International Journal of Research in Engineering, Science and Management
Volume-1, Issue-12, December-2018
www.ijresm.com | ISSN (Online): 2581-5792

Effect of Root Exudates on the Genomic Elements in Plant Growth Modulation in *Bacillus Aryabhattai* AB211

Subhajit Saha[1], Sanchita Debnath[2]
[1]*Student, Department of Bio-Technology, Bangalore University, Bangalore, India*
[2]*Student, Department of MLT in Microbiology, WBUHS, Kolkata, India*

***Abstract*: Bacillus aryabhattai AB211 is a plant growth promoting, Gram-positive firmicute, which has been isolated from the tea rhizosphere (Camellia sinensis) in Rohini tea estate, Darjeeling. From the whole genome data, it was revealed that this organism possesses a number of plant growth promoting traits, however, understanding the consecutive changes in growth pattern or gene expression of AB211 in association with plant roots is yet to be established. In rhizosphere, the root exudates play a major role in communication between the plant system and the resident microbiota, thus serve as a signal which may affect the microbes compelling changes in their gene expression according to changing the environment. This project has attempted to find the efficiency of the strain to get associated with the plant roots, so in turn to provide its beneficial effect on plant growth. Growth characteristics of the AB211 strain were evaluated in presence of root exudates. Also, the AB211 strain was exposed to maize root exudates for different time periods and several plant growth promoting genes, whose presence was evident from the genome data, were quantitatively studied by Real-time PCR.**

***Keywords*: SEM, Growth Curve Analysis, RT-PCR.**

1. Introduction

The rhizosphere is the narrow dynamic zone of soil influenced by plant roots where intense plant-microbe interaction is found. The plant rhizosphere is the major soil ecological environment for plant-microbe interactions involving colonization of different microorganisms in and around growing roots which may either result in associative, symbiotic, naturalistic, or parasitic interactions depending on plant nutrient status in soil, soil environment, plant defence mechanism, and the type of microorganism proliferating in the rhizosphere zone. PGPR is plant growth promoting rhizobacteria.

A. Mode of actions of PGPR

Different bacteria modulate plant growth in different ways. Here are six major ways that PGPR follow in order to support plant growth and better yields:

B. Nitrogen fixation

Legumes form root nodules that contain symbiotic bacteria (Rhizobium species). Other crops can use nitrogen that is made available from non-symbiotic soil bacteria.

C. Help in nodulation

The complex process of nodulation requires many hormones and enzymes produced by a wide variety of soil microbes, not just the Rhizobium species that ultimately form the root nodules.

D. Hormone production

Plant growth hormones produced by bacteria include auxins, gibberellins, and cytokines, and can result in plants with higher root weights, longer roots, as well as more and longer root hairs and lateral roots.

E. Nutrient uptake

PGPR can enhance the availability of nutrients in the soil and allow the nutrients to be taken up and used more efficiently by the plants.

F. Siderophore production

Siderophores are small compounds that bind to iron in the soil; this can deprive some of the disease-causing micro flora of the iron they otherwise need to survive.

G. Bio-control

Allowing microbes to colonize root systems of host plants can be environmentally-friendly alternative to expensive pesticides to combat weeds, as well as fungal and bacterial infections.

Table 1
General features of B. aryabhattai AB211 genome

General traits	Value
Size of the genome (BP)	5,403,108
GC content (mol %)	37.82
Total number of scaffold	23
Number of genes predicted	5731
Number of protein coding genes	5598
Number of characterized protein	4086
Number of putative protein	1612
Number of rRNA genes	11
Number of tRNA genes	118
Phage associated genes	11

2. Review of literature

Antoun H, Kloepper et al. [1], proposed that the plant growth-promoting rhizobacteria (PGPR) are soil bacteria that are able to colonize rhizosphere and to enhance plant growth by means of a wide variety of mechanisms like organic matter mineralization, biological control against soil-borne pathogens, biological nitrogen fixation, and root growth promotion mineralization, biological control against the soil borne pathogens, biological nitrogen fixation and root growth promotion. Arshad, Frankenberger et al. [2], presented that the enhanced amount of soluble macro- and micronutrients in the close proximity of the soil-root interface has indeed a positive effect on plant nutrition. They can also increase the bioavailability of iron and other amino acids, produce phyto-hormones and other plant growth regulating compounds like indole acetic acid (IAA), gibberellins, acetoin (3-hydroxy-2-butanone), 2,3-butanediol, and cytokinin. B.G. Kang et al. [3], proposed that the local changes within the rhizosphere include the growth and development of neighboring plant species and microorganisms. Upon encountering a challenge, roots typically respond by secreting certain small molecules and proteins in the form of root exudates. Root secretions may play symbiotic or defensive roles, depending on the other elements of its rhizosphere. Bhattacharyya et al. [4], told that it has already been found that Bacillus aryabhattai has the plant growth promoting abilities. Here, we have performed experiments to understand the effect of root exudates on the AB211 strain. H. Marschner et al.[5], proposed that the ability to secrete a vast array of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots, with nearly 5% to 21% of all photosynthetically fixed carbon being transferred to the rhizosphere through root exudates. R.Hayat et al. [6], proposed that the plant rhizosphere is the major soil ecological environment for plant–microbe interactions involving colonization of different microorganisms in and around growing roots which may either result in associative, symbiotic, neutralistic, or parasitic interactions depending upon plant nutrient status in soil, soil environment, plant defense mechanism, and the type of microorganism proliferating in the rhizosphere zone. S. Nardi et al. [7], says that the compounds secreted by plant roots serve important roles as chemical attractants and repellents in the rhizosphere, the narrow zone of soil immediately surrounding the root system. The chemicals secreted into the soil by roots are broadly referred to as root exudates. Through the exudation of a wide variety of compounds, roots may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbioses, change the chemical and physical properties of the soil, and inhibit the growth of competing for plant species. Walker et al. [8], presented that the root exudates play an important role in the interaction of the plants with the microbes in the soil. In addition to the classical roles of providing mechanical support and allowing water/nutrient uptake, roots also perform certain specialized roles, including the ability to synthesize, accumulate, and secrete a diverse array of compounds.

3. Materials and methods

SEM analysis was performed to assess the ability of AB211 to colonize the plant root surface-

A. Materials required

M9 minimal media (100 ml)- Disodium hydrogen phosphate-1.28g, Di-hydrogen potassium phosphate-0.3g; Sodium chloride-0.05g, Ammonium chloride -0.1g. After autoclaving of M9 media, filter sterilization was performed to add the following compound- 0.2 ml of 1M $MgSO_4$, 0.01 ml of 1M $CaCl_2$, 0.5 gm of glucose.M8 buffer preparation (For 500ml)-
1. 22 mM Na_2HPO_4, 22 mM KH2PO4, 100 mM NaCl, (pH 7.0).

1) Protocol

The strain AB211 was inoculated in 50 ml of complete M9 medium. Then the culture was then allowed to incubate at 37^0C overnight under shaking conditions. After that cells were harvested by centrifugation at 6000rpm for 5 minutes. The harvested cells were washed twice in M8 buffer and were re-suspended again in 250 ml of M8 buffer. Then roots were washed continuously with sterile distilled water to get rid of the attached soil particles. The washed roots were then soaked in bacterial suspension under aseptic conditions for 1 hour. Then they were transferred to sterile bottles. A control was also set up which consisted of M8 buffer but without the cells. Then both the test and the control setups were transferred to plant growth chamber at 28^0C (16hr of light followed by 8hr of darkness). The roots were then cut and fixed in 2.5% glutaraldehyde in 0.075M Phosphate buffer. These roots were kept overnight and further were processed for dehydration and visualization under Scanning Electron Microscopy (SEM).

B. Analysis of the effect of root exudates on the growth curve of AB211

1) Collection of root exudates

For the collection of root exudates, 50 maize seedlings were prepared. The 7days old seedlings were taken out and the plant roots were washed several times with sterile double distilled water very gently to get rid of the attached soil particles. The seedlings were divided in 5 sets and in each set 10 plants were placed into a 250ml flask and the roots were submerged in 100ml sterile double distilled water. All 5 sets were placed in a plant growth chamber for 24 hours which consisted of 16 hours of the light period followed by 8 hours of darkness at 28^0C with gentle shaking at 80 rpm. The combined solution of 500 ml was divided into 2 parts-The first half (250 ml) was lyophilized and stored for future purpose. The other half (250 ml) solution was lyophilized and then dissolved in sterile double distilled water to achieve 50 x concentrations. This was filter sterilized and used for the further experimentations.

2) Bacteria growth curve analysis

An overnight grown culture of AB211 was inoculated in M9 media (supplemented with 0.5%w/v glucose) adjusting the O.D

(at 600nm) to be 0.1. Media was supplemented with root exudates (1X). Control was kept untreated. Both the experimental sets were kept at 37°c in shaking condition. O.D. was checked at every 30mins interval. And putting the O.D. valued against time, the growth curve was generated.

C. Real-time PCR was performed to analyze the effect of root exudates on the expression pattern of genomic elements involved in plant growth modulation

1) Treatment of AB211 with root exudates

To assess the effect of root exudates on B. aryabhattai AB211, 25 ml of M9 minimal medium (supplemented with 0.5% w/v glucose) were inoculated with the strain AB211 along with the supplementation of root exudates(1X) and incubated for different time periods of 6 hours,12 hours and 24 hours. Control was taken as untreated with root exudates. After this the AB211 cells were harvested, RNA was isolated and cDNA was prepared.

2) RNA extraction from AB211 cells

First 50 ml of bacterial culture (optical density-1.0) were plated down. 500µl of Trizol reagent was mixed to the pellet and the pellet was re-suspended in the reagent. The mixture was kept at room temperature for 10-15 minutes. 100µl of chloroform was added to the mixture and it was vortex for 1 minute. The mixture was kept for 5 minutes at room temperature. Then it was centrifuged at 12000g for 15 minutes. After centrifugation, the clear supernatant was transferred to another tube. An equal volume of isopropanol was added to the clear supernatant and it was gently mixed and was kept for 20 minutes at room temperature. Then again the mixture was centrifuged at 16000g for 15 minutes at 4^0C. After that the supernatant was allowed to decant and it was washed with 200µl of 70% ethanol. Then centrifugation at 16000g for 5 minutes was carried out and it was washed again. The supernatant was allowed to decant and the pellet was air dried inside the Laminar Air Flow (LAF). 20µl of Nuclease-Free water was added to the pellet and it was kept at 65^0C in the heating block for 10 minutes. Then it was snap chilled. From the RNA solution, 0.5µlwas taken and 24.5µl of Nuclease-Free water was added to dilute it 50 times. The concentration of the sample was measured in the nano-drop machine. Then 2µl of Dnase1 was added to the remaining concentration of RNA solution and was kept at 37^0C for 30 minutes. After that 500µl of Trizol reagent was added to this mixture and the steps were repeated from (1-11). The pellet was dissolved in 15µl of NF water. It was heated at 65^0C in the heat block for 10 minutes. Then it was again snap chilled and concentration of RNA was measured. A control PCR (Polymerase Chain Reaction) was carried out to check the absence of DNA. It was checked by performing Agarose Gel Electrophoresis.

D. cDNA preparation

1) Materials required

Primer (random hexamer: 20pmol), RNA-5µg, dNTPs (2.5mM)-4µl, NF Water, 5X Buffer-4µl, RNase inhibitor-1 µl, Reverse Transcriptase-1µl

2) Protocol

The PCR mix was made by adding the components in the following amounts:

- Primers (20pmol)-4µl
- RNA is 5µg (volume added depending on concentration). Then2.5mM dNTP- 4µl .Volume was made up to 14µl by adding water.

The following protocol was set in the PCR machine:

To this mixture, the following components were added.

- 5X Buffer-4µl
- RNase inhibitor-1 µl
- Reverse Transcriptase-1µl

Then the cDNA which was thus obtained was subjected to Real-Time PCR to study the effect of root exudates on various genes of Bacillus aryabhattai AB211.

E. Real-time PCR for genes

Study the effect of root exudates on the expression of plant growth promoting genes in Bacillus aryabhattai AB211. Since the whole genome sequencing has already been performed, so we have selected 11 genes from there. The genes whose expressions are to be determined are as follows: Nitrilase, Indole-3-glycerol phosphate synthase, Siderophore biosynthesis diaminobutyrate-2-oxoglutarate aminotransferase ABC-type Fe(³⁺)siderophore transport system, permease component, Phosphate transport system regulatory protein, PhoU, Alkaline phosphatase synthesis transcriptional regulatory protein PhoP, Exopolyphosphatase, Phosphate ABC transporter, periplasmic phosphate-binding protein PstS, 2, 3-butanediol dehydrogenase, R-alcohol forming,(R) and (S)-acetoin specific, Acetolactate synthase large subunit, Acetoin dehydrogenase E1 component beta-subunit.

F. Materials required:

- Forward primer (5µM): 1.2µl
- Reverse primer (5µM): 1.2µl
- CDNA: 1µl
- Nuclease-Free Water: 6.6µl
- SyBr Green: 10µl

1) Procedure:

The Real-Time Reaction Mix was prepared by combining all the non-enzymatic components except the fluorescent dye SyBr Green and the samples. Now the respective samples and the fluorescent dye was added carefully and were mixed well. The contents were transferred to respective wells of a 96-Well Reaction Plate. The plate was sealed and transferred to the thermal cycler block. Then Real Time was performed under the following conditions: An initial cycle at 95^0C for 3mins, followed by 40 cycles of 95^0C for the 30s, 49^0C for 45s and 72^0C for 30s. Then those replicates were carried out for each target gene. After that quantification was analyzed based on the threshold cycle (Ct) values.

4. Results and discussion

A. *Root association via Scanning Electron Microscopy (SEM)*

The main feature which was observed about Bacillus aryabhattai AB211 is its surface attachment i.e. its ability to efficiently colonize the plant root surface, thereby, in turn, exerting its beneficial effects on the plants. Through the whole genome sequencing, it has already been elucidated that the genes responsible for biofilm formation and extracellular polysaccharide (EPS) formation necessary for adhesion and colonization on the plant root surface are present in its genome. It was evident from the SEM data that the AB211 strain can effectively adhere to the root surface, thus successful colonization on maize roots was observed.

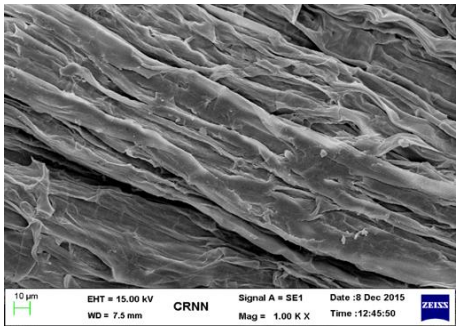

Fig. 1. SEM micrograph of control maize root

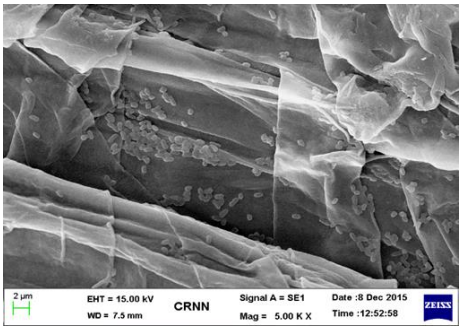

Fig. 2. SEM micrograph of control maize root colonized

B. *Growth characteristics in effect of root exudates*

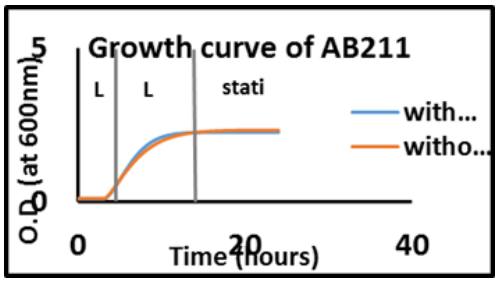

Fig. 3. Growth curve

Root exudates are composed of many amino acids, organic acids, phenol compounds, and many other secondary metabolites. Thus it is an excellent source of nutrients to the rhizobacteria. In the growth pattern of Bacillus aryabhattai AB211, there was not much of significant difference with the supplementation of root exudates. However, it was evident from the data that the strain can utilize the root exudates and grow in presence of it.

C. *Real-time PCR results:*

As it has been earlier said that the whole genome sequence of Bacillus aryabhattai AB211 has already been performed, so we have selected 11 genes supposed to be involved in plant growth promotion. The primers for the respective genes were constructed and the quantitative study of these genes was carried out by performing Real-Time PCR of the individual genes respectively. 16S rRNA gene was taken as an internal control. The relative expressions of the following genes were studied and the patterns found are discussed as follows:

- The acetolactate synthase is the enzyme involved in the two-step pathway of acetoin formation which converts pyruvate to acetoin. Acetoin is a volatile organic compound which is found to be produced by very few microorganisms which impart great plant growth promotion and also serves as a plant defense. It was found that with respect to control, there was (0.93±0.335) fold change at 6hours, (1.337±0.601) fold change at 12hours and (0.048±0.001) fold change at 24 hours (Fig. 4). So from this data, it can be concluded that the gene encoding for acetolactate synthase is being up-regulated starting from 6 hours till 12 hours indicating that the root exudates has exerted its effect on this gene enabling the interaction of the bacteria with the plant root surface and increase in acetoin production in initial phase but after reaching at 24 hours, the gene expression was down regulated so less acetoin formed and lesser impact on plant growth promotion.

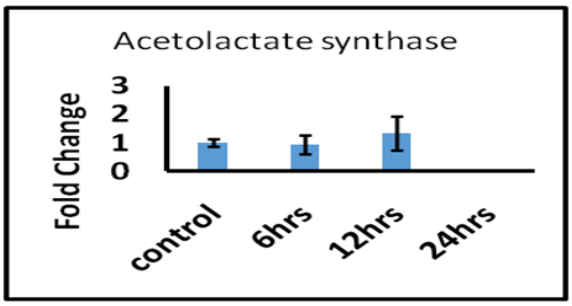

Fig. 4. Acetolactate synthase

- The acetoin dehydrogenase is induced in presence of acetoin. The E1 component of the beta subunit of this enzyme is involved in catalyzing the formation of acetate and acetyl CoA from acetoin. It was found that with respect to control, there was (1.463±0.407) fold change at 6 hours, (1.748±0.366) fold change at 12 hours, (Fig. 5) indicating the gene encoding for acetoin dehydrogenase is being up-regulated during this period in presence of the root exudates has started

exerting its effect on this gene thus catalyzing the previously formed acetoin further into acetyl CoA and acetate, but after reaching at 24 hrs, it reduced to 0.047±0.011 fold.

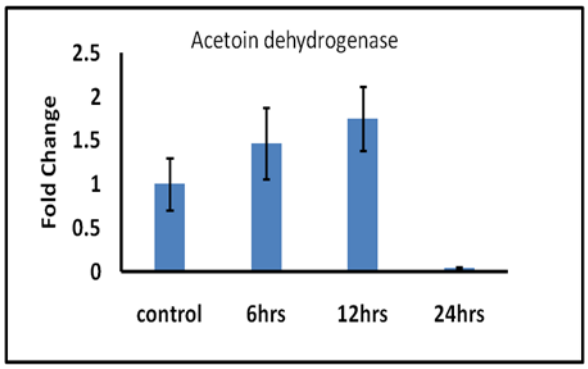

Fig. 5. Acetoin dehygrogenase

- 2,3-butanediol dehydrogenase or BDH catalyzes a reversible reaction between acetoin and 2,3-butanediol. In addition, the enzyme usually catalyzes the non-reversible conversion of diacetyl to acetoin. It was found that with respect to control, there was (0.246±0.001) fold change at 6 hrs, (0.481±0.089) fold change at 12 hrs and (0.315±0.013) fold change at 24 hours (Fig. 6). The data shows, the root exudate doesn't have much effect on the formation of 2, 3-bunadiol, the volatile compound which acts as a plant defense system.

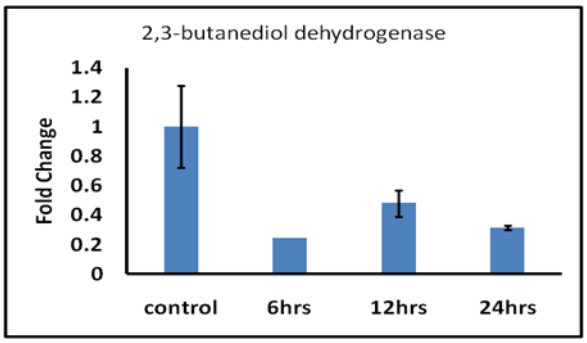

Fig. 6. 2, 3- butanediol dehydrogenase

- The nitrilase enzyme facilitates plant hormone synthesis (IAA) and nitrogen utilization. It is involved in the formation of IAA in a tryptophan independent pathway. It was found that with respect to control, there was (1.157±0.276) fold change at 6 hrs, (3.389±0.082) fold change at 12 hrs and (0.186±0.0492) fold change at 24 hours, (Fig. 7) indicating the gene encoding for this enzyme is being up-regulated during the log phase and late log phase of the bacteria, when the root exudates have exerted their maximum effect on this gene during this period, thus the formation of IAA has started, but at stationary phase, the gene expression was significantly down-regulated.

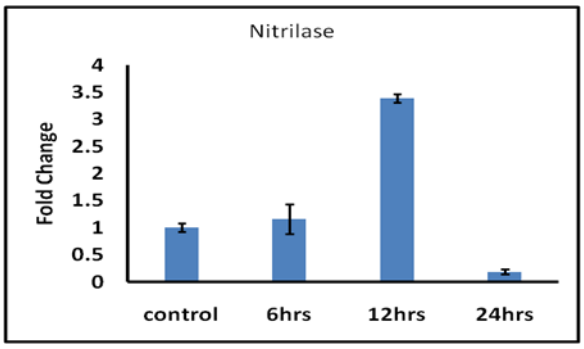

Fig. 7. Nitrilase

- The Indole-3-glycerol phosphate synthase synthesizes indole-3-glycerol phosphate, which plays an important role in the synthesis of aromatic amino acids like phenylalanine, tyrosine and tryptophan biosynthesis. It was found that with respect to control, there was (1.626±0.389) fold change at 6 hrs, (5.708±0.278) fold change at 12 hrs and (0.139±0.008) fold change at 24 hrs, (Fig. 8). So, the data suggests, that the root exudates are helping the bacteria to synthesize the amino acids during the 6-12hrs. However, that interaction decreases after that.

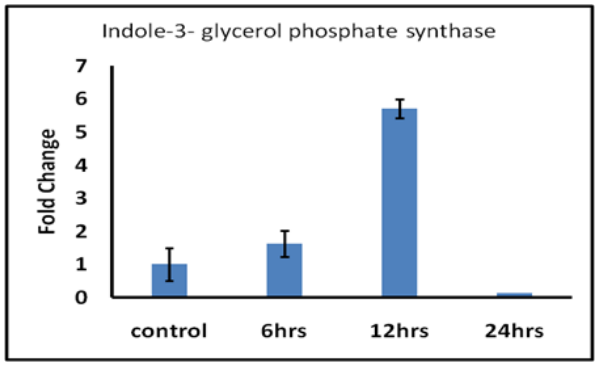

Fig. 8. Indole-3- glycerol phosphate synthase

- The siderophore diaminobutyrate-2-oxoglutarate aminotransferase enzyme is involved in the synthesis of siderophore. It was found that with respect to control there was (0.642±0.511) fold change at 6 hrs, (2.426±0.119) fold change at 12 hrs and (0.032±0.001) fold change at 24 hrs (Fig. 9). The data shows the gene encoding for this enzyme is being upregulated in presence of root exudates during the initial hours and the maximum synthesis of siderophore during this period enabled the greater scavenging of iron which is present in insoluble form and in trace amounts in the soil, but after reaching at 24 hrs, the gene expression was significantly down-regulated.

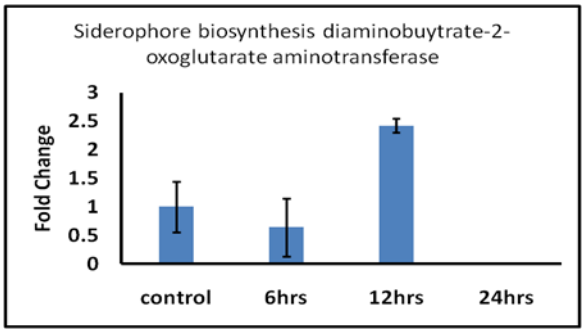

Fig. 9. Biosynthesis

- The ABC-type Fe3+ siderophore transport system, permease component helps in the transport and uptake of Fe3+ from the surrounding environment. It was found that with respect to control there was (1.802±0.329) fold change at 6 hrs, (2.063±0.242) fold change at 12 hrs and (0.057±0.019) fold change at 24 hrs (Fig. 10). This signifies this gene is being upregulated till the early stationary phase of the bacteria enabling more uptake of iron which is an essential nutrient. However, at 24 hrs, the gene expression was lower.

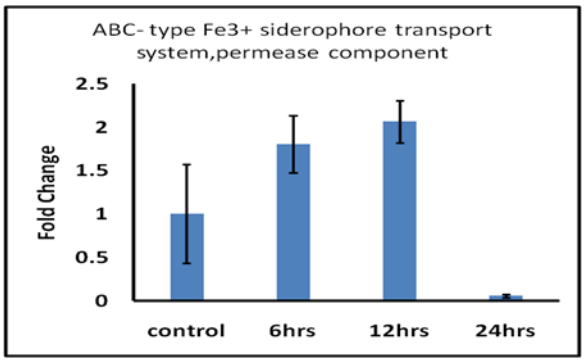

Fig. 10. ABC-type Fe3+ siderophore transport system, permease component

- The alkaline phosphatase synthesis transcriptional regulatory protein pho P, member of two components regulatory system Pho P/Pho R, is involved in the regulation and uptake of phosphorus which is present in low concentration in nature in the inorganic state. It has been found that with respect to control there was (0.547±0.113) fold change at 6 hrs time, (1.215±0.621) fold change at 12 hrs and (0.071±0.037) fold change at 24 hrs (Fig. 11). So the data represents, the root exudates is helping AB211 enabling more uptake of phosphorus which is an essential nutrient during its growth phase, but after reaching stationary phase, the gene expression was significantly down-regulated indicative of the fact that the interaction of the root exudates with bacteria has been lessened.

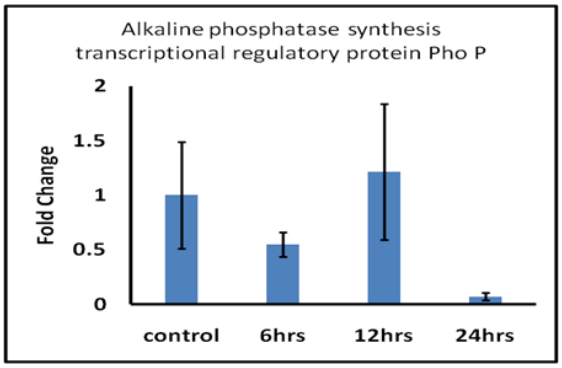

Fig. 11. Alkaline phosphatase synthesis transcriptional regulatory protein pho P

- The phosphate transport system regulatory protein, pho U is involved in the uptake of free inorganic phosphate which illustrates that Pho U participates in Pi transport and plays the regulatory role of the phosphate-specific transport system. It was found that with respect to control there was (1.753±0.207) fold change at 6 hrs of time, (2.063±0.242) fold change at 12 hrs of time and (0.774±0.147) fold change at 24 hrs of time (Fig. 12). So this can be concluded that root exudates are enabling specific regulation of phosphate transport in AB211 during its growth, but after reaching at 24 hrs, its effect is lessening.

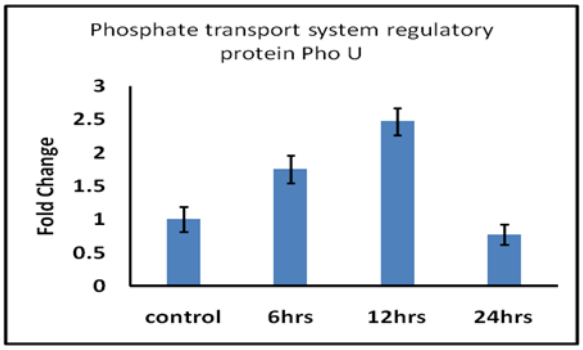

Fig. 12. Phosphate transport system regulatory protein, pho U

- The phosphate ABC transporter, periplasmic phosphate binding protein (Pst S) is involved in phosphate import and this protein is accompanied by an ATP binding protein(Pst B) and two permease proteins (Pst C and Pst A).This protein accumulation is enhanced under phosphate starvation conditions. It was found that with respect to control, there was (0.952±0.014) fold change at 6 hrs, (2.661±0.655) fold change at 12 hrs and (0.044±0.012) fold change at 24hrs of time(Fig. 13). So from this data, it can be concluded that the gene encoding for this enzyme is being up-regulated during the initial time points, indicating that root exudates have exerted its effect on the phosphate transport pathway.

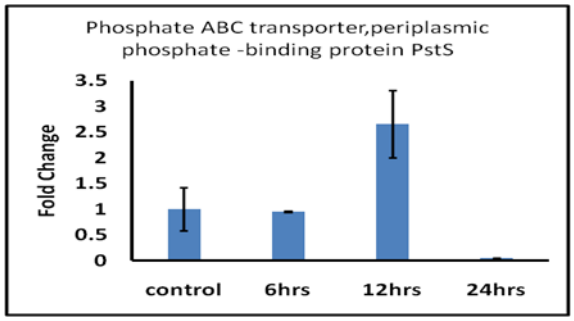

Fig. 13. Phosphate ABC transporter, periplasmic phosphate binding protein (Pst S)

- The exopolyphosphatase is a phosphatase enzyme which catalyzes the hydrolysis of inorganic phosphate and plays an important role in maintaining the appropriate levels of intracellular polyphosphate. It was found that with respect to control there was (1.578±0.849) fold change at 6 hrs, (1.613±0.348) fold change at 12 hrs and (0.035±0.005) fold change at 24 hrs of time (Fig. 14). The data signifies the effect of root exudates is high at the initial growth stage of AB211 on its phosphate solubilization pathway; however, the effect gets reduced with time.

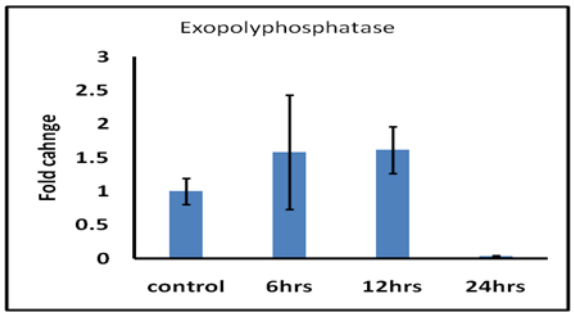

Fig. 14. Exopolyphosphatase

5. Conclusion

Bacillus aryabhattai strain AB211 is a plant growth promoting rhizobacteria which are capable of association with plant roots and establish interaction with the plants. The root exudate which acts as a medium of communication between them can influence the genomic elements of AB211 in their plant growth modulation pattern. It was found that the bacterial strain can utilize the root exudates as a nutrient source and grow successfully. The root exudate can up regulate the siderophore formation and phosphate solubilization pathways in AB211significantly, suggesting that these pathways are more influenced by the root exudates. It was proposed previously that strain AB211 can produce IAA in a tryptophan independent pathway with the help of the enzyme Nitrilase. The expression of nitrilase gene was found to be increased in presence of root exudate, confirming the presence of the alternate pathway and its upregulation during an interaction and association with plant roots. However, the genes involved in volatile compound formation (acetoin and 2, 3- butanediol) were found less affected upon addition of root exudates and possibly require direct interaction with plant roots. Further studies are indeed essential to better understand the molecular basis of the interaction between stain AB211 and plant roots, followed by plant growth modulation. Finally, the genes involved in the formation of volatile compounds were less affected upon addition of root exudates possibly requiring more direct interaction with plant roots.

References

[1] Antoun H, Kloepper JW. Plant growth promoting rhizobacteria. In: Brenner S, Miller JH, editors. Encyclopedia of Genetics. Academic; New York: 2001. pp. 1477–1480.
[2] Arshad, M., and Frankenberger, W. T. (1991). Microbial-production of plant hormones. Plant Soil 133, 1–8.
[3] B.G. Kang, W.T. Kim, H.S. Yun, S.C. Chang. Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol. Rep., 4 (2010), pp. 179-183.
[4] .Bhattacharyya C., Bakshi U., Mallick I., Mukherji S., Bera B., Ghosh A. (2017). Genome-guided insights into the plant growth promotion capabilities of the physiologically versatile Bacillus aryabhattai strain AB211. Frontiers in Microbiology.
[5] H. Marschner. Mineral Nutrition of Higher Plants, Academic Press, London (1995).
[6] R. Hayat, S. Ali, U. Amara, R. Khalid, I. 5.AhmedSoil beneficial bacteria and their role in plant growth promotion: a review Ann Microbiol., 60 (2010), pp. 579-598.
[7] S. Nardi, G. Concheri, D. Pizzeghello, A. Sturaro, R. Rella, G. Parvoli. Soil organic matter mobilization by root exudates, Chemosphere, 5 (2000), pp. 653-658.
[8] Walker, H.P. Bais, E. Grotewold, J.M. Vivanco. Root exudation and rhizosphere biology, Plant Physiol., 132 (2003), pp. 44-51.