# Gene Expression Profile of a Plant Growth Promoting Rhizobacterium *Bacillus atrophaeus* UCMB-5137 in Response to Maize Root Exudate Stimulation

Liberata Mwita, Wai Yin Chan, Oleg N. Reva
Department of Biochemistry
University of Pretoria
Pretoria, South Africa
E-mails: libemwi@yahoo.co.uk,
annie.chan@fabi.up.ac.za,oleg.reva@up.ac.za

Sylvester Lyantagaye
Department of Molecular Biology and Biotechnology
University of Dar-es-salaam
Dar-es-salaam, Tanzania
E-mail: lyantagaye@gmail.com

Svitlana V. Lapa, Lilija V. Avdeevad
Department of Antibiotics
D.K Zabolotny Institute of Microbiology and Virology
Kiev, Ukraine
E-mails: slapa@ukr.net, avdeeva@imv.kiev.ua

*Abstract*— **Plant Growth Promoting Rhizobacteria (PGPR) are widely used in agriculture as an ecologically safe replacement of chemical pesticides and fertilizers. In this work, gene expression regulation under stimulation by maize root exudate was studied in a PGPR strain *Bacillus atrophaeus* UCMB-5137. A strong up-regulation of synthesis of (p)ppGpp (guanosine pentaphosphate) alarmone was observed. This alarmone is associated with the stringent response in bacteria, but its involvement in regulation of plant colonization has never been reported. Comparison of the profiles of gene expression at the classical stringent response and in the current experiment showed only a partial overlap that allowed us to make a conclusion that the tested bacteria had not been starved but responded specifically to the root exudate stimulation and (p)ppGpp alarmone is involved in this complex response. Comparison of the gene expression regulation in *B. atrophaeus* UCMB-5137 with a report obtained on a similar experiment with another PGPR strain *B. amyloliquefaciens* FZB42 showed that these two closely related organisms had used different strategies of plant colonization.**

***Keywords—Bacillus atrophaeus; plant growth promoting; gene expression; transcriptional regulation.***

## I. INTRODUCTION

Application of beneficial microorganisms residing in the rhizosphere to promote plant growth is a promising and efficient method of biocontrol of plant pathogens; it is safe for human health, other soil microorganisms and the environment. Plant growth promoting rhizobacteria (PGPR) is a diverse group of microorganisms living in the plant rhizosphere. They exert beneficial direct or indirect effects on plant growth [1]. Till now, the gene regulation in PGPR *Bacillus* during root colonization was studied only in a few bacteria [13], and it remains unclear to which extend other PGPR bacteria follow the same pattern of gene regulation. Introduction of new generation sequencing techniques (NGS) made it possible to investigate gene expression profiles under similar experimental conditions in multiple taxonomically related PGPR bacteria by using the RNA-Seq approach. Here, we want to present our very first result in a planned series of experiment. *Bacillus atrophaeus* is one of PGPR able to colonize plants by forming thick colonies on the root surface. This study aimed at investigating the gene expression profile in UCMB-5137 stimulated by maize root exudate and compare the results to those obtained for PGPR *B. amyloliquefaciens* [13]. Understanding gene regulation underlying interaction between this bacteria and plants will broaden our understanding on how the PGPR bacteria belonging to *Bacillus subtilis* taxonomic clade can be effectively used to promote plant growth taking into consideration the commonalities and differences in gene regulation in these bacteria. The next section will provide information on the previous related researches. Then, the section on the methods is followed by a discussion of results obtained in this study and a conclusion section.

## II. STATE OF THE ART

Use of Bacilli as biocontrol agents is an advantage over other bacteria because they form thermostable and chemically resistant endospores [12]. Complete genome sequence of *Bacillus atrophaeus* UCMB-5137 showed

 ISBN: 978-1-61208-409-1 

multiple horizontally acquired unique genes, which were hypothesized as possible source of an extraordinary activity in plant root colonization [2]. This strain has shown a significant potential of promoting plant growth in greenhouse trials (ongoing research). It was therefore interesting to study gene regulation mechanisms taking place as it interacts with plant. The interaction between *Bacillus amyloliquefaciens* FZB42 under maize root exudate stimulation was studied previously using a method developed by Fan et al. [13]. It is demonstrated in this publication that the maize root exudate added to liquid medium may simulate plant derived signals stimulating root colonization behavior in PGPR bacteria. We used their approach except for transcriptomic profiling, where we used RNA-Seq. RNA-Seq is a sequence based approach, which has several advantages over microarray techniques including a broader dynamic range of expression levels [14]. Our expectation was that the gene expression profiles obtained by both methods will be comparable and it will be possible to identify similarities and differences in plant colonization strategies of these two PGPR strains. *B. atrophaeus* UCMB-5137 was selected for this study because being as active PGPR bacterium as *Bacillus amyloliquefaciens* FZB42, it showed some alterations in root colonization behaviour, which will be discussed in the conclusion.

## III. MATERIALS AND METHODS

Root exudate was extracted as it was described previously [13]. *B. atrophaeus* UCMB-5137 was grown up on the liquid medium (1 C medium containing 0.1% glucose) with (experiment) and without (control) 0.25 mg/ml maize root exudate. RNA from two independent experiment samples and three control samples were extracted using ZR Fungal/ Bacteria RNA Mini Prep kit and sequenced by MiSeq 500 Illumina in Inqaba (Pretoria, South Africa). They were quality controlled and trimmed, and then mapped against the available complete genome sequence of UCMB-5137 [2] using CLC Genomics Workbench 7. EDGE statistics approach was used to identify significantly up- and down- regulated genes (at least 3 folds difference) with a p-value $\leq 0.01$.

## IV. RESULTS AND DISCUSSION

Tiny amounts of root exudate caused a significant up and down regulation of many genes. A remarkable up-regulation was observed for genes responsible for the following mechanisms: stress response, biofilm formation, transcription and translation regulation, heme synthesis, ribosome proteins, cell division, vitamin synthesis and quorum sensing. Meanwhile, down-regulation of genes belonging to the following functional categories: transport proteins, DNA replication, purines and pyrimidines synthesis, sporulation and secondary metabolite biosynthesis were observed. Remarkable, it was the complete silencing of multiple phage associated genes. Approximately, 70% of the genes regulated by root exudates were annotated with known functions whilst 30% were annotated as hypothetical, putative or unknown proteins. An overview of regulated genes is shown in Table 1.

TABLE I. GENES REGULATED BY ROOT EXUDATE

| Pathway | # up-regulated | # down-regulated |
|---|---|---|
| Aerobic respiration | 4 | 1 |
| Amine and polyamine synthesis | 2 | 1 |
| Amino acid synthesis | 1 | 18 |
| Anaerobic respiration | 1 | 3 |
| Antibiotic and bacteriocin synthesis | 1 | 7 |
| Aromatic compound synthesis | 1 | 1 |
| Biofilm formation and regulation | 1 | 2 |
| Carbohydrate synthesis and degradation | 3 | 19 |
| Cell division | 4 | 0 |
| Cell wall protein synthesis | 12 | 5 |
| Chemotaxis and motility | 1 | 5 |
| Co-factor and vitamin synthesis and utilization | 8 | 12 |
| DNA replication | 2 | 2 |
| Fatty acid synthesis and degradation | 0 | 6 |
| Formaldegyde assimilation | 1 | 1 |
| Nucleotide synthesis | 0 | 9 |
| Other pathways of degradation of complex compounds | 2 | 4 |
| Protein maturation and activation | 14 | 3 |
| Ribosomal proteins | 10 | 4 |
| Sporulation regulation | 3 | 3 |
| Stress response and detoxication | 28 | 5 |
| Transcriptional regulation | 31 | 2 |
| Transport and uptake | 5 | 21 |
| Urea degradation | 0 | 2 |

One of the important observation was a strong up-regulation of synthesis of the alarmone (p)ppGpp (guanosine pentaphosphate). This alarmone is known to be responsible for the stringent response in bacteria, usually associated with starvation [3]. It controls many metabolic reactions including inhibition of protein synthesis, DNA replication and transcription when bacteria experience a shortage of nutrients. Surprisingly, this alarmone activation was in response to the root exudate stimulation while the control bacteria did not experience any shortage of nutrients. It was hypothesized that the stringent response in this PGPR bacterium was needed to prepare the organism for root colonization. It was interesting to compare genes expression profiles under root exudate stimulation against the classical stringent response described by Eymann et al. [3]. One similarity was that the general stress response pathways were

 ISBN: 978-1-61208-409-1 

up regulated in both cases. Another similarity was down-regulation of the amino acid biosynthesis, DNA replication, thiamine biosynthesis, fatty acid biosynthesis and nucleotide biosynthesis metabolic pathways. Silencing of phage-related genes was also reported in both cases. However, a number of discordances were also observed. Pathways of cell wall biosynthesis, ribosome proteins, protein translation, maturation, activation and utilization were negatively controlled by stringent response, but they were up-regulated in this study. Contrary, cross-membrane transportation, urea degradation and preparation for sporulation were up-regulated by the stringent response but down-regulated by the root exudate.

Stress related proteins were up-regulated by maize root exudates. A stress state in bacteria was defined by Lengeler et al. [4] as any change in the environment that provokes a significant change in cell physiology. Up-regulated stress related proteins included heat stress induced protein, manganese superoxide dismutase, organic hydroperoxide resistance reductase B, alkyl hydroperoxide reductases, cold shock protein CspA and integral membrane protein YggT responding to heat, oxidative, cold and osmotic stresses respectively. Bacteria may produce multiple stress resistance proteins to anticipate for future stress [5]. Some of these stress resistance proteins are detoxifying enzymes, e.g., superoxide dismutase and thiol peroxidase, which are used to neutralize oxidative burst from plants [6].

Transcriptional regulators mediate bacteria adaptive responses to continuous changes in their environment [7]. In this study, many transcriptional regulators responsible for variety of responses were up regulated. Among them there were SinR regulator and transition state regulatory protein AbrB, which regulate post exponential phase responses (competence, sporulation and biofilm formation); transport and uptake regulators Fur (iron uptake) and Zur (zinc uptake); peroxide stress regulator PerR; thiol specific oxidative response regulator Spx; and RsbR positive regulator of sigma-B. Sigma-B is a general stress transcription top level regulator, which is activated when bacteria is exposed to environmental stresses.

Remarkably, most of carbohydrate, amino and fatty acid biosynthesis and degradation metabolic pathways were down regulated by the root exudate. This might be connected to the observed up-regulation of the GntR family transcriptional regulator, which represses the general metabolism.

Communication within and between bacterial occur through autoinducers, among which the most studied are quorum sensing autoinducers [8]. Bacteria use quorum sensing to control their cell population density and sense metabolic potential of the environment [4]. Up regulation of *luxS* gene, which is involved in synthesis of autoinducer 2 (AI-2), was observed. AI-2 is used for interspecies cell-to-cell communication. Up-regulation of *luxS* may be important to enable communication between bacteria cells and plant cells during root colonization and biofilm formation. Biofilm is an extracellular matrix produced by complex aggregate of cells that adhere to each other [9]. Bacteria produce biofilm to attach and maintain contact with the host plant during bacteria-plant interaction [10]. It was observed in previous research that *B. atrophaeus* UCMB-5137 colonize plants by forming biofilm on the root surface. Up-regulation of Veg protein, which stimulates biofilm formation [9] and YMCA protein, – a master regulator for biofilm formation, – is in agreement to this observation.

## V. CONCLUSION AND FUTURE WORK

Even though (p)ppGpp was up regulated and some pathways might have been controlled by the stringent response, we concluded that the bacteria have not been starved in our experiment and the gene transcription was regulated by several other signalling pathways. Anyway, the involvement of (p)ppGpp alarmone regulation in plant root colonization have never been reported before including the recently published paper on gene expression regulation by root exudate in a PGPR strain *B. amyloliquefaciens* FZB42 [11]. In general, the gene expression profile in *B. amyloliquefaciens* FZB42 was quite different to that in *B. atrophaeus* UCMB-5137 with strong up-regulation of motility proteins and antibiotic biosynthesis. Also, dissimilar was the pattern of root colonization observed by luminescent microscopy (Figure 1).

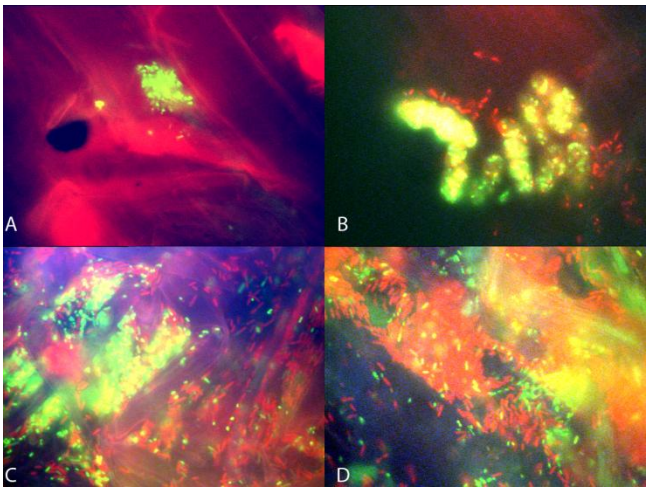


Figure 1. *Colonization of barley roots by B. atrophaeus* UCMB-5137 *(A and B) and by B. amyloliquefaciens* UCMB-5113 *(C and D).*

*B. atrophaeus* had formed thick colonies on the root surface, while *B. amyloliquefaciens* had been characterized by active penetration into plant tissues and an endophytic growth [12]. This difference in behavioural strategies may explain the observed striking differences in the root exudate stimulated gene expression profiles in these two relative PGPR bacteria. In the future, we want to repeat this experiment with a number of selected PGPR *Bacillus*, belonging to closely relates species *B. amyloliquefaciens*, *B. subtilis* and *B. mojavensis*, which have been sequenced recently (see NCBI bio-projects with the reference numbers: 176685, 176687, 176696, 176688, 176703 and 176701).

 ISBN: 978-1-61208-409-1 

## ACKNOWLEDGMENT

This work was supported by the funding from the Southern African Biochemistry and Informatics for Natural Product [http://www.sabina-africa.org/], and by a grant provided by the Genomic Research Institute (GRI) at the University of Pretoria for genome sequencing.

## REFERENCES

[1] B. Lugtenberg and F. Kamilova, "Plant Growth Promoting Rhizobacteria," Annu. Rev. Microbiol., vol. 63, 2009, pp. 541-56, doi:10.1146/annurev.micro.62.081307.162918.

[2] W. Y. Chan et al. "Draft genome sequence of Bacillus atrophaeus UCMB-5137, a plant growth-promoting rhizobacterium," Genome Announc., vol. 1, 2013, pp. e00233-13, doi: 10.1128/genomeA.00233-13.

[3] C. Eymann, G. Homuth, C. Scharf, and M. Hecker, "Bacillus subtilis functional genomics : global characterization of the stringent response by proteome and transcriptome analysis," J. Bacteriol. vol. 184, 2002, pp. 2500-2520.

[4] J. W. Lengeler, G. Drews, and H. G. Schlegel, "Biology of Prokaryotes," Blackwell Science Ltd, Germany, 1999, pp. 652.

[5] C. W. Price, "Protective function and regulation of the general stress response in Bacillus subtilis and related gram positive bacteria," *In* G. Storz and R. Hengge-Aronis (ed), Bacteria stress responses. ASM press, Washington, D.C, 2000, pp. 179-197.

[6] R. B. Abramovitch, J. C. Anderson, and G. B. Martub, "Bacteria elicitation and evasion of plant innate immunity," Nat Rev Mol Cell Biol., vol. 7, 2006, pp. 601-611.

[7] J. L. Ramos et al. "The TetR family of transcriptional repressors," Microbiology and Molecular Biology Reviews, 2005, pp. 326–356, doi:10.1128/MMBR.69.2.326–356.2005.

[8] M. B. Miller and B. L. Bassler, "Quorum sensing in bacteria," Annu. Rev. Microbiol., vol. 55, 2001, pp. 165-99.

[9] Y. Lei, T. Oshima, N. Ogasawara, and S. Ishikawa, "Functional Analysis of the Protein Veg, which stimulates biofilm formation in Bacillus subtilis,"Journal of Bacteriology, vol. 195, 2013, pp. 1697-1705.

[10] B. E. Ramey, M. Koutsoudis, S. B. von Bodman, and C. Fuqua, "Biofilm formation in plant microbe associations," Current Opinion in Microbiology, vol. 7, 2004, pp. 602–609.

[11] K. Kierul et al. "Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium Bacillus amyloliquefaciens FZB42," Microbiology, vol. 161, 2015, pp. 131-147, doi: 10.1099/mic.0.083576-0.

[12] B. Fan, R. Borriss, W. Bleiss, and X. Wu, "Gram-positive rhizobacterium Bacillus amyloliquefaciens FZB42 colonizes three types of plants in different patterns," J. Microbiol., vol. 51, 2013, pp. 544, doi: 10.1007/s12275-012-1439-4.

[13] B. Fan et al. "Transcriptomic profiling of Bacillus amyloliquefaciens FZB42 in response to maize root exudates," BMC. Microbiol., vol. 12, 2012, pp. 116.

[14] Z. Wang , M. Gerstein, and M. Synder, "RNA-Seq : a revolutionary tool for transcriptomics," Nat Rev Genetics, vol. 10, 2009, pp. 57-63.

 ISBN: 978-1-61208-409-1 