mechanism would be to increaseplant growth by supplying the plant with nutrients and hormones.Indirect mechanism on otherhand ,include reducedsusceptibilityto diseases ,and activating a form of defense referred to as induced systematic resistance(Yang, et. al.,2009).Example of bacteria which have been found to enhance plant growth,including *Pseudomonas,Enterobacter* and*Arthobacter(Saharan, et.al.,2011).*

Plant growth promotion activities:

1.IAA (Indole-acetic-acid) production

2.siderophore production

3.phosphoroussolubilization

4.N2-Fixation

Function of the plant growth promotion activities:

PGPA	**PRODUCED**	**FUNCION**
IAA production	Indole acetic acid /Auxin	1.Reduced lateral root elongation 2.Balance the growth of Root/Shoot Ratio
Siderophore production	Iron	1.Increase availability of p
Phosphorous solubilization	P(inorganic and organic form)	1.Flowering and fruting 2Development of early stage of embryonic plants
N_2-Fixation	Nitrogen	1.Promotes vegetative growth

Plant growth promoting Rhizobacteria:

Rhizosphere microbial communities are increasingly understood to interact extensively with plants,and this association is very crucial to the overall plant health and development. Beneficial free-swimming planktonic plant plant growth promoting rhizobacteria (PGPR)have long been used as biofertilizer (sturz and Christie,2003;Glick,2012).However ,their effects in the field are inconsistent,Which has limited commercial application.This is probably caused by the inoculants' inability to complete with exixting endogenous microbial communities (Gupta et. al.,2015).To address this issue ,Scientists have begun to investigate the use of biofilm PGPR(B-PGPR) as alternative inoculants (Trivent et. al.,2012).

Table 1: Plant association and growth-promoting characteristics of PGPR in Agriculture

Vegetation type	Host plant	Dominant exudation	Identifidmicrobiota	References
Legumes	Soybean [*Glycine max.* (L.) Merr.]	Phosphate solubilization, N-fiationsiderophores production, protease production	*Bacillus amyloliquefaciens* LL2012, *Bradyrhizobium japonicum*	Masciarelli et al.(2014)
	Chickpea (*Cicer arietinum*L.)	(*arietinum*L.) Siderophores production, chitinase activity, ACCdeaminase activity, exopolysaccharide production, phosphate solubilization, HCN production	*Serratiamarcescens* (SF3) and *Serratia*spp. (ST9) + *M. ciceri*	Shahzad et al.(2014)
	Mungbean (*Vignaradiata*	ACC-deaminase activity, Auxin production, phosphate solubilization antibiotic	*Pseudomonas fragi*P5, *Pseudomonas jesseni*	Iqbal et al. (2012)

	L.)	resistance	P10 and *Rhizobium leguminosarum*Z22	
Cereals	*Wheat (Triticum aestivumL.),*	IAA,HCN, siderophores	*Serratiamarcescens*	Selvakumaret al. (2008)
	maize (Zea mays L)	Acid phosphatase, alkaline phosphatase, IAA productioni	*Azospirillumbrasilense CNPSo 2083*, *Rhizobium tropici CIAT 899*	Marks et al.(2015)
	Rice (Oryza sativa L.)	IAA production, gibberellic acid production	*Enterobacter*spp. and *Azospirillium*spp.	Hasan et al. (2014)
	Oat (Avena sativa L.) and barley (Hordeum vulgareL.)	IAA production, siderophores production, phosphate solubilization	*Sinorhizobiummeliloti L3Si*, *Pseudomonas* sp. LG *Azotobacter chroococcum*AV, *Enterobacter sp. E1*,	Stajković-Srbinović et. al. (2014)
	Oat (Avena sativa L.)	ACC deaminase, HCN, IAA production, phosphate solubilization	*Sinorhizobiummeliloti*, *Azotobactersp.*, *Pseudomonas sp.*	Delić et. al. (2012)
	Sugarcane (Saccharum offiinarumL.)	Production of IAA, phosphate solubilization, Induced systemic resistance,	*Azospirillumsp.*	Moutia et al.(2010)
	Sugarcane (Saccharum offiinarumL.)	Phosphate solubilization, HCN production, IAA production	*Bacillus megaterium*	Sundara et al.(2002)

Oil seed	Turnip mustard (*Brassica rapa* L.)	IAA, ACC deaminase, Siderophores	*Pseudomonas sp.*	Poonguzhaliet al. (2008)
	Mustard (*Brassica campestris*L .)	HCN production, IAA production	*Mesorhizobium loti MP6*	Chandra et al.(2007)
	Canola (*Brassica napus* L.)	Siderophores,IAA, Salicylic acid, ACC deaminase	*Dyellaginsengisoli*, *Burkholderiakururiensis*, *Pandoraeasp*. ATSB30	Anandham et al.(2008)
	Sunflwer (*Helianthus annuus*L.)	Siderophores production and IAA production	*Pseudomonas florescens* biotype F and *Pseudomonas florescens* CECT 378T	Shilev et al.(2012)

Vegetation type	Host plant	Dominant exudation	Identifidmicrobiota	References
Trees	*Pinus roxburghii*	Siderophores production and IAA production	*Bacillus subtilis*	Singh et al. (2008)
	Italian stone pine (*Pinus pinea*L.)	Phosphate solubilization, IAA, exopolysaccharide production, organic acid production	*Bacillus licheniformis CECT 5106 and Bacillus pumilus CECT 5105*	Probanza et al. (2001)
	Teak (*Tectona Grandis*) and	Nitrogen fiation, phosphate	*Azotobacter sp. DCU26 and Bacillus*	Aditya et al.(2009)

	Indian redwood (*Chukrasia Tabularis*)	solubilization, siderophores production	*megaterium A3.3*	
Grasses	Canary grass (*Phalaris minor* L.)	IAA production, Nitrogen fiation, HCN production	*Azotobacter*and *Azospirillum*	Zaefarianet al. (2012)
	Bermuda grass (*Cynodon dactylon*L.)	Phosphate solubilization, Exopolysaccharide production, ACCdeaminase activity, HCN production,	*Serratia sp.*—TRY2 and *Bacillus sp.*—TRY4	SarathambalandIlamurugu (2013)
	Barnyard grass (*Echinochloa crus-galli*L.), Italian ryegrass (*Lolium multiflrum*L.)	Phosphate solubilization, HCN production, IAA production, antifungal, HCN production,	*Bacillus*, *Arthrobacter*, *Stenotrophomonas*, *Acinetobacter*, and *Pseudomonas*	Sturz et al.(2001)
	Nut grass (*Cyperus rotundus*L.)	Phosphate solubilization, Organic acids production, siderophores production, HCN production	*Enterobacter*sp. Arh 1, *Pseudomonas* sp. Bro 5	Diogo et al. (2010)
Vegetables	Red pepper (*Capsicum annuum*L.)	Gibberellic acid, IAA production	*Bacillus cereus* MJ-1	Joo et al.(2005)
	Mint (*Mentha piperita*L.)	Phosphate solubilization, siderophores production, IAA production	*Agrobacterium rubi*A16, *Burkholderiagladii*BA7, *P. putida*BA8, *B. subtilis*	Kaymaket al. (2008)

			OSU142, *B. megaterium* M3	
	Cabbage (*Brassica oleracea*L.)	IAA production, Phosphate solubilization, HCN production, Organic production	*Bacillus megaterium* TV-91C, *Pantoea agglomerans*RK-92 *and B. subtilis*TV-17C	Turan et al.(2014)
	Tomato (*Solanum lycopersicum* L.)	IAA production, antagonistic behavior, HCN \production, siderophores production, Gibberellic acid production	*Pseudomonas putida,P. florescens*, *Serratia marcescens*, *Bacillus subtilis*, *B. amyloliquefaciens*, and *Bacillus cereus*	Almaghrabiet al. (2013)
	Cucumber	Antagonistic effect, HCN production, siderophores production, Phosphate solubilization,	*Bacillus sp*	Stout et al. (2002)
	Bitter gourd (*Momordica charantiaL.)*	Phosphate solubilization, Nitrogen fixation, siderophores production,HCN production,ACC deaminase activity	*Azospirillum, Pseudomonas florescens, and Bacillus subtilis*	Kumar et al.(2012)

Plant Growth Promotion Activities Of Biofilm Producing Bacteria:

1.Indole acetic acid production:

Indole-3-acetic acid (IAA) accumulation in culture supernatants of rhizosphere bacterial strains was quantified by high-performance liquid chromatography with two selective detectors. Twelve of 14 rhizobacterial strains produced detectable levels of IAA in culture filtrates. Two strains, 7SR5 and 7SR13, produced large concentrations of IAA (5-10 μg ml^{-1}), reduced root elongation, and increased shoot:root ratios of sugar beet when applied as seed inoculants. A significant linear relationship was observed correlating IAA accumulation of rhizobacterial strains with decreased root elongation and increased shoot:root ratios of sugar beet seedlings.

Table 2: production of IAA from biofilm producing bacteria(*pseudomonas)*

pgp traits	IAA (μg ml−1)
Ps1	209.25 ±1.15
Ps2	149.21± 1.16
Ps3	169.31 ± 1.26
Ps4	159.21±1.16
Ps5	189.41±1.26
Ps6	139.91±1.66
Ps7	179.81±1.06

Table 3: Effect of inoculating wheat seedling with exopolysaccharide(EPS-) producing bacteria on different yield parameters

Treatment	RS[a]DW[b] (g/ pot)	Root DW (MG/pot)	Shoot DW (MG/Pot)	RS /Ratio Ratio	EPS-bacteria on rhizoplane (CFU/gDW)	Saccharides in RSc (mg/pot)	Water insoluble Na+ in RS (μmol g −1 DW)
Control	0.50 e[d](34.6a)[e]	19d	45d	26.2c	8.2^{07} c	2.48 d	19.6 a
Aeromonas	3.21c(11.0 d)	91b	149b	35.7b	3.1^{08}b	8.63 b	18.0a
hudrophila/caviae MAS765	2.86c(16.7c)	82b	139b	35.9b	2.5^{08}b	6.67 bc	15.5a
Bacillus insolitusMAS617	1.38 d(23.8b)	48c	83c	29.2bc	1.1^{08}c	5.99 c	19.0a
Bacillus sp. MAS620	3.96b(17.0 c)	83b	136b	48.3a	2.4^{08}b	11.22 a	18.0 a
Bacillus sp. MAS820	4.45a(14.8c)	120a	171a	38.1b	4.0^{08}a	12.33 a	16.7 a
LSD 5%	0.43(2.25)	4.12	15.37	8.33	8.7^{07}	2.31	6.87
LSD 1%	0.59(3.03)	9.01	20.70	11.21	1.2^{08}	3.12	9.60

aRhizosphere soil

b Dry weight Sources:Ashraf et. al.,2004

c Water-insoluble saccharides ,measured as glucose equivalents

d In each column ,figures by followed by different letters(s) are significantly Duncan's multi range test (p< .05

e Figures in parentheses represent % moisture content of the rhizosphere soil

2.Siderophore production:

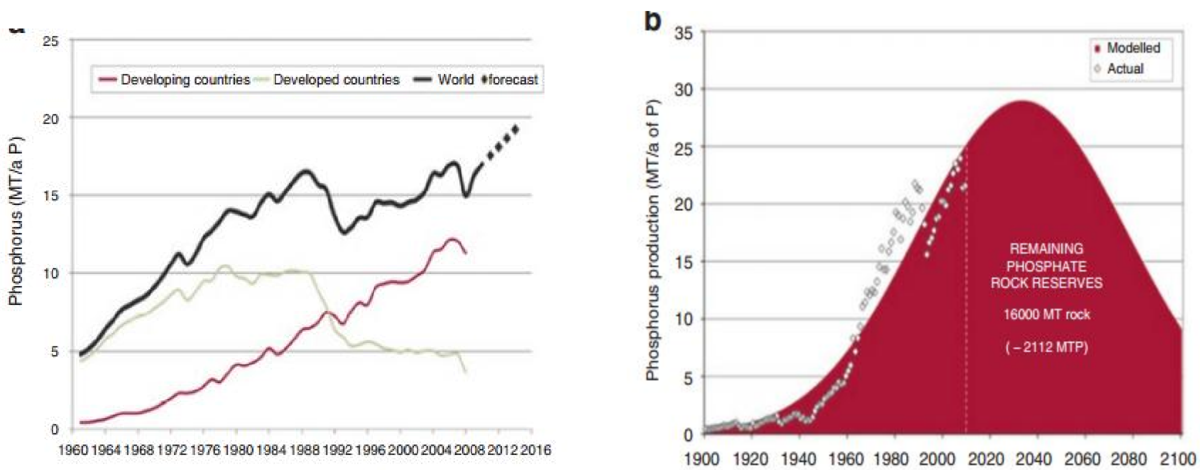
Siderophore are low molecular weight metal chealating agents which are produced by plants and microorganisms in Fe-limiting conditions.Different type of siderophore producing bacteria producing bacteria produced siderophore at different time and with different percentage (chaudhary . et. al.,2017).Iron is essential element for the growth of the living microorganisms as it acts as a catalyst in enzymatic process,oxygenmetabolism,electrontransfer,andDNAand RNA synthesis(Aguado-Santacruz et. al.,2012)Iron is also important in biofilm formation as it regulates surface motility and stabilizes the polysaccharide matrix (Weinberg, 2004: chhibber et. al.,2013).In an environment deficient of iron ,the microbial surface hydrophobicity decreases which alter the surface protein composition and thus limit the biofilm formation(Simoes et. al., 2007).

Table 4: production of siderophorefrom biofilm producing bacteria(*pseudomonas*)

PgP traits	Siderphore((µg ml−1)
Ps1	+ve (23.54 ± 1.36)
Ps2	+ve (16.52 ± 1.16)
Ps3	+ve (12.42 ± 1.06)
Ps4	+ve (17.32 ± 1.02)
Ps5	+ve (13.39 ± 1.32)
Ps6	+ve (20.49 ± 1.52)
Ps7	+ve (10.49 ± 1.32)

3.Significance of phosphorus for plant:

Phosphorus (P) is an essential element to all life forms of the earth ecosystem. In particular, phosphorus is key primary macronutrient necessary for plant growth and development along with nitrogen and potassium. P entry into plant is facilitated by root hairs, root tips, and the outermost layer of root cells. Plants typically take up P in inorganic form either as primary orthophosphates ($H_2PO_4^{-}$) or secondary orthophosphates (HPO_4^{2-}) ion from soil solution. Phosphorus also exists as phytin, a major P reserve of seeds and fruits, required for seed formation and early developmental stages of embryonic plant.



(a) (b)

Sources: Arif et. al.,(2017)

Figure 1: (a) Global phosphorus fertilizer consumption between 1961 and 2006 (in million tons p)The figure indicates that while demand in the developed world reached a plateau and then declined around 1990,fertilizer demand has been steadily increasing in the developing world(IFA 2009). (b) Peak in production by 2033,derived from the Us geological Survey and industrial data (Cordell et. al.,2009)

Phosphate solubilizing:

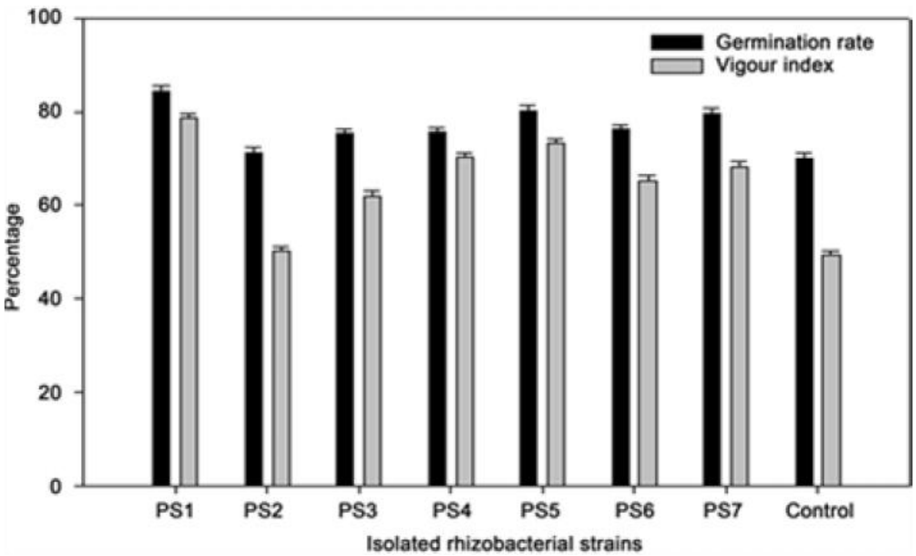
Phosphorous(p) which is an essential plant nutrient element is second most important element after nitrogen.It is unavailable to plants because in the soil it ismostly present in the fixed form.Soil bacteria having the phosphate solubilizing capacity are called as phosphate Solubilising Bacteria (PSB).The convert the insoluble phosphate into soluble from through the production of organic acid and make it available for plant uptake and nutrition.They are also useful as biofertilizer as they belong to the plant growth promoting *Rhizobacteria.* P is abundant in soils in both organic and inorganic forms,its availability is restricted to plants as it occurs mostly insoluble forms (Pradhan and Sukla,2005).Thus the release of these fixed and insoluble forms as soluble forms is a veryimportant factor in increasing soil p availability.Soil microorganisms play a key role in soil p dynamics and subsequent availability of phosphate to plants (Khosro mohammadi,2012)

Table 5 : Production of Phosphorus from phosphorus solubilizing bacteria (*pseudomonas*)

Pgp traits	Phosphate solubilization(µg ml−1)
Ps1	+ve (170.45 ± 1.22)
Ps2	+ve (120.40 ± 1.12)
Ps3	+ve (129.42 ± 1.22)
PS4	+ve (119.32 ± 1.32)
Ps5	+ve (123.22 ± 1.42)
Ps6	+ve (153.32 ± 1.52)
Ps7	+ve (163.42 ± 1.32)

Effect of Bacterial seed treatment on germination and vigor index in wheat:

The effect of bacterial (rhizobacterial) treatment upon seed germination and vigor index of wheat varied with different strains of bacteria.. The FAP1 stain had a significant effect on germination and vigor index was 78.25±1.36% with the treatment of FAp1 compared with which are not inoculated by bacteria or control.Although ,other isolates were also reveled different pattern of seed germination and vigor index as deicted in figure (Ahmed et. al.,2018)



Source: Ansari et. al.; (2018)

Figure 2 : Effect of Rhizobacterial treatment on the germination rate and seedling vigor index

4.N_2-Fixation:

The nitrogen-fixing symbiosis is the result of a complex interaction by which a legume plant and a type of bacteria (*rhizobia*) both obtain nutritional benefit.The bacteria supply the plant with reduced nitrogen from atmoshpheric sources that are not directly available to the plant;while the bacteria obtain carbon compounds from the plant within the protected root nodule.(Gage et. al.,2004).Specifically *Sinorhizobiummeliloti* (S. meliloti),under nitrogen limitation conditions,is able to engage in a symbiotic association with the agriculturally significant legume *Medicago sativa*(alfalfa).(Jones et. al.,2007).S. meliloti produces two different EPSs commonly known as EPS I (*succinoglycan*) and EPS II (glactoglucan).(Janczarek et. al.,20012),Which are both able to promote symbiosis.EPSI,the best-understood symbiotically important EPS,is required for the invasion of alfalfa roots by S. meliloti strain Rm1021. (Cheng et.al., 1998).

Chapter IV

CONCLISION

Biofilm are exopolysaccarides that is self -replicating.It take part in plant growth promoting activities by different pathway like IAA production, Siderophoreproduction,N-fixation ,phosphorussolubilization and many other ways.Bacteria plays important role in the plant growth activities.for plant growth ,different type physiological activities occures in plant physiology and need different raw materials like auxin (IAA), N ,P ,Fe and others. Each one of these product are important for plant root and shoot growth, development of embryonic stage, fruting ,flowering etc.SO lack of nutrient in soil excess fertilizer and pesticides are used in agricultural land .it is harmfull for the environment as well as human health.From the above discussion and information we can understand that biofilm producing bacteria has capability to synthesize nutrient element from the nature and capable of surviving in the stress condition.If it is used in producing biofertilizer and it can reduced using higher amount of synthetic fertilizer.It also Known about the plant promototingRhizobacteria and their wide range of interaction with crops like cereals,oilseed,trees,grasses ,vegetables etc.

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