MONITORING OF COBALT ADAPTATION OF ENVIRONMENTAL BACTERIAL ISOLATES USING ATR-FTIR SPECTROSCOPY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

JANUARY 2014

Approval of the thesis:

MONITORING OF COBALT ADAPTATION OF ENVIRONMENTAL BACTERIAL ISOLATES USING ATR-FTIR SPECTROSCOPY

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ABSTRACT

MONITORING OF COBALT ADAPTATION OF ENVIRONMENTAL BACTERIAL ISOLATES USING ATR-FTIR SPECTROSCOPY

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February 2014, 90 pages

This thesis investigates the effects of cobalt acclimation in three distinct bacteria. Heavy metals at high concentration can be toxic for bacteria. However, upon prolonged exposure bacteria can acclimate to grow in the presence of heavy metals. As a result of the acclimation, bacteria alter their metabolism and molecular structures.

Aim of this study was to examine these alterations in cobalt acclimated bacteria via attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy on viable samples. *Bacillus* sp., and *Pseudomonas* sp., isolated from a temperate shallow lake, and a well-established strain of *E. coli* were investigated. Results hinted drastic changes in cell membrane and wall dynamics of *Bacillus* sp. and *E. coli*, including a decrease in peptidoglycan content of *Bacillus* sp. and the increased lipid ordering of the membrane in

both bacteria. Furthermore, a decrease in RNA and protein concentrations of *Bacillus* sp. is measured. All three bacteria studied showed a decrease in conformational freedom of proteins in their cobalt acclimated status. Interestingly, both *Bacillus* sp. and *E. coli* showed slight but significant alterations in their DNA conformations which might imply a methylation mediated memory formation leading to epigenetic modulation for cobalt adaptation.

Keywords: ATR-FTIR spectroscopy, heavy metal adaptation, cobalt acclimation

ÖZ

ATR-FTIR SPEKTROSKOPİSİ İLE ÇEVRESEL BAKTERİ İZOLATLARINDA KOBALT ADAPTASYONUNUN İNCELENMESİ

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Şubat 2014, 90 sayfa

Bu çalışma, kobalt aklimasyonunun üç farklı bakteri üzerindeki etkilerini incelemiştir. Ağır metaller yüksek konsantrasyonlarda bakteriler için toksiktir. Ancak metallere uzun süreli maruz kalma sonucunda, bakteriler ağır metallerin varlığında büyümeye uyum sağlayabilir. Bu uyumun sonucu olarak, bakteriler metabolizmaları ve moleküler yapılarında değişiklikler gösterir.

Bu çalışmanın amacı kobalta alışmış bakterilerdeki bu değişimleri ATR-FTIR spektroskopisi ile canlı örnekler üzerinden incelemektir. Ilıman sığ bir gölden izole edilmiş olan *Bacillus* sp. ve *Pseudomonas* sp. ile beraber, iyi bilinen bir *E. coli* suşu incelenmiştir. Sonuçlar *Bacillus* sp. ve *E. coli*'nin hücre zarı ve duvar dinamiklerinde büyük değişimler olduğunu göstermiştir; *Bacillus* sp.'da peptidoglikan içeriğinde bir düşüş ve iki bakteride de zar yağlarının dizilmesinde sıklaşma görülmüştür. Dahası, *Bacillus* sp.'da RNA ve protein

konsantrasyonlarında bir düşüş gözlenmiştir. Kobalta alışmış olan üç bakteri de, proteinlerinin konformasyonal bağımsızlığında bir düşüş sergilemiştir. İlginç bir şekilde, hem *Bacillus* sp., hem de *E. coli* DNA konformasyonlarında ufak ancak önemli değişimler göstermiştir. Bu ise metilasyona dayanan bir hafıza oluşumuna ve kobalt adaptasyonunun epigenetik yollarla kontrol edildiğine işaret ediyor olabilir.

Anahtar Kelimler: ATR-FTIR spektroskopisi, ağır metal adaptasyonu, kobalt aklimasyonu

To My Parents

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor Assoc. Prof. Dr. Ayşe Gül Gözen and co-supervisor Prof. Dr. Feride Severcan for their guidance, advice, criticism, encouragements and insight throughout the research.

I would like to thank to Tuğba Özaktaş, Nihal Şimşek Özek, Emrulla Spahiu for their suggestions, guidance and help with the experimental studies.

I would also like to extend my thanks to Ahmet Yücel, Elif Erdoğan, Kasım Emre Kardaş for their help, support, and sincere friendship.

Finally, I would like to send my ultimate appreciation to my mother Emine Kardaş, my father Ali Kardaş, my sister Esra Kardaş and my brother Fatih Kardaş for their endless patience, encouragement, support and love.

TABLE OF CONTENTS

ABSTRACT v

ÖZ vii

ACKNOWLEDGEMENTS x

TABLE OF CONTENTS xi

LIST OF TABLES xiv

LIST OF FIGURES xvi

CHAPTERS

1. INTRODUCTION 1

1.1 Heavy Metal Acclimation in Bacteria 1

1.2 Infrared (IR) Spectroscopy 2

1.2.1 Fourier Transform Infrared (FTIR) Spectroscopy .. 2

1.2.2 Attenuated Total Reflectance (ATR) FTIR Spectroscopy 3

1.2.3 Use of ATR-FTIR as a Tool for the Bacteria Studies 3

1.3 Scope and Aim of This Study 4

2. MATERIALS AND METHODS 5

2.1 Bacteria Isolates 5

2.2 Growth Conditions 5

2.3 Finding Minimum Inhibitory Concentration (MIC) for Cobalt 6

2.4 Cobalt Acclimation 6

2.5 Sample preparation for ATR-FTIR spectroscopy studies 7

2.6 ATR-FTIR spectroscopy and data analysis 7

2.7 Statistics 8

3. RESULTS AND DISCUSSION 9

3.1 Minimum Inhibitory Concentration (MIC) for Cobalt 9

3.2 Acclimation to Cobalt 10

3.3 General FTIR Spectrum and Band Assignment of Bacteria 11

3.4 FTIR Spectra Analysis 14

3.4.1 Effects of Cobalt Acclimation on Proteins 22

3.4.2 Effects of Cobalt Acclimation on Lipids 24

3.4.3 Effects of Cobalt Acclimation on the Cell Wall 26

3.4.4 Effects of Cobalt Acclimation on Nucleic Acids 27

4. CONCLUSION 31

REFERENCES 35

APPENDICES

A. FTIR DATA FOR *PSEUDOMONAS* SP. (n = 20) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p)..... 41
B. FTIR DATA FOR *BACILLUS* SP. (n = 20) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p)..... 61
C. FTIR DATA FOR *E. COLI* (n = 10) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p) . 81

LIST OF TABLES

TABLES

Table 1. Acclimation to Cobalt in terms of Growth Times and Number of Passages 10

Table 2. General Band Assignments of Bacteria in the Related Literature 12

Table 3. The band frequencies with significant differences between controls and acclimated bacteria at $p<0.05$ 19

Table 4. The band areas with significant differences between controls and acclimated bacteria at $p<0.05$ 20

Table 5. The bandwidths values with significant differences between controls and acclimated bacteria at $p<0.05$ 21

Table 6. Frequency data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them.....41

Table 7. Band area data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them 49

Table 8. Band area ratio of certain bands for control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them 57

Table 9. Bandwidth data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them.....59

Table 10. Frequency data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them 61

Table 11. Band area data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them 69

Table 12. Band area ratio of certain bands for control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them 77

Table 13. Bandwidth data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them 79

Table 14. Frequency data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them 81

Table 15. Band area data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them 85

Table 16. Band area ratio of certain bands for control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them 89

Table 17. Bandwidth data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them 90

LIST OF FIGURES

FIGURES

Figure 1. A general representative FTIR spectrum of a bacterium with band assignments adapted from *E. coli* spectrum (A). The average spectra of (a) *Pseudomonas* sp. (n= 20), (b) *Bacillus* sp. (n= 20) and (c) *E.coli* (n= 10) samples in the 3700–900 cm^{-1} region normalized with respect to the amide A band (B) 11

Figure 2. The average spectra of *Pseudomonas* sp. (n= 20) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B) 14

Figure 3. The average spectra of *Bacillus* sp. (n= 20) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B) 15

Figure 4. The average spectra of *E. coli* (n= 10) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B) 16

CHAPTER 1

INTRODUCTION

1.1 Heavy Metal Acclimation in Bacteria

Cobalt (Co^{2+}) is an important heavy metal trace element found as cofactor for several enzymes and vitamins in cells. However, at high concentrations heavy metals are known to form unspecific compounds with biomolecules and lead to toxicity (Nies, 1999). Bacteria that are inhibited by a given concentration of a heavy or toxic element can acclimate themselves to grow in that concentration upon prolonged exposure. This acclimation occurs by the emergence of resistant populations that maintain essential microbial processes and can only occur after a minimum threshold concentration of the pollutant is reached (Spain *et al.*, 1980). Mechanisms which play a role in the acclimation process involve; induction of specific enzymes after exposure to toxicant, genetic selection favoring new metabolic abilities and increase in the number of microbes that can lessen the effect of the toxicant (Liebert *et al.*, 1991).

As a result of acclimation, bacterial populations can develop adaptations with varying degrees to high levels of heavy metals using resistance mechanisms. These include exclusion, permeability barrier, sequestration of the metal ions inside or outside the cell, active transport efflux pumps and detoxification of metal ions to a less toxic state (Bruins *et al.*, 1999; Nies, 1999). The

mechanisms are mediated through a variety of chromosomal and plasmid based systems (Silver, 1996).

1.2 Infrared (IR) Spectroscopy

Infrared region (14.000 cm^{-1} to 4 cm^{-1}) of the electromagnetic spectrum is divided into three regions: near infrared region (14.000 cm^{-1} to 4.000 cm^{-1}), mid infrared region (4.000 cm^{-1} to 400 cm^{-1}) and far infrared region (400 cm^{-1} to 4 cm^{-1}) (Smith,1999). Applications of infrared spectroscopy concerning biological samples use mid infrared region.

If the electric dipole moment of a molecule changes upon infrared radiation, the molecule shows infrared absorption and chemical bonds of the molecule displays vibrations. These vibrations can be classified in two major groups: stretching and bending vibrations. If the movement of the atoms in molecules changes relative to each other, bond lengths can vary, which means a stretching; or one atom can move out of its present plane, which means a bending (Stuart, 1997). These alterations in vibration modes correspond to the changes in the conformation, structure and function of the related functional group.

1.2.1 Fourier Transform Infrared (FTIR) Spectroscopy

In an FTIR spectrometer, the radiation originating from a source needs to pass through an interferometer and the sample before reaching a detector. After the signal amplification by eliminating the high frequency contributions with a filter, the data are converted to a digital form by a converter and transferred to the computer for Fourier transformation, which is a mathematical process. FTIR spectroscopy makes use of a Michelson interferometer with a movable

mirror, which produces an interferogram by the interference of radiation between two beams. An interferogram signal is produced when the change of path length between the two beams is measured (Stuart, 1997).

1.2.2 Attenuated Total Reflectance (ATR) FTIR Spectroscopy

ATR-FTIR spectroscopy utilizes the total internal reflection phenomenon. When an infrared beam is directed onto an optically dense crystal with a high refractive index and passes through it at a certain angle, it gets internally reflected. However, an evanescent wave can pass through a few micrometers beyond the surface of ATR crystal into the contacted sample. Penetration rate of the evanescent wave into the sample is typically between 0.5 and 2 micrometers. When energy is absorbed by the sample, the evanescent wave gets attenuated and this is picked up by the detector to form an IR spectrum (Stuart, 1997).

For ATR crystal, diamond, amorphous material transmitting IR (AMTIR) or silicon material might be used, however; most commonly used ones are zinc selenide (ZnSe) and germanium (Ge). ZnSe is preferred, as it is water resistant, cost effective and easily cleanable (Perkin Elmer Life Sciences, 2005; Kazarian & Chan, 2006).

1.2.3 Use of ATR-FTIR as a Tool for the Bacteria Studies

Bacteria while acclimating to environmental stress caused by high level heavy metal exposure alter their metabolism. As a result, composition and structure of cellular constituents oftentimes change substantially (Harrison *et al.*, 2007; Kamnev, 2008). These changes can be measured in terms of the total and specific chemical composition of lipids, proteins, nucleic acids and

polysaccharides by using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy due to its nondestructive, rapid and sensitive nature (Naumann *et al.*, 1991; Schuster *et al.*, 1999; Belfer *et al.*, 2000; Melin *et al.*, 2004). As mentioned above, even very small changes in bond lengths and angles of macromolecules can be observed with this technique (Toyran *et al.*, 2006). Shifting of peak positions, changes in bandwidth and intensities of bands obtained via FTIR analysis provide structural and functional information about the molecules of interest (Çakmak *et al.*, 2003). The use of ATR module in FTIR spectrometer shortens the sample preparation steps and allows for examination of the live samples (Winder & Goodacre, 2004).

1.3 Scope and Aim of This Study

The aim of this study was to determine the extent of molecular changes occurring in bacteria acclimated to grow in inhibitory concentrations of cobalt. In experiments, two bacteria previously isolated from a temperate shallow lake (*Bacillus* sp., *Pseudomonas* sp.*)* and a well-established strain of *E. coli* were used. After acclimating the bacteria to grow in inhibitory levels of cobalt, resulting changes in macromolecules were measured via ATR-FTIR spectroscopy. Even though, the cobalt detoxification mechanisms in gram positive and gram negative bacteria are well documented (Nies, 1999), compositional, structural and functional changes taking place in molecules of bacteria which acclimated to grow in toxic levels of cobalt have not been reported so far. This study will provide information at a molecular level, bringing insight into how different kinds of bacteria develop adaptations to survive in heavy metal exposed environments.

CHAPTER 2

MATERIALS AND METHODS

2.1 Bacteria Isolates

Two of the bacteria used in this study, *Bacillus* sp. and *Pseudomonas* sp. had been previously isolated from Lake Mogan, situated in Ankara (Özaktaş *et al.*, 2012). Their 16S rRNA sqeunces can be found in NCBI GenBank database under accession numbers of JF421727 and JF421699, respectively. Additionally, a non-environmental strain of *E. coli ATCC* 8739 was investigated.

2.2 Growth Conditions

Nutrient broth (peptone from meat 5 g., meat extract 3 g. per liter, Merck) and nutrient agar were used as growth medium for all applications. Stock solutions of Cobalt Chloride in hexahydrate form ($CoCl_2 \cdot 6H_2O$ from Sigma-Aldrich) were prepared by dissolving in H_2O and filter sterilization (0.22-micron filters, Pall, USA). The stock solutions were stored in the dark at 4^0C. The cobalt solution was incorporated into broth or agar media followed by autoclaving and cooling down to 45-50^0C. All passages and media preparations were done in the laminar flow chamber (Esco, USA).

Bacterial isolates used in this study were grown under aerobic conditions and incubated at 28˚C in a shaker incubator at 200 rpm (Özaktaş *et al.*, 2012). Bacteria were cultured in 5 ml nutrient broth in test tubes. In passages, a 90 µl aliquot from the cultures was inoculated into fresh media.

2.3 Finding Minimum Inhibitory Concentration (MIC) for Cobalt

All three bacteria samples were tested for growth inhibiton in the presence of cobalt using the broth dilution method according to the guidelines established by EUCAST (2003). For *Bacillus* sp. and *Pseudomonas* sp. cobalt concentrations ranging between 10-160 µg/ml and for E.coli between 10-320 µg/ml were tested. The cultures were grown as mentioned above.

2.4 Cobalt Acclimation

In the cobalt acclimation studies, each of the three bacteria were grown at their corresponding MIC for cobalt.

The three bacteria were acclimated to cobalt through multiple passages in an attempt to find a constant growth duration. In other words, when they have fully grown in the presence of minimum inhibitory concentration of cobalt, they were transferred to a fresh growth medium with the corresponding MIC of cobalt and were allowed to re-grow, then transferred again until they started to reach full growth in a fix time period. When constant growth times were achieved without major fluctuations for 7 consequent passages, it was assumed that the bacteria were acclimated to grow at growth inhibitory cobalt concentration. In the acclimation process, controls were used to make visual assessments of turbidity.

2.5 Sample preparation for ATR-FTIR spectroscopy studies

For FTIR analysis, the concentrations of bacteria were calibrated in a way that an absorbance value of 0.5 OD in 1 ml of total volume corresponded to 10^9 CFU mL^{-1} per sample. Upon calibration of concentrations, samples were centrifuged (Sigma 1-14 Microfuge, SciQuip, UK) for 10 minutes in 10,000 g. The supernatants were removed and pellets were re-suspended in 15 µl of distilled water. In total, the 20 independent replicates for each of *Pseudomonas* sp. and *Bacillus sp*. and the 10 independent replicates of *E. coli* cobalt acclimated groups and their controls were subjected to ATR-FTIR analysis.

2.6 ATR-FTIR spectroscopy and data analysis

A total of 5 µl replicate samples were applied onto the ATR-Diamond crystal of Spectrum 100 FT-IR Spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA). The 5 µl sample volume was achieved at sequential applications of 1 µl volume on the crystal and drying with a mild N_2 flux for 5 minutes before the following 1 µl volume applied.

Scanning was done within the range of 4000-650 cm^{-1} with 4 cm^{-1} resolution, and 50 scans at room temperature. Since original bacterial suspensions were 15 µl per sample, 3 independent scan sets were obtained (5 µl each) for each replica. For the spectral data of one analyzed sample, the average of the three was used in order to minimize signal/noise ratio.

Analysis of the spectral data, which included averaging of spectra within samples, smoothing, baseline corrections, functional group assignments, band area and bandwidth calculations of the bands were performed with PerkinElmer Spectrum One software (PerkinElmer, US).

2.7 Statistics

Results were presented as mean ± standard deviation. Mann–Whitney U-test was performed between each isolate's control and cobalt acclimated samples to test the significance levels. The p value of less than 0.05 was considered statistically significant. Degrees of significance were denoted as $p < 0.05$*, $p < 0.01$** and $p < 0.001$***.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Minimum Inhibitory Concentration (MIC) for Cobalt

Minimum inhibitory concentrations for cobalt were measured as 75 µg/ml for both *Pseudomonas* sp. and *Bacillus* sp. and 225 µg/ml for *E. coli*. The results indicated that there is an obvious difference in their ability to grow in the presence of cobalt. The fact that *E. coli* seems to have a much greater MIC (225 µg/ml) than the other two (75 µg/ml) might point to a higher level of innate resistance.

Reported values of MIC for cobalt in related literature vary between different bacteria. For *E. coli* K-12, MIC was reported as 90 µg/ml. On a side note, the addition of ferric citrate into the media was reported to increase the tolerance up to more than 130 µg/ml concentration of cobalt (Ranquet *et al.*, 2007). On a different study, for *E. coli* K12, MIC of cobalt was 175 µg/ml, for *P. aeruginosa* between 350-700 µg/ml changing among different strains and 45 µg/ml for *B. thuringiensis*, each of which isolated from polluted environments (Hassen *et al.*, 1998). As it can be seen, reported MIC values for related bacteria vary greatly between species and even in strain levels. The cause of variations in MIC values between previous records and ours may be due to differences in environments where the samples had been collected, slight differences in media composition and growth conditions.

3.2 Acclimation to Cobalt

Table 1. Acclimation to Cobalt in terms of Growth Times and Number of Passages

Isolate name	First full growth after inoculation with MIC of cobalt	Number of passages after which constant growth time was obtained	Constant Growth Time (Acclimated)
Pseudomonas sp.	8 days	6 passages	2 days
Bacillus sp.	10 days	9 passages	1 day
E. coli	4 days	8 passages	1 day

Cobalt acclimation of the three bacteria grown under conditions as specified in material and methods varied in time it takes to attain heavily turbid growth and the number of passages before they reached to constant culture growth time as it can be seen in the **Table 1**. For *Pseudomonas* sp. first heavily turbid growth (with respect to its control) after inoculation with its MIC of cobalt was achieved after 8 days, for *Bacillus* sp. and *E. coli* on the other hand, these were observed 10 and 4 days later followed by inoculation with cobalt, respectively. The constant growth time and the number of passages after which it was reached differed among three bacteria. As it is seen, through consequent passages, all three of them tend to grow faster. The constant growth time in the presence of cobalt adequately showed that they were acclimated to grow at an inhibitory concentration that they were subjected to. It is worth to note, however, these variations might not indicate different mechanisms of cobalt adaptation in bacteria.

3.3 General FTIR Spectrum and Band Assignment of Bacteria

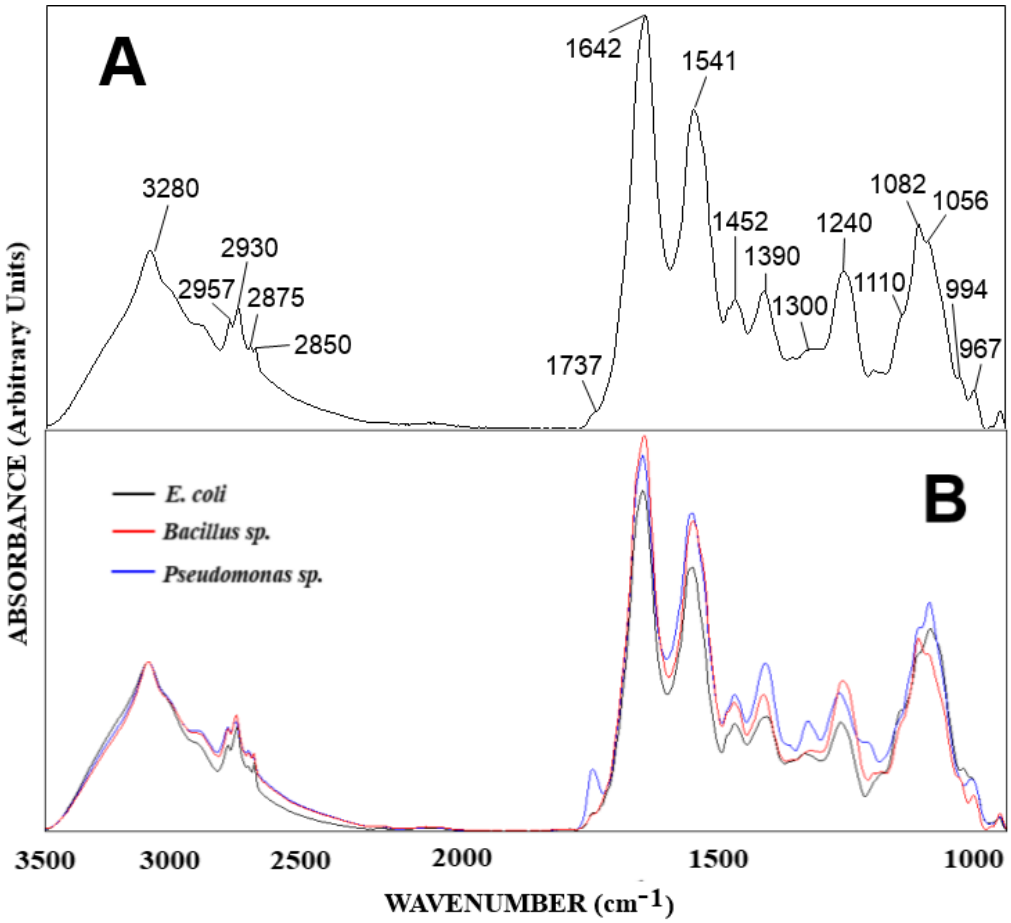

Figure 1. A general representative FTIR spectrum of a bacterium with band assignments adapted from *E. coli* spectrum (A). The average spectra of (a) *Pseudomonas* sp. (n= 20), (b) *Bacillus* sp. (n= 20) and (c) *E. coli* (n= 10) samples in the 3700–900 cm^{-1} region normalized with respect to the amide A band (B).

Figure 1A shows a general representative FTIR spectrum of a bacterium as an example in the spectrum of *E. coli.* The band assignments were done according to related reports as presented in the **Table 2**. The **Figure 1B** compares the average spectra of *Pseudomonas* sp., *Bacillus* sp. and *E. coli* in 3700-900 cm^{-1} region, in which analysis of the bands were performed.

Table 2. General Band Assignments of Bacteria in the Related Literature

Wavenumber (cm-1)	Definition of the spectral assignments	References
~3300	Amide A: N–H and O–H stretching vibration of proteins with little contribution from polysaccharides	Melin *et al.*, 2000; Görgülü *et al.*, 2007; Garip *et al.*, 2009
2957	CH_3 asymmetric stretching of fatty acids	Takahashi *et al.*, 1991; Naumann, 2001; Maquelin *et al.*, 2002
2930	CH_2 asymmetric stretching of mainly lipids	Naumann, 2001; Çakmak *et al.*, 2006; Özek *et al.*, 2014
2875	CH_3 symmetric stretching of mainly proteins	Naumann, 2001; Çakmak *et al.*, 2006; Özek *et al.*, 2014
2850	CH_2 symmetric stretching of mainly lipids	Naumann, 2001; Çakmak *et al.*, 2006; Özek *et al.*, 2014
1737	C=O stretching of triglycerides and cholesterol esters	Naumann, 2001; Çakmak *et al.*, 2006; Özek *et al.*, 2014
1693–1627	Amide I mainly C=O stretching of proteins	Haris & Severcan, 1999; Naumann, 2001; Özek *et al.*, 2014
1541	Amide II - N–H bending, C–N stretching of proteins	Dwivedi *et al.*, 1984; Naumann, 2001; Kong & Yu, 2007; Özek *et al.*, 2014
1452	CH_2 bending of lipids	Çakmak *et al.*, 2006; Kamnev, 2008
1390	COO^- symmetric stretching of amino acid side chains and fatty acids	Jackson *et al.*, 1998; Naumann, 2001
1300	Amide III band components of proteins	Naumann, 2001; Maquelin *et al.*, 2002
1240	PO_2 asymmetric stretching of mainly nucleic acids	Naumann, 2001; Çakmak *et al.*, 2006; Özek *et al.*, 2014
1110	CC symmetric stretching of ribose	Banyay *et al.*, 2003

Table 2 (continued)

Wavenumber (cm-1)	Definition of the spectral assignments	References
1082	PO_2 symmetric stretching of nucleic acids and phospholipids	Naumann, 2001; Garip *et al.*, 2009; Özek *et al.*, 2014
1056	C–O–C, P–O–C symmetric stretching of polysaccharides on capsule and peptidoglycan	Quiles *et al.*, 2010
994	Ribose skeleton	Banyay *et al.*, 2003; Quiles *et al.*, 2010
967	RNA and DNA backbone CC stretching of nucleic acids	Dovbeshko *et al.*, 2000; Banyay *et al.*, 2003; Garip *et al.*, 2009

3.4 FTIR Spectra Analysis

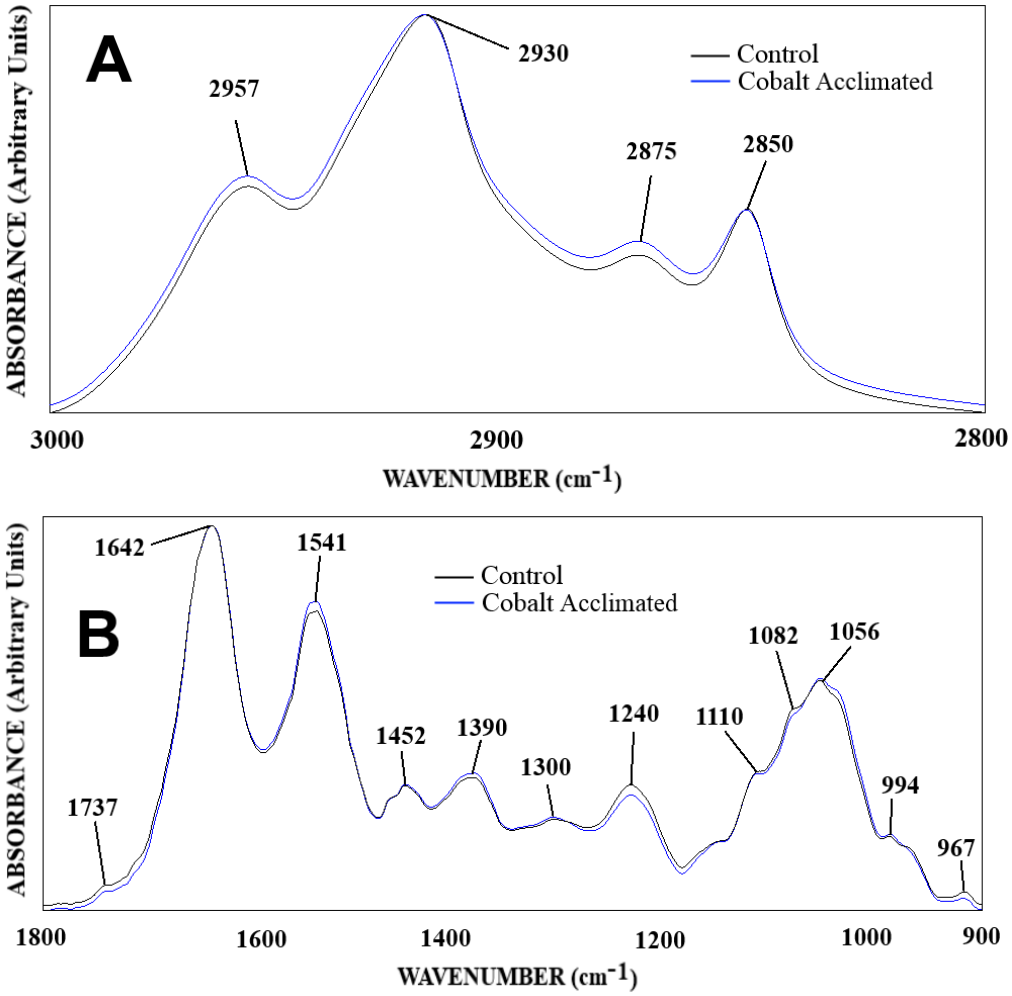

Figure 2. The average spectra of *Pseudomonas* sp. (n= 20) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B).

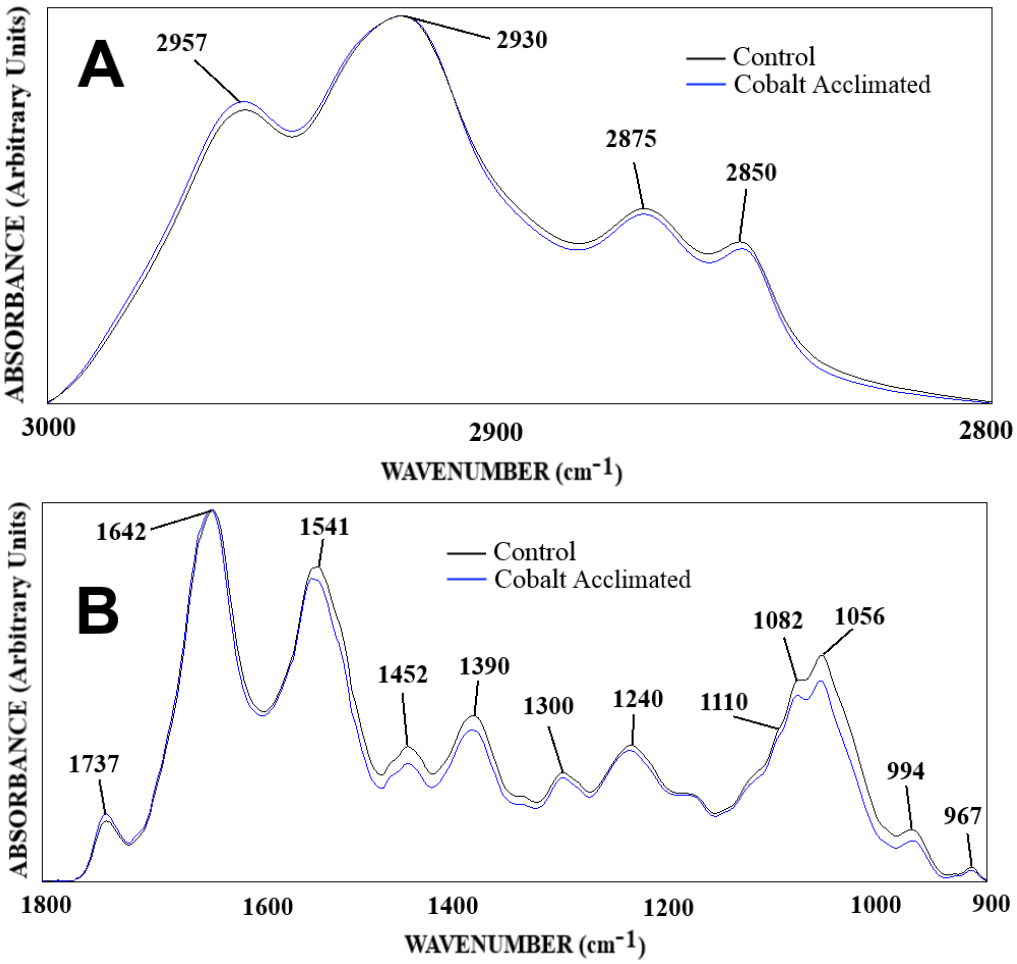

Figure 3. The average spectra of *Bacillus* sp. (n= 20) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B).

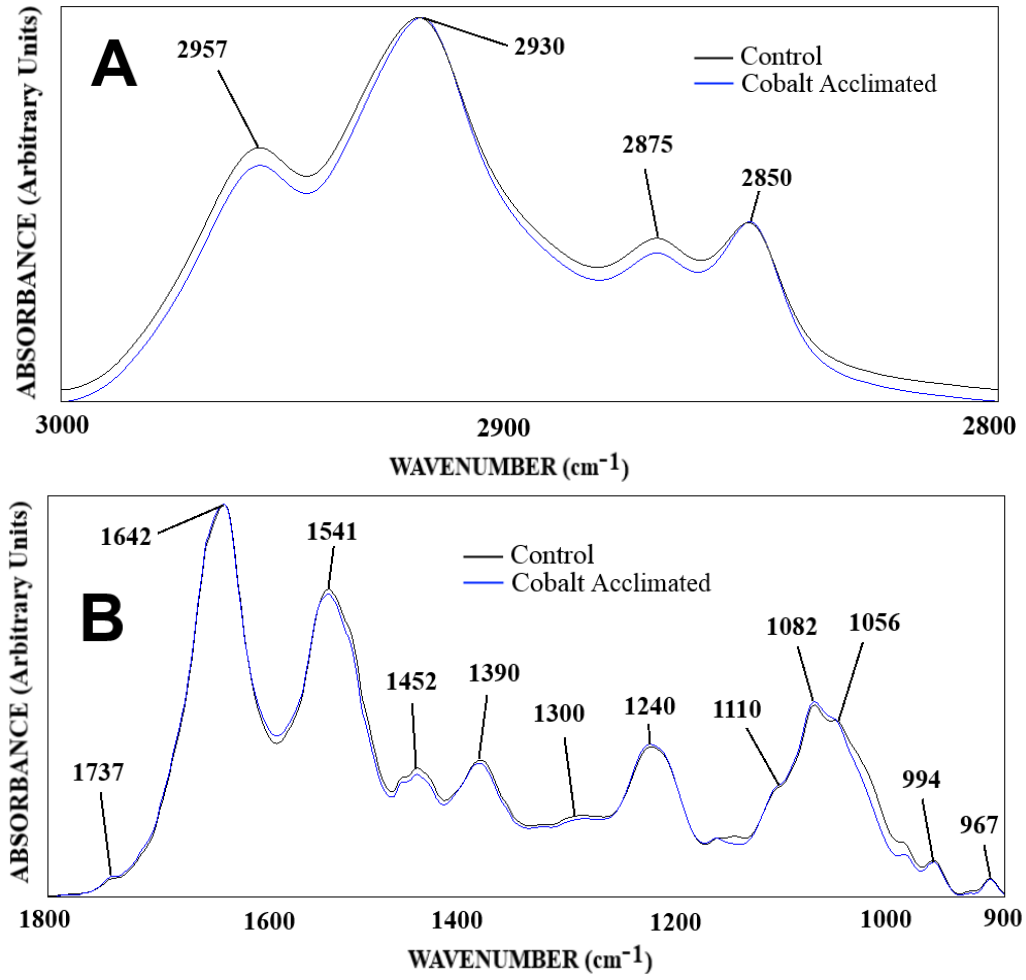

Figure 4. The average spectra of *E. coli* (n= 10) in the 3700-2800 cm^{-1} region normalized with respect to the CH_2 asymmetric stretching band (A) and in the 1800-900 cm^{-1} region normalized with respect to the amide I band (B).

The average spectra of acclimated bacteria and their controls are given in **Figures 2, 3** and **4.** Spectral analysis results had significant differences in frequency, band area values, and bandwidth of certain bands between control and cobalt acclimated groups. Only the statistically significant band differences

between controls and acclimated samples are shown in the **Table 3.** Differences with p values greater than 0.05, were not discussed.

Frequency values of infrared bands give information about structure, conformation and intermolecular interactions (Garip *et al.*, 2009; Özek *et al.*, 2014). The intensity or more accurately the area under the bands corresponds to concentrations of the functional group that the peak is assigned to according to the Beer-Lambert Law. The bandwidth in general is related to conformational freedom and flexibility. While bandwidths of C-H stretching bands give information about the membrane lipid dynamics (Schultz and Naumann, 1991), bandwidths of amide I and II bands give information about conformational freedom of proteins (Wharton, 2000; Barth & Zscherp, 2002). Protein-to-lipid ratios were calculated using the ratios of the area of lipid and protein based bands (Szalontai *et al.*, 2000; Özek *et al.*, 2014).

Table 3. The band frequencies with significant differences between controls and acclimated bacteria at $p<0.05$

Functional Groups	Frequency values (cm^{-1})		% change[a]	p-value
	Control	Cobalt acclimated		
Pseudomonas sp. (n=20)				
CC symmetric stretching	1119.33 ± 0.60	1119.96 ± 1.03	+0.06	0.05*
Bacillus sp.(n=20)				
Amide A	3271.02 ± 7.90	3278.34 ± 7.42	+0.22	0.01**
CH_2 symmetric stretching	2851.77 ± 0.52	2851.53 ± 0.52	-0.01	0.05*
C=O stretching	1736.82 ± 0.60	1738.07 ± 0.54	+0.07	0.001***
Amide I	1640.10 ± 1.53	1641.45 ± 1.65	+0.08	0.05*
Amide II	1538.15 ± 1.22	1539.74 ± 1.93	+0.1	0.001***
CH_2 bending	1450.13 ± 0.31	1450.58 ± 0.41	+0.03	0.001***
CC symmetric stretching	1100.78 ± 0.38	1101.21 ± 0.33	+0.04	0.001***
PO_2 symmetric stretching	1082.81 ± 0.41	1083.39 ± 0.59	+0.05	0.05*
C–O–C, P–O–C symmetric	1056.55 ± 0.70	1057.11 ± 0.81	+0.05	0.05*
Ribose skeleton	994.39 ± 0.31	994.84 ± 0.54	+0.04	0.05*

Table 3 (continued)

Functional Groups	Frequency values (cm^{-1})		% change[a]	p-value
	Control	Cobalt acclimated		
E. coli (n=10)				
CH_2 asymmetric stretching	2925.74 ± 0.09	2925.02 ± 0.31	-0.02	0.001***
CH_2 symmetric stretching	2852.35 ± 0.07	2852.21 ± 0.17	-0.01	0.05*
Amide I	1638.18 ± 0.21	1638.68 ± 0.49	+0.03	0.01**
CH_2 bending	1451.40 ± 0.61	1451.88 ± 0.50	+0.03	0.05*
PO_2 symmetric stretching	1082.20 ± 0.82	1083.15 ± 0.30	+0.09	0.05*
C–O–C, P–O–C symmetric	1056.40 ± 0.47	1057.29 ± 0.33	+0.08	0.001***
Nucleic acid CC stretching	965.95 ± 0.26	966.23 ± 0.36	+0.03	0.05*

Table 4. The band areas with significant differences between controls and acclimated bacteria at $p<0.05$

Functional Groups	Band area (cm^{-1})		% change	p-value
	Control	Cobalt acclimated		
Bacillus sp.(n=20)				
Amide A	59.31 ± 4.62	56.46 ± 4.71	-4.82	0.056
CH_3 asymmetric stretching	4.76 ± 0.62	4.40 ± 0.56	-7.56	0.05*
CH_2 asymmetric stretching	5.51 ± 0.71	5.02 ± 0.65	-8.75	0.05*
CH_3 symmetric stretching	1.96 ± 0.29	1.77 ± 0.26	-9.83	0.05*
CH_2 symmetric stretching	1.75 ± 0.28	1.56 ± 0.25	-10.5	0.05*
Amide I	25.43 ± 2.21	23.99 ± 2.50	-5.68	0.05*
Amide II	23.84 ± 3.56	20.76 ± 3.95	-12.9	0.05*
CH_2 bending	5.87 ± 1.19	4.05 ± 0.77	-30.8	0.001***
COO^- symmetric stretching	9.84 ± 1.83	8.42 ± 1.77	-14.5	0.01**
C–O–C, P–O–C symmetric	11.6 ± 2.64	9.14 ± 2.72	-21.5	0.05*
Ribose skeleton	1.11 ± 0.35	0.77 ± 0.32	-30.4	0.01**
Nucleic acid CC stretching	1.60 ± 0.52	1.14 ± 0.49	-28.7	0.05*
	Band area ratio value			
Amide I/Amide I + Amide II	0.518 ± 0.019	0.539 ± 0.024	+4.05	0.01**

Table 4 (continued)

Functional Groups	Band area (cm^{-1}) Control	Cobalt acclimated	% change	p-value
E. coli (n=10)				
CH_2 asymmetric stretching	6.20 ± 0.30	6.40 ± 0.15	+3.26	0.05*
C=O stretching	0.31 ± 0.02	0.37 ± 0.01	+22.8	0.001***
Ribose skeleton	1.22 ± 0.17	1.00 ± 0.14	-18.6	0.01**
	Band area ratio values			
Amides I + II/ester C=O stretching	177.24 ± 5.12	144.73 ± 10.2	-18.3	0.001***
C=O/lipid	0.066 ± 0.002	0.0797 ± 0.005	+20.8	0.001***
RNA/protein	0.0226 ± 0.003	0.0184 ± 0.003	-18.5	0.01**

Table 5. The bandwidths values with significant differences between controls and acclimated bacteria at $p<0.05$

Functional Groups	Bandwidth (cm^{-1}) Control	Cobalt acclimated	% change	p-value
Pseudomonas sp. (n=20)				
Amide II	25.14 ± 0.81	24.54 ± 0.89	-2.36	0.05*
Bacillus sp.(n=20)				
Amide II	27.17 ± 0.89	24.52 ± 1.33	-9.78	0.001***

Table 5 (continued)

Functional Groups	Bandwidth (cm^{-1}) Control	Cobalt acclimated	% change	p-value
E. coli (n=10)				
Amide II	29.45 ± 0.96	27.42 ± 2.19	-8.20	0.05*
CH_2 symmetric stretching	4.27 ± 0.10	4.62 ± 0.9	+6.89	0.001***

3.4.1 Effects of Cobalt Acclimation on Proteins

Amide A band, belong to N–H and O–H stretching of proteins and glycogen. In addition, there might be a contribution from bound water to amide A, and this can be seen as a shoulder around 3500 cm^{-1}. In *Bacillus* sp. **(Table 3)**, there was a significant frequency increase in cobalt acclimated groups of amide A band ($p < 0.01$). This shift was irrelevant for the backbone conformation of proteins, however, it indicates a strengthening of the hydrogen bonds in cobalt acclimated groups dealing with N-H stretching (Krimm & Bandekar, 1986). In cobalt acclimated *Bacillus sp*. band area significantly decreased ($p = 0.056$). This decrease indicates some alterations in the concentration of proteins and polysaccharides as supported by changes measured in related bands discussed below (Jackson *et al.*, 1997). CH_3 symmetric stretching of proteins at 2873 cm^{-1} was not significantly different in terms of frequency among the three bacteria. In *Bacillus* sp., on the other hand, **(Table 4)**, the band area of CH_3 symmetric stretching of proteins at 2873 cm^{-1} band in cobalt acclimated group was significantly lower ($p < 0.05$) than that of the control group indicating a decrease in protein concentration. At the same time, again in *Bacillus sp*. **(Table 4)**, there was a significant ($p < 0.05$) decrease in band area values of amide I and amide II in cobalt acclimated group. This was also supported by the ratio of amide I to amide I + amide II ($p < 0.01$). These results indicate a

decrease in protein concentration of bacteria. It was reported in a study done in *E. coli* that in the presence of cobalt, bacteria tend to decrease the synthesis of several Fe/S-containing enzymes, as high levels of cobalt can compete out iron and impair Fe/S biogenesis (Fantino *et al.*, 2010). Although, *E. coli* strain that was used in the experiments did not show a decrease in protein concentration, *Bacillus* sp. showed a significant decrease. In other words, under cobalt exposure the *Bacillus* sp. strain behaved like previously studied *E. coli* strains by reducing protein synthesis. In *Bacillus sp*. **(Table 3)**, there was a significant ($p < 0.05$) increase in frequency of the cobalt acclimated group's amide I band. In *E. coli* samples **(Table 3)**, on the other hand, the shift to a higher frequency in cobalt acclimated group was very highly significant ($p < 0.01$). This indicates conformational and structural changes in cell proteins (Haris & Severcan, 1999). In *Bacillus* sp. **(Table 3)**, the frequency of amide II, which shows much less conformational sensitivity than amide I (Dwivedi *et al.*, 1984; Naumann, 2001; Kong & Yu, 2007), was significantly ($p < 0.05$) higher in the cobalt acclimated group, when compared to the control group. It points to conformational changes in proteins, as confirmed by amide I band shift. Therefore, it seems that, in *Bacillus sp*., general protein concentration is lower than un-acclimated counterparts and at the same time, structurally modified proteins possibly arise to compensate for cobalt stress as the frequency shift suggests. Furthermore, bandwidth of the amide II band had significant differences in all three of the sample groups. In *Pseudomonas* sp. **(Table 5)**, bandwidth of the cobalt acclimated group was significantly lower ($p < 0.05$) than the control group. The same significance was valid for *E. coli* **(Table 5)** ($p < 0.05$), with a lower bandwidth in cobalt acclimated samples than in the control group. *Bacillus* sp. **(Table 5)** had a ($p < 0.001$) very significantly lower bandwidth in the cobalt acclimated group than in the control group. These reductions in the bandwidth of amide II are indicative of a general restriction of conformational freedom in proteins, and it had also been suggested that this restriction in flexibility might be due to molecules that bind to proteins

(Wharton, 2000; Barth & Zscherp, 2002). In other words, if there is an increase in protein binding, flexibility decreases and bandwidth narrows. This might be suggestive of an increased number of cobalt binding proteins or might be the cause of other protein bindings due to cobalt acclimation.

3.4.2 Effects of Cobalt Acclimation on Lipids

Alterations taking place in lipid structures as a result of cobalt acclimation were also evaluated. The frequency shift of the CH_2 stretching bands gives information about lipid order/disorder which is a structural parameter; however, bandwidths of these bands give dynamics information about the membrane which is a functional parameter (Severcan & Cannistraro, 1990; Boyar & Severcan, 1997; Çakmak *et al.*, 2006). In *E. coli* **(Table 3)** frequency value of CH_2 asymmetric stretching of lipids at 2925 cm^{-1} was remarkably lower in the cobalt acclimated group with respect to control ($p < 0.001$). This might indicate a decrease in hydrocarbon chain conformational disorder. In other words, since the frequency is lower in cobalt acclimated group; while acyl chain flexibility decreases, the membrane lipid order increases (Severcan, 1997; Liu *et al.*, 2002). Supporting this, in cobalt acclimated *Bacillus* sp. and *E. coli* groups **(Table 3)** CH_2 symmetric stretching band also shifted to a lower frequency value ($p < 0.05$).

The band area analysis of CH_2 symmetric and asymmetric bands revealed significant decreases ($p < 0.05$) in cobalt acclimated *Bacillus sp.* **(Table 4)** group, which point to a lower concentration of saturated lipid content or synthesis of shorter chain lipids in the membranes, thus, might mean more fluidity. In *E. coli* **(Table 5)** frequency of the CH_2 asymmetric band shifts to lower values suggested an increase in membrane lipid ordering. However, the

bandwidth of this band was significantly higher ($p < 0.001$) in the cobalt acclimated group, which indicated a higher level of membrane fluidity (Çakmak *et al.*, 2006)

Frequency and band area results seem to be inconsistent with respect to bandwidth for CH_2 asymmetric band in *E. coli*, since an increase in lipid order is not accompanied by a decrease in membrane fluidity. This may imply the existence of domain formation in the cell membrane, as suggested by other studies (Korkmaz & Severcan, 2005; Severcan *et al.*, 2005; Özek *et al.*, 2014).

The frequency of C=O stretching of triglycerides and cholesterol esters at 1738 cm^{-1} had a significant difference ($p < 0.001$) between control and cobalt acclimated groups in *Bacillus* sp. **(Table 3)**. Cobalt acclimated group had a much higher frequency than the control. This shows that the number of hydrogen bonds between C=O groups of lipids and polysaccharides and other chemicals were lower (Görgülü *et al.*, 2007). In *E. coli* **(Table 4)**, the area of this band of cobalt acclimated group was significantly higher ($p < 0.001$) than in control groups, indicating that the cobalt adapted group had a higher concentration of triglyceride and cholesterol esters. In *E. coli* samples **(Table 4)**, there was a highly significant ($p < 0.001$) decrease in protein-to-lipid ratio and an increase in C=O stretching/lipid ratio in cobalt acclimated group. Since the concentration of C=O stretching of triglycerides and esters in *E. coli* was significantly higher than control and there were no significant changes in protein concentrations calculated from both amide I and II and no changes in lipid content of *E. coli*, C=O stretching was the main reason for these changes. Increased intensity of C=O stretching band was reported to have been induced by heavy metals leading to the accumulation of polyester compounds in other bacteria (Kamnev, 2008), which might be similar in *E. coli* as well. CH_2

bending of lipids and esters at 1453 cm^{-1}, on the other hand, mostly arises from polyesters like Polyhydroxybutyrate (PHB) which was shown to be produced in response to stress in bacteria (Kamnev, 2008). The difference in frequency between experimental groups was not significant in *Pseudomonas* sp. ($p = 0.065$). However, in *E. coli* and in *Bacillus* sp. **(Table 3)**, its frequency differed between the control and cobalt acclimated groups, with cobalt acclimated group having a higher frequency at a high level of significance ($p < 0.05$ and $p < 0.001$, respectively). This might be due to the production of structurally or conformationally different form of polyesters, perhaps as a result of prolonged cobalt exposure and an outcome of acclimation in *Bacillus* sp. and *E. coli*. Furthermore, the area of this particular band in *Bacillus* sp. **(Table 4)** was significantly lower in the cobalt acclimated group ($p < 0.001$) when compared to the control group. This finding suggests a decrease in the concentration of polyesters in *Bacillus* sp.. However, when considered along with the frequency shift, it might mean that, instead of increasing polyester concentrations in the cobalt acclimated groups, structurally or conformationally different versions of polyhydroxyalkanoates might have been preferably synthesized.

In *Bacillus sp*. **(Table 4)**, the band area of COO^- symmetric stretching at 1393 cm^{-1} was significantly lower ($p < 0.01$) in cobalt acclimated group, indicating a decrease in the concentration of amino acids and fatty acids having side chains with COO^- stretching. Whereas in *Pseudomonas* sp. the difference was not significant ($p = 0.06$).

3.4.3 Effects of Cobalt Acclimation on the Cell Wall

Infrared spectroscopy can also provide information about cell wall peptidoglycan (Naumann *et al.*, 1982). In terms of changes in the cell wall, in

Bacillus sp. and *E. coli* **(Table 3)**, the cobalt acclimated group had a significantly ($p < 0.05$ and $p < 0.001$, respectively) higher frequency value than control group in C–O–C, P–O–C symmetric stretching of polysaccharides on capsule and peptidoglycan. This indicates alteration of peptidoglycan structure of cell wall (Santivarangkna *et al.*, 2007). Furthermore, in *Bacillus sp*. band area values of the cobalt acclimated group was lower than control ($p < 0.05$), indicative of a decrease in the number of cell wall polysaccharides of *Bacillus sp*.

3.4.4 Effects of Cobalt Acclimation on Nucleic Acids

Alterations in nucleic acids were evaluated through the examination of the bands at 1119 cm^{-1}, 1083 cm^{-1}, 994 cm^{-1} and 966 cm^{-1}. In *Bacillus* sp. **(Table 3)**, the frequency of PO_2 symmetric stretching of nucleic acids at 1083 cm^{-1} was significantly higher ($p < 0.05$) in the cobalt acclimated group than the control group. It was the same case for *E. coli* **(Table 3)**, the frequency of this band in the cobalt acclimated group was higher than control group with the same level of significance ($p < 0.05$). Therefore, it appears that there was possibly a change or modification affecting the backbone of nucleic acids in both *Bacillus* sp. and *E. coli.* In previous studies, it has been reported that cations can induce transitions in DNA conformation states on synthetic polynucleotides at high salt concentrations (Behe & Felseld, 1981). For example, a Co(III) complex of $[Co(NH_3)_6]^{+3}$ was shown to induce a variety of conformational products at very low concentrations. It could form Z-DNA even at 4 μg/ml (Shin *et al.*, 1988). Furthermore, it has also been reported that in methylated DNA, the transition from B to Z could occur at even lower salt concentrations, close to physiological (Behe & Felseld, 1981). Another study reported a decrease in DNA flexibility as a result of methylation, especially at the phosphodiester backbone at flanking regions of methylation (Derreumaux

et al., 2001). It appears that the PO_2 symmetric stretching change measured in this study could be the product of two influences either separately or combined. One of the influences could be the direct effect of the cobalt ions associating with the backbone of DNA, and the other may be the methylation extent of the DNA due to cobalt acclimation. However, it should be noted that, in acclimated bacteria conformation shifted slightly, and the presence of the band at 966 cm^{-1} in the spectrum means that DNA was still in the B helix form, but perhaps with reduced flexibility. These results were interpreted in the light of the previous records on conformational changes in DNA as following: When the bacteria were first exposed to cobalt inhibitory concentrations, its direct effects on macromolecules would be evident. In time, however, the bacteria modulated themselves to grow under cobalt stress. Some elements of this module may cause DNA flexibility change as a function of methylation. Thus, it might mean that bacteria were forming a memory in culture through inherited metylation of DNA. Therefore, cobalt acclimation of the culture was most probably achieved epigenetically. Of course, this is only an inference and yet to be proven.

As mentioned above, the fact that there was only a single band at 966 cm^{-1} in all groups indicates that DNA was in B-form (Banyay *et al.*, 2003) as its normal state. In *E. coli* **(Table 3)**, the frequency of the band in cobalt acclimated group was significant ($p = 0.05$) supporting some of the alterations seen in nucleic acids. In *Bacillus sp*. **(Table 4)**, on the other hand, band area values of the cobalt acclimated group was significantly ($p < 0.05$) lower than the control groups pointing to a decrease in RNA amounts.

On a different note, frequency of the CC symmetric stretching of ribose at 1119 cm^{-1} was significant in *Pseudomonas* sp. ($p < 0.05$) and *Bacillus* sp. ($p < 0.001$)

(Table 3-A). In *Bacillus* sp., the position of this band had shifted to a very low value, around 1100 cm^{-1}, and cobalt acclimated group had a significantly higher frequency value than control group ($p < 0.001$). For both bacteria, this might point to a modification affecting the ribose of cobalt acclimated group. In addition, in *Bacillus sp*. **(Table 3)**, the frequency of ribose-phosphate main chain vibrations of the ribose skeleton at 994 cm^{-1} was significantly higher in cobalt acclimated group ($p < 0.05$), which as well might indicate a modification on RNA structure, affecting skeletal conformation. Cobalt acclimated group's band area, however, was significantly ($p < 0.01$) lower than that of control group in *Bacillus sp*. **(Table 4)**. The lower band area value measured here indicates a decrease in concentrations of RNA in *Bacillus sp*., as also supported by area values at 966 cm^{-1} band. RNA/protein ratio also decreased significantly ($p < 0.05$) in cobalt acclimated (0.017 ± 0.01) *Bacillus sp*. with respect to control (0.022 ± 0.01). This decrease was observed since decrease in RNA amounts were more pronounced than decrease seen in protein concentrations. As lower RNA and protein levels often corresponds to slower growth, apparently in *Bacillus* sp. a reduction in total metabolic activity was a strategy in the process of cobalt acclimation. In *E. coli* **(Table 4)**, band area values of the vibrations of ribose skeleton of the cobalt acclimated group was also significantly lower ($p < 0.01$) than the control group. Since RNA concentration had decreased while there were no significant changes in protein concentrations, there was also a decrease in RNA/protein amounts **(Table 4)**. These decreases in concentration are in tandem with the general concentration decrease seen in some of the important biological polymers in cobalt acclimated groups. It was reported previously that under stress conditions like starvation, cold shock and entry into stationary phase bacteria can produce increased levels of RNase R protein which is important for quality control of rRNA and tRNA as well as in the decay of mRNAs with considerable secondary structure (Chen & Deutscher, 2005). These high RNase R levels indicate a remodeling of RNAs in the bacteria. Growth inhibitory cobalt

concentrations that were applied may have had bacteria to modulate their activities through mechanisms described by Chen & Deutscher (2005).

CHAPTER 4

CONCLUSION

- The changes in the molecular concentration and composition of cellular constituents caused by cobalt acclimation seem to have varying effects in three distinct bacteria we have studied

- In cobalt acclimated bacteria (*Bacillus* sp., *Pseudomonas* sp., *E. coli*), conformational freedom in proteins lessened which might arise from binding to certain compounds synthesized in the presence of cobalt or due to sequestration of cobalt cations by proteins

- In *Bacillus* sp., hydrogen bonds of the proteins and polysaccharides had been strengthened

- In *Bacillus* sp., protein concentrations decreased as well as the concentration of amino acids and fatty acids having side chains with COO^- stretching

- In *Bacillus* sp., the ordering of the membrane increased, while the number of hydrogen bonds between C=O groups of lipids and other chemicals containing O–H groups decreased. In *E. coli*, the concentration of lipids with ester groups increased, acyl chain flexibility decreased and saturated lipids increased. However, increased

bandwidth observed in the CH_2 symmetric stretching band pointed out to the existence of domain formation in the cell membrane, thus an increase in membrane fluidity

- In *E. coli* protein-to-lipid ratio decreased due to increased lipid concentration

- In *Bacillus* sp., polyester concentration decreased. In both *Bacillus* sp. and *E. coli*, data hinted the production of structurally or conformationally different form of polyhydroxyalkanoates

- In *Bacillus* sp., the concentration of polysaccharides in cell wall decreased

- In *Bacillus* sp. and *E. coli*, there was a change in the backbone of their nucleic acids, leading to formations of DNA and RNAs with slightly different conformations but not a transition to Z form. The results implied either cobalt itself or cobalt induced DNA conformation influencing factors such as methylation changed the flexibility of DNA. This led to speculation that the flexibility change might hint to memory formation through methylation, and the presence of epigenetic mechanisms in play

- In *Bacillus* sp. RNA levels decreased, additionally, in both *Bacillus* sp. and *Pseudomonas sp*., a modification affecting ribose was detected

- The results of this study present a guide map for further research of the physiological and biochemical changes taking place as a consequence of acclimation to growth inhibitory compounds

- Among others, like drastic changes in cell membrane dynamics, general protein levels and cell wall constituents, more detailed further examination of the changes seen in nucleic acid compositions and protein dynamics might shed more light into how these different responses play a role in general picture of adaptation to heavy metal stress in bacterial populations

- Furthermore, since *Bacillus* sp. and *Pseudomonas* sp. were isolated from a freshwater lake, they can also be helpful in understanding heavy metal adaptation process of bacteria in aquatic systems

- Lastly, ATR-FTIR approach along with genetic analysis of memory factors would be useful in tracking the progression or results of epigenetic mechanisms which are the consequences of memory formation

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APPENDIX A

FTIR DATA FOR *PSEUDOMONAS* SP. (n = 20) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p)

Table 6. Frequency data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	3283,4	2959,83	2922,82	2873,5
Control-2	3283,72	2959,49	2921,31	2873,49
Control-3	3282,61	2960,42	2922,97	2873,38
Control-4	3283,42	2959,51	2922,75	2873,63
Control-5	3283,12	2960,58	2923,75	2873,64
Control-6	3283,65	2959,95	2921,95	2873,48
Control-7	3281,31	2960,03	2922,4	2873,41
Control-8	3282,52	2960,5	2923,96	2873,4
Control-9	3282,73	2960,28	2923,93	2873,58
Control-10	3283,41	2960	2924,5	2873,68
Control-11	3280,23	2960,69	2924,42	2875,74
Control-12	3280,7	2958,83	2924,57	2876,77
Control-13	3281,91	2958,08	2923,28	2876,62
Control-14	3285,01	2957,2	2917,54	2875,04
Control-15	3282,02	2957,15	2919,37	2876,37
Control-16	3280,61	2956,63	2917,97	2874,83
Control-17	3280,61	2957,51	2921,09	2877,04
Control-18	3281,82	2958,33	2926,07	2875,88
Control-19	3283,81	2958,28	2926,72	2877,12
Control-20	3284,35	2958,14	2926,4	2876,85
Mean	3282,548	2959,0715	2922,8885	2874,8725
STD	1,359797199	1,305640698	2,514016026	1,500497724

Table 6 (continued)

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Acclimated-1	3282,2	2960,58	2922,4	2873,79
Acclimated-2	3282,81	2960	2921,42	2873,46
Acclimated-3	3278,67	2960,6	2922,5	2873,46
Acclimated-4	3282,56	2959,82	2921,1	2873,53
Acclimated-5	3283,4	2958,87	2921,31	2873,4
Acclimated-6	3282,95	2960,27	2922,98	2873,48
Acclimated-7	3281,22	2960,76	2923,42	2873,64
Acclimated-8	3282,99	2960,67	2923,6	2873,55
Acclimated-9	3282,62	2960,21	2923,26	2872,64
Acclimated-10	3284,34	2960,84	2923,93	2873,49
Acclimated-11	3278,36	2957,92	2925,1	2876,57
Acclimated-12	3277,72	2957,93	2924,71	2876,96
Acclimated-13	3276,44	2958,2	2924,26	2877,2
Acclimated-14	3278,01	2957,88	2921,09	2876,85
Acclimated-15	3280,96	2957,07	2918,24	2875,49
Acclimated-16	3280,26	2957,45	2921,44	2876,96
Acclimated-17	3283,73	2957,18	2919,92	2876,56
Acclimated-18	3282,8	2958,96	2929,09	2873,16
Acclimated-19	3285,16	2958,73	2928,14	2873,04
Acclimated-20	3285,94	2958,77	2928,49	2873,34
Mean	3281,657	2959,1355	2923,32	2874,5285
STD	2,632885349	1,307880869	2,807780543	1,648943122
p-value	0.384	0.818	0.944	0.303

Table 6 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Control-1	2850,8	1739,79	1651,77	1639,02
Control-2	2850,49	1739,55	1651,86	1640,09
Control-3	2850,82	1739,8	1651,08	1638,81
Control-4	2850,71	1739,63	1651,12	1639,47
Control-5	2851,25	1740,07	1651,34	1639,48
Control-6	2850,58	1739,86	1651,62	1640,01
Control-7	2850,49	1741,65	1651,16	1637,85
Control-8	2850,82	1743,21	1651,5	1638,24
Control-9	2850,93	1742,18	1651,35	1638,81
Control-10	2850,86	1741,94	1651,36	1639,23
Control-11	2852,69	1739,79	1651,23	1638,7
Control-12	2853,41	1739,55	1651,57	1640,28
Control-13	2853,26	1739,8	1651,58	1639,17
Control-14	2850,13	1739,63	1650,87	1640,8
Control-15	2851	1740,07	1650,93	1640,44
Control-16	2850,42	1739,86	1650,82	1638,81
Control-17	2851,47	1741,65	1651,53	1638,77
Control-18	2854,77	1743,21	1651,42	1638,82
Control-19	2854,9	1742,18	1651,43	1638,53
Control-20	2855,11	1741,94	1651,35	1639,27
Mean	2851,7455	1740,768	1651,3445	1639,23
STD	1,642496415	1,301511672	0,28474319	0,764790993

Table 6 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Acclimated-1	2850,59	1739,38	1651,43	1638,45
Acclimated-2	2850,41	1739,8	1651,38	1639,51
Acclimated-3	2850,59	1741,72	1651,17	1635,66
Acclimated-4	2850,48	1739,32	1651,06	1639,09
Acclimated-5	2850,76	1739,61	1651,69	1638,81
Acclimated-6	2850,82	1739,8	1651,49	1637,48
Acclimated-7	2850,75	1741,3	1651,18	1637,91
Acclimated-8	2850,64	1740,75	1651,52	1639,1
Acclimated-9	2850,73	1742,8	1651,65	1638,55
Acclimated-10	2850,4	1743,5	1651,61	1640,38
Acclimated-11	2853,72	1739,38	1651,19	1638,03
Acclimated-12	2854,57	1739,8	1651,62	1637,97
Acclimated-13	2853,1	1741,72	1651,55	1639,56
Acclimated-14	2851,47	1739,32	1651,25	1639,2
Acclimated-15	2850,4	1739,61	1651,16	1640,18
Acclimated-16	2851,84	1739,8	1651,02	1638,57
Acclimated-17	2851,16	1741,3	1651,48	1639,89
Acclimated-18	2852,14	1740,75	1651,5	1637,86
Acclimated-19	2852,12	1742,8	1651,35	1639,48
Acclimated-20	2852,07	1743,5	1651,37	1639,95
Mean	2851,438	1740,798	1651,3835	1638,7815
STD	1,200813759	1,451379017	0,203295869	1,114031441
p-value	0.535	0.674	0.617	0.271

Table 6 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Control-1	1538,88	1453,37	1390,59	1232,82
Control-2	1539,72	1453,46	1390,7	1233,42
Control-3	1539,12	1453,31	1390,67	1233,12
Control-4	1539,71	1453,67	1390,44	1233,75
Control-5	1539,37	1453,49	1389,14	1233,39
Control-6	1539,81	1453,72	1389,68	1234,4
Control-7	1538	1453,37	1389,82	1235,89
Control-8	1536,61	1453,67	1389,48	1235,84
Control-9	1537,16	1453,42	1389,11	1235,27
Control-10	1537,69	1453,17	1390,15	1236,72
Control-11	1536,29	1453,84	1390,15	1234,45
Control-12	1538,02	1453,44	1391,96	1235,11
Control-13	1537,35	1453,33	1392,25	1236,03
Control-14	1539,84	1453,15	1392,17	1234,69
Control-15	1538,66	1452,93	1391,38	1234,95
Control-16	1537,16	1453,16	1390,12	1233
Control-17	1536,4	1453,89	1390,59	1234,15
Control-18	1536,77	1454,29	1386,3	1235,29
Control-19	1536,95	1454,4	1384,71	1236,01
Control-20	1537,15	1453,75	1387,65	1236,98
Mean	1538,033	1453,5415	1389,853	1234,764
STD	1,247060122	0,371232301	1,865289875	1,255180423

Table 6 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Acclimated-1	1539,86	1453,13	1391,5	1232,47
Acclimated-2	1539,52	1453,04	1391,98	1235,05
Acclimated-3	1538,84	1452,77	1395,13	1233,9
Acclimated-4	1539,96	1453,46	1391,1	1232,62
Acclimated-5	1538,31	1452,9	1391,15	1233,61
Acclimated-6	1538,92	1453,1	1390,58	1234,28
Acclimated-7	1537,4	1453,03	1387,98	1233,92
Acclimated-8	1537,67	1453,1	1390,49	1234,95
Acclimated-9	1536,88	1453,32	1389,54	1234,51
Acclimated-10	1537,71	1452,89	1390,34	1235,62
Acclimated-11	1536,66	1453,66	1388,73	1235,84
Acclimated-12	1536,04	1453,62	1390,06	1233,49
Acclimated-13	1537,94	1453,61	1391,81	1235,76
Acclimated-14	1538,49	1453,12	1390,72	1234,27
Acclimated-15	1539,26	1452,91	1391,65	1234,19
Acclimated-16	1537,53	1453,8	1388,61	1234,98
Acclimated-17	1538,94	1453,26	1389,56	1234,71
Acclimated-18	1536,85	1454,02	1382,31	1240,39
Acclimated-19	1538,73	1454,16	1382,37	1241,05
Acclimated-20	1538,1	1454,24	1382,25	1240,75
Mean	1538,1805	1453,357	1389,393	1235,318
STD	1,096739066	0,442791385	3,405178873	2,503188703
p-value	0.617	0.065	0.992	0.944

Table 6 (continued)

Experimental Groups	CC symmetric stretching of ribose	PO_2 symmetric stretching of nucleic acids and phospholipids	C–O–C P–O–C symmetric stretching of polysaccharides on peptidoglycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Control-1	1118,65	1083,97	1055,59	988,92	965,6
Control-2	1118,82	1084,24	1056,16	989,19	965,88
Control-3	1118,69	1084	1055,78	988,92	965,72
Control-4	1118,83	1084,22	1056,15	989,1	966,06
Control-5	1118,9	1083,6	1055,76	988,81	965,73
Control-6	1118,96	1084,43	1055,96	989,13	966,01
Control-7	1119,24	1083,95	1056,05	987	969,24
Control-8	1118,72	1083,85	1056,61	987,25	967,49
Control-9	1118,8	1084,03	1056,71	987,15	967,76
Control-10	1119,17	1084,07	1056,19	987,42	967,48
Control-11	1118,88	1084,11	1055,94	987,25	967,45
Control-12	1119,82	1084,3	1055,58	987,75	967,04
Control-13	1119,03	1083,85	1054,63	988,23	966,68
Control-14	1120,03	1084,71	1054,8	987,6	967,33
Control-15	1120,26	1084,52	1054,75	987,3	967,09
Control-16	1120,26	1084,36	1054,63	987,04	967,64
Control-17	1119,65	1084,44	1055,49	987,45	967,4
Control-18	1119,44	1084,12	1055,26	986,69	964,2
Control-19	1120,21	1084,02	1055,16	986,62	969,47
Control-20	1120,14	1084,19	1055,13	987	966,24
Mean	1119,325	1084,149	1055,6165	987,791	966,8755
STD	0,593273	0,26275664	0,628433935	0,89360	1,248086

Table 6 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C P–O–C sym. stretching of poly-saccharides on peptido-glycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Acclimated-1	1120,02	1084,3	1054,59	988,43	965,34
Acclimated-2	1119,42	1084,46	1056,24	988,53	966,26
Acclimated-3	1118,87	1084,08	1055,2	988,32	965,22
Acclimated-4	1118,66	1084,33	1056,08	988,7	965,87
Acclimated-5	1118,92	1084,27	1055,83	988,25	965,59
Acclimated-6	1119,04	1084,39	1055,88	988,25	965,88
Acclimated-7	1118,89	1083,36	1055,88	987,38	966,99
Acclimated-8	1118,97	1083,63	1056,32	988,45	966,86
Acclimated-9	1118,82	1083,93	1056,27	987,6	966,04
Acclimated-10	1118,69	1084,01	1056,77	988,6	966,91
Acclimated-11	1120,84	1083,94	1055,82	985,89	972,24
Acclimated-12	1120,15	1084,01	1055,28	986,77	965,89
Acclimated-13	1120,95	1084,21	1055,84	987	967,65
Acclimated-14	1120,7	1084,58	1054,64	986,96	967,37
Acclimated-15	1120,6	1084,55	1054,59	987,08	967,33
Acclimated-16	1120,68	1084,37	1054,98	986,4	971,17
Acclimated-17	1120,74	1084,52	1055,07	986,3	969,57
Acclimated-18	1121,2	1083,11	1055,72	985,72	975,31
Acclimated-19	1121,43	1083,51	1055,99	985,9	975,28
Acclimated-20	1121,54	1083,33	1055,78	986	975,08
Mean	1119,95	1084,0445	1055,6385	987,3265	968,3925
STD	1,03130	0,4430097	0,621994034	1,0555481	3,474811
p-value	0.05*	0.794	0.849	0.13622	0.0703

Table 7. Band area data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	71,0827	4,0775	5,8348	1,2926
Control-2	67,5746	3,84	5,6544	1,2233
Control-3	70,7291	4,1151	5,8629	1,305
Control-4	70,2926	3,968	5,6904	1,2478
Control-5	70,0291	4,0574	5,7422	1,2854
Control-6	67,14	3,9484	5,7675	1,2645
Control-7	69,5385	4,1489	5,9975	1,337
Control-8	68,9534	4,2189	5,9689	1,3538
Control-9	63,8561	3,9462	5,6283	1,2695
Control-10	67,5157	4,1147	5,8026	1,3114
Control-11	64,246	3,8453	5,4556	1,2264
Control-12	62,6226	3,885	5,3342	1,2027
Control-13	65,193	4,313	6,0757	1,3867
Control-14	51,944	3,2586	5,7587	1,1439
Control-15	55,1612	3,5099	5,4138	1,155
Control-16	57,8318	3,7531	6,5495	1,3262
Control-17	61,2279	3,9524	5,8976	1,2903
Control-18	68,9379	3,8423	5,4127	1,2148
Control-19	69,3618	3,8683	5,4239	1,2311
Control-20	66,4162	3,8997	5,5011	1,2393
Mean	65,48271	3,928135	5,738615	1,265335
STD	5,376393853	0,237883282	0,288275466	0,063127242

Table 7 (continued)

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Acclimated-1	67,3043	4,4702	6,3765	1,4592
Acclimated-2	65,5194	4,1711	6,1777	1,3625
Acclimated-3	65,1663	5,1778	7,2348	1,7228
Acclimated-4	68,2385	4,0319	5,9457	1,2897
Acclimated-5	67,6563	4,0519	6,0288	1,3105
Acclimated-6	66,5297	4,1086	5,8964	1,3194
Acclimated-7	69,7071	4,2957	6,0583	1,373
Acclimated-8	67,0313	4,0203	5,7333	1,2696
Acclimated-9	66,0798	4,182	6,0145	1,3513
Acclimated-10	64,5034	3,9279	5,5881	1,2384
Acclimated-11	66,8002	4,0917	5,7756	1,3242
Acclimated-12	67,2781	4,3051	6,0093	1,3768
Acclimated-13	63,2456	4,2601	5,9725	1,3624
Acclimated-14	64,7926	3,9513	5,8323	1,2839
Acclimated-15	53,7483	3,445	5,6529	1,1766
Acclimated-16	61,6483	3,7855	5,805	1,2652
Acclimated-17	55,4036	3,4324	5,4324	1,1494
Acclimated-18	70,3859	3,8917	5,3389	1,2523
Acclimated-19	68,0869	3,7754	5,3066	1,2366
Acclimated-20	66,1591	3,6148	5,0374	1,1576
Mean	65,264235	4,04952	5,86085	1,31407
STD	4,187371285	0,383095862	0,457347429	0,124319614
p-value	0.490	0.284	0.250	0.155

Table 7 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Control-1	1,8489	0,2231	3,1441	25,4527
Control-2	1,7978	0,2386	2,9337	23,6399
Control-3	1,8704	0,22	3,2506	25,2141
Control-4	1,788	0,2236	2,6688	24,9687
Control-5	1,8192	0,2103	3,1823	25,2354
Control-6	1,8471	0,2366	3,2315	24,2872
Control-7	1,8841	0,1883	3,2278	25,7028
Control-8	1,8683	0,1706	3,2043	26,6373
Control-9	1,7704	0,1915	3,2794	24,2985
Control-10	1,8042	0,1719	3,2515	25,9769
Control-11	1,7012	0,166	2,9333	26,1246
Control-12	1,6541	0,1534	2,9906	23,9332
Control-13	1,9356	0,1567	2,9351	26,0307
Control-14	1,9611	0,2818	2,9258	18,5771
Control-15	1,7424	0,2142	2,8515	20,9589
Control-16	2,1967	0,3078	2,5982	23,3399
Control-17	1,8661	0,2059	2,3957	25,3132
Control-18	1,6073	0,1504	2,1364	26,0193
Control-19	1,6074	0,1324	2,9268	26,0487
Control-20	1,6311	0,174	2,7657	26,3788
Mean	1,81007	0,200855	2,941655	24,706895
STD	0,138390866	0,044563125	0,309527107	1,966979415

Table 7 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Acclimated-1	2,1078	0,2644	3,0895	25,2958
Acclimated-2	1,9945	0,2883	3,0027	26,5324
Acclimated-3	2,511	0,2655	3,1205	23,5206
Acclimated-4	1,9075	0,3658	2,8133	25,2645
Acclimated-5	1,9137	0,3134	3,0581	25,3688
Acclimated-6	1,885	0,2834	3,3109	25,2476
Acclimated-7	1,9121	0,2199	3,3117	25,7994
Acclimated-8	1,7556	0,2393	3,2944	26,2285
Acclimated-9	1,893	0,2293	3,2734	25,3448
Acclimated-10	1,7096	0,2371	3,2975	25,0155
Acclimated-11	1,805	0,1593	2,2532	26,4479
Acclimated-12	1,8884	0,1682	2,7001	26,9494
Acclimated-13	1,8902	0,175	2,9462	25,8042
Acclimated-14	1,8636	0,1867	3,01	23,9155
Acclimated-15	1,8876	0,2503	2,9745	19,8252
Acclimated-16	1,8381	0,2024	2,8433	23,917
Acclimated-17	1,7326	0,2185	2,8211	20,5713
Acclimated-18	1,5802	0,0722	2,9101	26,0694
Acclimated-19	1,5754	0,0906	2,3336	24,5814
Acclimated-20	1,4536	0,0943	2,6776	24,5606
Mean	1,855225	0,216195	2,952085	24,81299
STD	0,216746614	0,075495036	0,301099912	1,824623557
p-value	0.238	0.238	0.756	0.880

Table 7 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Control-1	19,8806	2,9705	7,086	8,2877
Control-2	18,1045	2,7455	6,6012	7,637
Control-3	19,8224	3,0191	7,2715	8,2694
Control-4	19,1244	2,854	6,9151	8,1025
Control-5	19,2705	2,9711	7,1432	8,2615
Control-6	18,6148	2,8915	7,0287	8,1576
Control-7	22,5148	3,5819	8,6568	7,4197
Control-8	23,5863	3,7098	8,8284	7,982
Control-9	20,9579	3,331	7,8166	7,1136
Control-10	22,2261	3,4606	8,1971	7,4942
Control-11	22,7023	3,5668	8,2628	7,7254
Control-12	19,648	3,1118	6,9945	6,6223
Control-13	22,8685	3,6959	8,6919	7,9368
Control-14	14,9781	2,5325	5,9147	5,7426
Control-15	17,309	2,8603	6,655	6,1411
Control-16	19,992	3,3285	7,912	7,6423
Control-17	21,3862	3,4452	7,9703	7,7248
Control-18	21,9593	3,3358	8,0805	7,2517
Control-19	22,2372	3,4115	8,4346	7,2046
Control-20	21,7727	3,349	8,1374	7,5326
Mean	20,44778	3,208615	7,629915	7,51247
STD	2,175565831	0,337153895	0,813288623	0,694312435

Table 7 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Acclimated-1	21,6042	3,349	8,6959	8,297
Acclimated-2	25,1929	3,905	8,0179	7,7186
Acclimated-3	18,7234	2,7538	11,1311	8,7635
Acclimated-4	21,1961	3,1973	6,9354	7,7545
Acclimated-5	21,1553	3,3189	7,9238	8,1043
Acclimated-6	22,7183	3,5875	8,2815	8,2725
Acclimated-7	21,425	3,2751	8,5262	7,6671
Acclimated-8	22,4361	3,5247	7,7228	7,5493
Acclimated-9	20,6618	3,1784	8,3144	7,8303
Acclimated-10	20,7819	3,3339	7,4388	7,3906
Acclimated-11	24,0266	3,8728	9,2161	7,1455
Acclimated-12	23,6903	3,8702	8,9193	7,8658
Acclimated-13	22,0968	3,6243	8,3593	7,1442
Acclimated-14	20,4423	3,3777	7,8381	6,6191
Acclimated-15	16,727	2,8444	6,6312	6,0415
Acclimated-16	20,4809	3,3223	7,8963	6,7195
Acclimated-17	17,226	2,8368	6,7263	5,8552
Acclimated-18	23,9867	3,7927	9,68	6,5544
Acclimated-19	21,442	3,4297	8,7753	5,8961
Acclimated-20	21,3433	3,3611	8,4778	6,0186
Mean	21,367845	3,38778	8,275375	7,26038
STD	2,124530248	0,334320019	1,036072521	0,875822172
p-value	0.250	0.214	0.06	0.423

Table 7 (continued)

Experimental Groups	CC symmetric stretching of ribose	PO_2 symmetric stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharide on pepti-doglycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Control-1	3,7813	6,3411	13,8791	1,6192	1,584
Control-2	3,556	5,7514	11,8689	1,4306	1,2464
Control-3	3,7561	6,2828	13,4694	1,7182	1,4213
Control-4	3,7395	6,1439	12,6491	1,4533	1,3094
Control-5	3,7545	6,2703	13,5457	1,5735	1,4911
Control-6	3,6867	5,9645	12,0827	1,7213	1,3356
Control-7	4,313	5,8173	13,8215	1,7576	1,5019
Control-8	4,3226	5,9059	13,7439	1,3755	1,3912
Control-9	3,9289	5,3853	12,4831	1,6477	1,2736
Control-10	4,1109	5,4864	11,9999	1,4632	1,15
Control-11	3,765	5,3334	12,1046	1,7356	1,3842
Control-12	3,1117	4,4716	8,9927	2,143	0,8503
Control-13	3,7033	5,3959	11,0636	2,0014	0,9709
Control-14	2,6834	3,6906	7,5995	1,3187	0,8568
Control-15	3,0758	4,2098	8,8574	1,3668	0,9559
Control-16	3,7549	5,1875	11,533	1,0269	1,315
Control-17	3,825	5,3013	11,433	0,9295	1,2267
Control-18	4,3252	6,052	15,1208	1,2395	1,8766
Control-19	4,6234	6,3721	16,4566	0,9741	1,9082
Control-20	4,5173	6,2302	14,2493	1,5213	1,5472
Mean	3,816725	5,579665	12,34769	1,500845	1,329815
STD	0,484003	0,744429	2,140719	0,315674315	0,289478208

Table 7 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Acclimated-1	3,5577	6,3604	13,3023	1,6306	1,5373
Acclimated-2	3,7005	5,8534	11,2348	1,9184	1,0938
Acclimated-3	3,9081	6,5679	12,7864	1,3839	1,3454
Acclimated-4	3,344	5,9984	12,1693	1,5458	1,2295
Acclimated-5	3,7187	6,1657	12,7684	1,3818	1,4348
Acclimated-6	3,758	6,1526	12,1318	1,4338	1,2649
Acclimated-7	4,0326	6,2272	13,9333	1,2931	1,574
Acclimated-8	3,8775	5,7842	11,8067	1,5496	1,1888
Acclimated-9	4,154	6,2816	12,5066	1,3622	1,4131
Acclimated-10	3,7615	5,4932	10,5465	1,7746	0,9947
Acclimated-11	4,1906	5,6416	14,8207	1,9875	1,8013
Acclimated-12	3,9563	5,6471	13,413	2,3269	1,5533
Acclimated-13	3,8018	5,2189	10,9895	2,5658	1,0844
Acclimated-14	3,5931	5,0021	11,5328	1,3327	1,3242
Acclimated-15	3,0173	4,1796	9,0437	1,7776	1,0609
Acclimated-16	4,0615	5,4939	13,1946	1,1828	1,5426
Acclimated-17	3,3707	4,4874	10,3267	1,4981	1,2086
Acclimated-18	5,0398	6,3007	18,4084	1,374	2,0437
Acclimated-19	4,8459	6,058	16,8807	1,7972	1,7901
Acclimated-20	4,7418	5,7867	15,551	2,1614	1,5533
Mean	3,92157	5,73503	12,86736	1,66389	1,401935
STD	0,50215	0,624539	2,251220782	0,3722748	0,2767526
p-value	0.674	0.522	0.756	0.28462	0.490

Table 8. Band area ratio of certain bands for control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them

Experimental Groups	CH_3 symmetric stretching/CH_3 asymmetric stretching	Amides I + II/ester C=O stretching	Ester C=O stretching/ CH_3 asymmetric	RNA/ Amide I + II
Control-1	0,32	203,20	0,055	0,036
Control-2	0,32	174,96	0,062	0,034
Control-3	0,32	204,71	0,053	0,038
Control-4	0,31	197,20	0,056	0,033
Control-5	0,32	211,63	0,052	0,035
Control-6	0,32	181,33	0,06	0,04
Control-7	0,32	256,07	0,045	0,036
Control-8	0,32	294,39	0,04	0,027
Control-9	0,32	236,33	0,049	0,036
Control-10	0,32	280,41	0,042	0,03
Control-11	0,32	294,14	0,043	0,036
Control-12	0,31	284,10	0,039	0,049
Control-13	0,32	312,06	0,036	0,041
Control-14	0,35	119,07	0,086	0,039
Control-15	0,33	178,65	0,061	0,036
Control-16	0,35	140,78	0,082	0,024
Control-17	0,33	226,81	0,052	0,02
Control-18	0,32	319,01	0,039	0,026
Control-19	0,32	364,70	0,034	0,02
Control-20	0,32	276,73	0,045	0,032
Mean	0,322497581	237,81331	0,05155	0,0334
STD	0,01095695	64,600645	0,01390371	0,00722131

Table 8 (continued)

Experimental Groups	CH_3 symmetric stretching/CH_3 asymmetric stretching	Amides I + II/ester C=O stretching	Ester C=O stretching/ CH_3 asymmetric	RNA/ Amide I + II
Acclimated-1	0,33	177,38	0,059	0,035
Acclimated-2	0,33	179,41	0,069	0,037
Acclimated-3	0,33	159,11	0,051	0,033
Acclimated-4	0,32	127,01	0,091	0,033
Acclimated-5	0,32	148,45	0,077	0,03
Acclimated-6	0,32	169,25	0,069	0,03
Acclimated-7	0,32	214,75	0,051	0,027
Acclimated-8	0,32	203,36	0,06	0,032
Acclimated-9	0,32	200,64	0,055	0,03
Acclimated-10	0,32	193,16	0,06	0,039
Acclimated-11	0,32	316,85	0,039	0,039
Acclimated-12	0,32	301,07	0,039	0,046
Acclimated-13	0,32	273,72	0,041	0,054
Acclimated-14	0,32	237,59	0,047	0,03
Acclimated-15	0,34	146,03	0,073	0,049
Acclimated-16	0,33	219,36	0,053	0,027
Acclimated-17	0,33	172,99	0,064	0,04
Acclimated-18	0,32	693,30	0,019	0,027
Acclimated-19	0,33	507,98	0,024	0,039
Acclimated-20	0,32	486,79	0,026	0,047
Mean	0,324621896	256,41030	0,05335	0,0362
STD	0,006759569	145,70123	0,018488332	0,00787801
p-value	0.32218	0.4593	0.58232	0.56192

Table 9. Bandwidth data of control and cobalt acclimated *Pseudomonas* sp. with statistical significance (p-value) of difference between them

Experimental Groups	CH_2 asymmetric stretching of lipids	CH_2 symmetric stretching of lipids	Amide I	Amide II
Control-1	12,6281	4,6599	29,2492	24,947
Control-2	10,4049	4,7312	29,5666	24,0702
Control-3	12,6921	4,5835	25,527	24,7083
Control-4	11,1147	4,6779	25,5572	23,9161
Control-5	11,1632	4,5335	25,7189	24,3518
Control-6	10,7031	4,6948	26,1012	23,4469
Control-7	11,2732	4,3045	27,1707	25,0641
Control-8	12,2896	4,131	27,0409	25,973
Control-9	12,0434	4,3141	27,8524	26,2907
Control-10	12,6007	4,3499	26,7969	24,718
Control-11	12,39	4,18	27,84	26,08
Control-12	12,24	4,35	27,66	25,28
Control-13	11,8	4,32	26,72	25,05
Control-14	7,95	4,81	28,97	24,63
Control-15	9,48	4,64	28,74	25,43
Control-16	8,21	4,68	29,39	26,32
Control-17	10,82	4,53	29,15	26,19
Control-18	12,78	3,73	28,54	25,52
Control-19	13,52	3,21	28,72	25,24
Control-20	12,3	3,48	28,26	25,51
Mean	11,42015	4,345515	27,72855	25,136805
STD	1,497401363	0,430089903	1,335941228	0,814254717

Table 9 (continued)

Experimental Groups	CH_2 asymmetric stretching of lipids	CH_2 symmetric stretching of lipids	Amide I	Amide II
Acclimated-1	11,5214	4,4266	25,3322	22,9005
Acclimated-2	10,7302	4,5476	25,94	23,5852
Acclimated-3	11,1121	4,2201	21,1682	22,8676
Acclimated-4	10,1581	4,7171	25,4726	23,3875
Acclimated-5	10,531	4,6543	24,9937	24,684
Acclimated-6	11,3764	4,5479	25,4671	23,689
Acclimated-7	11,8502	4,2971	27,046	24,9414
Acclimated-8	12,5599	4,3481	27,2432	25,026
Acclimated-9	11,946	4,438	27,7538	24,8196
Acclimated-10	12,2985	4,3467	27,0152	24,8474
Acclimated-11	12,64	3,89	27,7	25,57
Acclimated-12	12,18	4,11	27,91	26,03
Acclimated-13	12,14	4,35	27,1	24,38
Acclimated-14	10,67	4,58	28,88	24,53
Acclimated-15	8,55	4,77	28,55	24,88
Acclimated-16	11,19	4,67	29,18	25,49
Acclimated-17	10,1	4,78	29,41	25,58
Acclimated-18	14,15	4,75	28,6	25,14
Acclimated-19	14,49	4,61	27,83	24,24
Acclimated-20	14,4	4,59	28,42	24,27
Mean	11,72969	4,482175	27,0506	24,54291
STD	1,495916307	0,236249749	1,923433354	0,887599893
p-value	0.992	0.465	0.262	0.0315*

APPENDIX B

FTIR DATA FOR *BACILLUS* SP. (n = 20) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p)

Table 10. Frequency data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	3277,3	2957,68	2926,21	2875,95
Control-2	3276,53	2957,45	2925,8	2875,45
Control-3	3281,03	2957,85	2928,48	2876,1
Control-4	3274,19	2957,85	2927,03	2875,54
Control-5	3275,67	2958,09	2927,25	2876,36
Control-6	3277,45	2957,94	2929,55	2877,1
Control-7	3284,33	2958,35	2929,36	2876,57
Control-8	3283,79	2958,36	2930,19	2877,04
Control-9	3280,89	2958,43	2930,96	2878,42
Control-10	3265,6	2957,73	2930,19	2876,42
Control-11	3266,9	2957,57	2929,67	2875,57
Control-12	3263,62	2957,57	2929,45	2875,38
Control-13	3263,96	2957,7	2929,52	2875,21
Control-14	3266,97	2957,7	2929,47	2875,49
Control-15	3261,8	2957,68	2929,58	2875,28
Control-16	3265,43	2957,35	2928,01	2875,36
Control-17	3264,49	2957,33	2927,42	2875,38
Control-18	3265,43	2957,31	2927,45	2875,27
Control-19	3261,71	2957,32	2927,78	2875,32
Control-20	3263,28	2957,32	2927,72	2875,23
Mean	3271,0185	2957,729	2928,5545	2875,922
STD	7,896524402	0,357416166	1,430114	0,844110245

Table 10 (continued)

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Acclimated-1	3284,29	2957,89	2927,35	2876,09
Acclimated-2	3287,25	2957,97	2925,86	2876,28
Acclimated-3	3281,22	2957,8	2927,35	2876,12
Acclimated-4	3281,26	2957,75	2927,32	2876,3
Acclimated-5	3280,31	2957,92	2927,33	2876,21
Acclimated-6	3282,57	2958,43	2930,54	2878,21
Acclimated-7	3285,41	2958,16	2931,14	2878,4
Acclimated-8	3289,86	2958,42	2931,54	2878,57
Acclimated-9	3294,27	2958,83	2932,42	2878,76
Acclimated-10	3272,41	2957,86	2930,06	2876,13
Acclimated-11	3277,45	2958,05	2928,29	2875,49
Acclimated-12	3274,66	2957,56	2927,61	2875,14
Acclimated-13	3269,32	2957,61	2929,55	2875,21
Acclimated-14	3278,04	2958,83	2929,56	2876,14
Acclimated-15	3269,06	2957,45	2926,95	2874,64
Acclimated-16	3276,14	2957,3	2927,17	2875,04
Acclimated-17	3270,99	2957,74	2929,05	2875,7
Acclimated-18	3272,22	2958,03	2930	2876,17
Acclimated-19	3271,41	2957,63	2928,6	2875,97
Acclimated-20	3268,65	2957,96	2929,64	2875,81
Mean	3278,3395	2957,9595	2928,8665	2876,319
STD	7,416736036	0,411741807	1,761549184	1,206425342
p-value	0.0058	0.056	0.68	0.337

Table 10 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of tri-glycerides	Amide I A	Amide I B	Amide II
Control-1	2850,76	1737,06	1651,33	1639,88	1535,58
Control-2	2850,64	1736,2	1651,46	1639,86	1537,03
Control-3	2851,43	1736,67	1651,71	1642,88	1537,03
Control-4	2851,18	1737,03	1651,75	1639,22	1537,03
Control-5	2851,1	1736,3	1651,68	1640,34	1537,03
Control-6	2851,32	1736,41	1649,35	1640,1	1539,78
Control-7	2851,72	1735,98	1651,73	1642,26	1539,78
Control-8	2852,04	1736,99	1651,55	1642,31	1540,06
Control-9	2852,27	1737,46	1651,84	1643,03	1540,63
Control-10	2852,02	1737,28	1652	1641,72	1538,85
Control-11	2852,31	1737,14	1652,01	1638,97	1538,85
Control-12	2852,16	1737,39	1652,02	1639,06	1538,09
Control-13	2852,18	1737,44	1651,91	1639,1	1538,09
Control-14	2852,24	1737,48	1651,92	1639,18	1538,09
Control-15	2852,08	1737,32	1652,03	1639,27	1538,09
Control-16	2852,08	1735,89	1652,02	1639,52	1537,62
Control-17	2851,83	1736,53	1651,72	1640,45	1537,62
Control-18	2851,88	1736,16	1651,62	1638,48	1537,94
Control-19	2852,15	1737,69	1651,38	1638,03	1537,78
Control-20	2852,07	1735,9	1651,49	1638,37	1538,02
Mean	2851,773	1736,816	1651,626	1640,1015	1538,1495
STD	0,516813	0,597700	0,58033928	1,533490257	1,224506238

Table 10 (continued)

Experimental Groups	CH_2 sym. stretching of lipids	Ester C=O stretching of tri-glycerides	Amide I A	Amide I B	Amide II
Acclimated-1	2850,8	1737,98	1651,66	1642,17	1540,05
Acclimated-2	2850,53	1737,38	1651,01	1642,77	1540,2
Acclimated-3	2850,79	1737,49	1651,09	1641,8	1540,2
Acclimated-4	2850,73	1737,87	1650,83	1642,76	1541,87
Acclimated-5	2850,73	1737,78	1651,43	1642,16	1541,87
Acclimated-6	2851,53	1737,56	1651,73	1641,2	1540,52
Acclimated-7	2851,97	1737,88	1651,36	1642,43	1540,52
Acclimated-8	2851,99	1737,91	1649,52	1643,85	1541,32
Acclimated-9	2851,79	1738,16	1651,06	1645,36	1544,71
Acclimated-10	2852	1737,92	1652,17	1639,52	1538,43
Acclimated-11	2851,37	1737,72	1652,06	1641,57	1539,62
Acclimated-12	2851,79	1738,12	1651,76	1641,37	1539,8
Acclimated-13	2852,11	1737,69	1652,39	1639,84	1537,02
Acclimated-14	2851,62	1738,53	1651,12	1641,18	1539,05
Acclimated-15	2851,88	1739,47	1650,41	1642,67	1541,01
Acclimated-16	2851,63	1739,37	1651,22	1640,71	1538,47
Acclimated-17	2851,8	1738,32	1651,74	1639,5	1537,26
Acclimated-18	2851,92	1738,05	1651,51	1639,52	1537,42
Acclimated-19	2851,88	1738,12	1651,7	1639,26	1537,57
Acclimated-20	2851,84	1738,08	1651,71	1639,36	1537,82
Mean	2851,535	1738,07	1651,374	1641,45	1539,7365
STD	0,516440	0,5377144	0,6461008	1,6513598	1,9299503
p-value	0.043*	0.0001***	0.082	0.015*	0.0001***

Table 10 (continued)

Experimental Groups	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	Amide III band components of proteins	PO_2 asymmetric stretching of nucleic acids
Control-1	1450,33	1387,9	1305,44	1240,34
Control-2	1449,81	1390,22	1305,95	1238,78
Control-3	1450,04	1388,71	1305,68	1238,66
Control-4	1450,25	1389,26	1305,29	1237,49
Control-5	1449,81	1389,38	1305,56	1239,24
Control-6	1449,98	1391,11	1306,5	1240,62
Control-7	1449,61	1391,13	1306,19	1241,3
Control-8	1449,92	1390,13	1305,79	1241,3
Control-9	1450,52	1389,22	1305,69	1242,43
Control-10	1450,58	1390,3	1305	1237,87
Control-11	1450,46	1390,66	1305,13	1237,3
Control-12	1450,39	1394,14	1305,13	1237,29
Control-13	1450,34	1390,03	1305,03	1237,64
Control-14	1450,58	1389,71	1305,22	1238,08
Control-15	1450,34	1390,31	1305,07	1237,61
Control-16	1450,24	1389,85	1305,07	1235,54
Control-17	1450,04	1389,49	1305,1	1235,66
Control-18	1449,82	1389,67	1305,12	1235,04
Control-19	1449,75	1389,59	1305,21	1235,74
Control-20	1449,77	1389,64	1305,27	1235,13
Mean	1450,129	1390,0225	1305,422	1238,153
STD	0,309190794	1,229338183	0,422718022	2,189097627

Table 10 (continued)

Experimental Groups	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	Amide III band components of proteins	PO_2 asymmetric stretching of nucleic acids
Acclimated-1	1450,82	1389,74	1305,54	1241,68
Acclimated-2	1450,96	1388,06	1306,15	1242,14
Acclimated-3	1450,28	1390,8	1305,91	1242,65
Acclimated-4	1451,04	1391,08	1305,94	1242,89
Acclimated-5	1451,04	1390,55	1305,59	1242,82
Acclimated-6	1450,88	1389,84	1305,69	1241,14
Acclimated-7	1450,95	1390,82	1305,8	1242,66
Acclimated-8	1450,05	1390,08	1305,87	1243
Acclimated-9	1450,69	1390,27	1305,7	1244,86
Acclimated-10	1451,1	1390,2	1305,3	1238,11
Acclimated-11	1449,97	1392,57	1306,19	1236
Acclimated-12	1449,83	1390,34	1306,36	1236,01
Acclimated-13	1450,92	1390,02	1305,19	1236,73
Acclimated-14	1450,16	1393,01	1306	1235,39
Acclimated-15	1450,45	1393,74	1306,65	1237,02
Acclimated-16	1450,85	1393,37	1306,82	1236,35
Acclimated-17	1450,69	1389,35	1304,8	1238,52
Acclimated-18	1450,04	1388,23	1304,8	1240,73
Acclimated-19	1450,44	1389,8	1305,12	1237,52
Acclimated-20	1450,49	1389,16	1304,7	1237,75
Mean	1450,5825	1390,5515	1305,706	1239,6985
STD	0,407377686	1,557678012	0,592908975	3,021693974
p-value	0.000933***	0.207	0.0784	0.101005

Table 10 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	CC stretching of nucleic acids
Control-1	1100,96	1082,7	1056,08	994,6	966,96
Control-2	1101,25	1082,83	1056,88	994,35	967,49
Control-3	1101,16	1082,57	1056,77	994,6	967,53
Control-4	1100,65	1082,32	1056,31	994,3	967,54
Control-5	1100,91	1083,07	1056,94	994,27	967,46
Control-6	1101,39	1083,55	1057,49	994,4	968,68
Control-7	1101,36	1083,65	1058,06	994,6	968,19
Control-8	1101,23	1083,55	1057,78	994,75	968,3
Control-9	1101,41	1083,48	1057,76	995,22	969,79
Control-10	1100,54	1082,68	1056,11	994,7	967,11
Control-11	1100,48	1082,7	1056,07	994,33	966,91
Control-12	1100,51	1082,52	1055,98	994,28	966,59
Control-13	1100,36	1082,7	1056,13	994,49	966,44
Control-14	1100,51	1082,62	1055,98	994,2	966,67
Control-15	1100,4	1082,55	1055,92	994,49	966,88
Control-16	1100,6	1082,54	1056,22	994	966,78
Control-17	1100,42	1082,56	1056,19	994,27	966,79
Control-18	1100,41	1082,51	1056,17	994,05	966,75
Control-19	1100,58	1082,64	1055,91	994,09	967,48
Control-20	1100,5	1082,55	1056,33	993,83	966,66
Mean	1100,7815	1082,8145	1056,554	994,391	967,35
STD	0,3818828	0,40897143	0,69646778	0,3102274	0,8402568

Table 10 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	CC stretching of nucleic acids
Acclimated-1	1101,5	1083,1	1056,8	995,52	968,16
Acclimated-2	1101,7	1083,11	1057,04	995,67	968,35
Acclimated-3	1101,6	1083,43	1057,34	995,22	968,45
Acclimated-4	1101,45	1083,71	1057,5	995,5	968,78
Acclimated-5	1101,37	1083,44	1057,22	995,8	969,13
Acclimated-6	1101,21	1083,42	1057,07	995,27	969,88
Acclimated-7	1101,49	1083,6	1057,6	994,86	968,55
Acclimated-8	1101,53	1083,74	1057,76	995	969,52
Acclimated-9	1101,54	1084,07	1057,93	995,08	970,22
Acclimated-10	1100,88	1083,49	1056,93	994,32	967,53
Acclimated-11	1100,8	1083,83	1057,98	994,48	968,84
Acclimated-12	1101,21	1083,72	1057,98	994,63	966,67
Acclimated-13	1100,75	1083,03	1056,37	994,22	967,04
Acclimated-14	1100,98	1084,05	1057,61	994,5	967,12
Acclimated-15	1101,12	1084,08	1058,14	994,4	967,27
Acclimated-16	1101,59	1084,13	1057,69	993,86	966,54
Acclimated-17	1100,74	1082,57	1055,76	994,44	967,25
Acclimated-18	1100,88	1082,22	1055,41	995,11	967,7
Acclimated-19	1101,09	1082,61	1056,09	994,42	966,27
Acclimated-20	1100,79	1082,39	1055,93	994,5	967,18
Mean	1101,211	1083,387	1057,1075	994,84	968,0225
STD	0,329575	0,582635	0,812435423	0,5401948	1,1396023
p-value	0.001***	0.0038**	0.0394*	0.0056**	0.0703

Table 11. Band area data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	58,7094	4,4331	4,9311	1,5145
Control-2	54,5474	4,1672	4,7634	1,7106
Control-3	56,9369	4,2231	5,6338	1,6522
Control-4	59,9882	4,7424	4,9299	1,9797
Control-5	53,7383	4,0507	4,9928	1,7164
Control-6	60,8021	4,3644	5,2794	1,724
Control-7	52,687	3,8378	5,852	1,8067
Control-8	49,5881	3,665	4,2829	2,1101
Control-9	50,9917	3,8125	4,1704	1,4965
Control-10	64,8106	5,2069	4,3833	1,4526
Control-11	63,2166	5,279	6,02	2,2064
Control-12	61,0005	5,2887	5,9435	2,1778
Control-13	60,9247	5,2946	6,0545	2,2668
Control-14	60,8002	5,2139	6,0465	2,2434
Control-15	61,3985	5,29	5,9965	2,2156
Control-16	62,4442	5,1812	6,2782	2,1815
Control-17	62,9766	5,1991	6,2291	2,1732
Control-18	63,2612	5,2719	6,1794	2,1933
Control-19	63,5165	5,3392	6,1076	2,1587
Control-20	63,9366	5,3735	6,0566	2,2076
Mean	59,313765	4,76171	5,506545	1,95938
STD	4,61988298	0,616401685	0,715680716	0,288991214

Table 11 (continued)

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Acclimated-1	51,5864	3,6607	4,0566	1,405
Acclimated-2	48,7516	4,1473	4,6194	1,6065
Acclimated-3	55,3211	4,1047	4,5761	1,5884
Acclimated-4	54,232	4,3261	5,016	1,7376
Acclimated-5	54,9913	4,292	4,9701	1,7215
Acclimated-6	56,897	4,3783	5,0839	1,7591
Acclimated-7	56,0277	3,5237	4,1853	1,3977
Acclimated-8	51,0092	3,9208	4,5508	1,5665
Acclimated-9	45,6448	4,9099	5,5298	1,9649
Acclimated-10	63,103	3,1278	3,4062	1,1656
Acclimated-11	55,0686	4,3618	5,4072	1,9293
Acclimated-12	55,322	5,1515	4,9337	1,7144
Acclimated-13	56,4565	4,4721	5,8934	2,138
Acclimated-14	57,99	4,304	5,1705	1,841
Acclimated-15	60,0782	5,1567	4,94	1,735
Acclimated-16	60,336	5,0883	5,8345	2,1118
Acclimated-17	60,8883	4,9333	5,7745	2,0807
Acclimated-18	61,5151	4,9882	5,543	1,9882
Acclimated-19	61,6353	4,5249	5,6853	2,0346
Acclimated-20	62,2978	4,6663	5,3173	1,8484
Mean	56,457595	4,40192	5,02468	1,76671
STD	4,715543921	0,558901786	0,65129053	0,260246401
p-value	0.056	0.0315*	0.0279*	0.0315*

Table 11 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of tri-glycerides	Amide I A	Amide I B	Amide II
Control-1	1,2946	1,4405	2,3388	24,7687	21,5447
Control-2	1,2485	1,5974	2,4779	22,8212	20,1601
Control-3	1,3083	1,4021	2,7682	26,522	25,1665
Control-4	1,4792	2,0417	2,5292	25,4484	22,3762
Control-5	1,4579	1,9217	2,9173	23,8001	21,6373
Control-6	1,7652	2,0357	2,7907	25,9834	23,3822
Control-7	1,4932	1,5054	2,61	26,7611	25,9926
Control-8	1,5536	1,37	2,8846	20,8922	16,3456
Control-9	1,6069	1,5517	2,8902	20,747	17,0752
Control-10	1,872	1,3822	2,3772	21,7646	17,9391
Control-11	1,9863	1,9731	2,8992	26,5607	25,9732
Control-12	1,9488	1,8392	2,904	26,5918	26,5682
Control-13	2,0577	1,8073	2,8974	26,5362	26,5097
Control-14	2,0362	1,7268	2,8906	26,6429	26,7874
Control-15	2,0095	1,6241	2,9665	27,4533	26,7156
Control-16	1,9748	1,7295	2,9099	27,2467	26,6369
Control-17	1,9489	1,7028	2,9799	27,3965	26,7688
Control-18	1,9737	1,7339	2,9794	27,3722	26,6975
Control-19	1,9343	1,418	2,9871	27,1386	26,332
Control-20	1,9874	1,8828	2,8725	26,2392	26,187
Mean	1,74685	1,684295	2,79353	25,43434	23,83979
STD	0,2830576	0,22206700	0,20735456	2,21309617	3,5653165

Table 11 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of tri-glycerides	Amide I A	Amide I B	Amide II
Acclimated-1	1,1956	1,9856	2,6106	20,9161	15,7081
Acclimated-2	1,375	2,0098	2,7711	24,1637	19,5005
Acclimated-3	1,3649	2,1815	2,7151	23,2352	18,7168
Acclimated-4	1,5509	2,1665	2,8261	24,2636	19,8593
Acclimated-5	1,5413	2,0842	2,4309	23,6554	19,3209
Acclimated-6	1,5718	2,2436	2,5235	24,9294	20,6043
Acclimated-7	1,2491	2,3262	2,9949	21,1989	16,2254
Acclimated-8	1,395	2,1452	2,0201	22,3129	18,1977
Acclimated-9	1,7251	1,7815	2,4551	27,1275	23,5852
Acclimated-10	0,9867	1,7806	2,7857	16,7965	11,9756
Acclimated-11	1,6562	1,0385	2,9776	24,2814	20,0743
Acclimated-12	1,7603	1,2447	2,5978	26,9413	26,6028
Acclimated-13	1,5104	1,1667	2,6306	23,1586	19,9947
Acclimated-14	1,9084	1,5518	2,9008	23,3274	19,5757
Acclimated-15	1,6531	1,1209	2,9388	26,3606	26,3826
Acclimated-16	1,545	1,1786	2,8554	26,8634	26,7413
Acclimated-17	1,8849	1,7581	2,9053	25,7993	24,9688
Acclimated-18	1,8452	1,5956	2,8101	26,3554	25,9228
Acclimated-19	1,7548	2,3432	2,5868	25,3576	21,4753
Acclimated-20	1,8101	1,9339	2,7263	22,7215	19,7522
Mean	1,56419	1,781835	2,70313	23,988285	20,759215
STD	0,2465144	0,4341237	0,23290981	2,5074716	3,9493287
p-value	0.0292*	0.219	0.164	0.0384*	0.0114*

Table 11 (continued)

Experimental Groups	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	Amide III band components of proteins	PO_2 asymmetric stretching of nucleic acids
Control-1	3,8095	6,5901	3,4053	6,4838
Control-2	3,9215	6,7364	3,4521	6,5431
Control-3	4,7667	8,2182	4,1638	7,8907
Control-4	4,7023	7,9889	4,0337	7,8177
Control-5	6,0067	10,158	4,9859	9,5366
Control-6	5,2077	8,7683	4,4944	8,8839
Control-7	5,055	8,6245	4,2756	8,1761
Control-8	5,3761	9,3489	4,9803	9,5787
Control-9	6,2622	10,792	5,2047	9,8091
Control-10	3,7536	6,4942	3,4526	6,5549
Control-11	6,7464	10,8497	5,0791	9,4978
Control-12	6,6938	11,4363	5,2998	9,7926
Control-13	6,5687	11,2717	5,3616	9,9923
Control-14	6,7465	11,4885	5,3849	9,9854
Control-15	6,6601	11,395	5,2987	9,9288
Control-16	6,534	11,2259	5,2372	9,833
Control-17	7,296	11,5339	5,3904	9,5691
Control-18	7,2477	11,6576	5,5245	9,729
Control-19	7,0752	11,1883	5,2346	9,4594
Control-20	6,9444	11,0872	5,2078	9,4902
Mean	5,868705	9,84268	4,77335	8,92761
STD	1,190493493	1,828717241	0,718485322	1,225483466

Table 11 (continued)

Experimental Groups	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	Amide III band components of proteins	PO_2 asymmetric stretching of nucleic acids
Acclimated-1	3,5004	6,209	3,4284	6,4216
Acclimated-2	4,349	7,7853	4,1395	7,7139
Acclimated-3	4,3708	7,7143	4,1503	7,8221
Acclimated-4	4,826	8,4336	4,4391	8,0841
Acclimated-5	4,6623	8,178	4,2435	7,707
Acclimated-6	4,9934	8,7895	4,6313	8,4946
Acclimated-7	3,9576	6,8517	3,9409	7,3222
Acclimated-8	4,3759	7,8224	4,164	7,6383
Acclimated-9	5,6838	9,8852	4,9354	10,05
Acclimated-10	2,5942	4,7013	2,604	4,8398
Acclimated-11	3,0692	7,1655	3,5161	7,8652
Acclimated-12	4,4857	10,988	5,2507	6,7341
Acclimated-13	3,2655	7,6086	3,5151	7,5068
Acclimated-14	3,1021	7,2729	3,5145	10,235
Acclimated-15	4,4508	11,096	5,4385	6,825
Acclimated-16	4,4055	11,039	5,3479	7,1884
Acclimated-17	4,1816	10,482	5,4106	10,095
Acclimated-18	4,2948	10,799	5,3642	10,177
Acclimated-19	3,3865	7,9204	3,7393	9,8046
Acclimated-20	3,3105	7,6302	3,5382	10,288
Mean	4,06328	8,418595	4,265575	8,140635
STD	0,770456424	1,772961506	0,819191397	1,519085297
p-value	0.00004***	0.0099**	0.119	0.263

Table 11 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 symmetric stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	CC stretching of nucleic acids
Control-1	1,831	2,7221	6,7939	1,1541	0,7029
Control-2	1,8125	2,697	6,705	0,4734	0,6517
Control-3	2,1855	3,3021	6,4815	0,4964	0,8539
Control-4	2,2455	3,4738	8,1655	0,4746	1,1564
Control-5	2,7631	4,4093	9,7508	0,6199	1,8004
Control-6	2,5817	3,9594	13,498	0,8457	1,4411
Control-7	2,3238	3,5681	11,366	1,3096	1,2511
Control-8	2,7886	4,2454	10,028	1,0352	1,7485
Control-9	2,74	4,3834	12,621	0,908	1,7152
Control-10	1,867	2,7579	12,695	1,1825	0,7078
Control-11	2,7645	4,4038	13,7669	1,3382	2,0905
Control-12	2,7568	4,4178	13,7029	1,2565	1,9858
Control-13	2,7552	4,4391	13,3509	1,4657	1,9974
Control-14	2,7376	4,4512	13,2886	1,4097	1,9643
Control-15	2,6745	4,426	13,2472	1,4908	1,9156
Control-16	2,689	4,4045	13,4552	1,4555	1,9964
Control-17	2,6823	4,3726	13,0247	1,4138	1,8407
Control-18	2,7033	4,4043	13,6555	1,3349	2,0599
Control-19	2,7474	4,4252	13,4483	1,2917	1,9944
Control-20	2,7239	4,4828	13,7665	1,2874	2,1416
Mean	2,51866	3,98729	11,64057	1,11218	1,60078
STD	0,342975	0,6485845	2,639431478	0,3517261	0,5196717

Table 11 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	CC stretching of nucleic acids
Acclimated-1	1,7941	2,9295	6,2315	0,4344	0,9867
Acclimated-2	2,1362	3,1236	7,4832	0,5334	0,9364
Acclimated-3	2,1853	4,4471	8,2724	0,65	0,8533
Acclimated-4	2,2726	1,9547	8,3404	0,6259	1,0059
Acclimated-5	2,1634	2,6177	7,7965	0,5802	1,0619
Acclimated-6	2,3837	3,1526	8,726	0,6747	1,1261
Acclimated-7	2,0144	3,2341	8,0371	0,6791	1,3289
Acclimated-8	2,1198	3,3768	8,4329	0,738	0,4729
Acclimated-9	2,843	3,2022	11,163	0,9168	0,68
Acclimated-10	1,3661	3,5185	4,464	0,2859	0,8084
Acclimated-11	2,2264	3,4392	7,9175	0,5352	0,8453
Acclimated-12	1,94	2,9633	12,488	0,6095	1,6139
Acclimated-13	2,1168	3,3032	7,0652	1,3843	0,7422
Acclimated-14	2,8822	4,6202	7,6464	1,2446	0,8336
Acclimated-15	1,8837	2,9328	13,583	1,3469	2,061
Acclimated-16	2,0039	3,1027	13,121	1,2535	1,7861
Acclimated-17	2,7806	4,4863	13,668	0,664	2,1381
Acclimated-18	2,8256	4,5739	13,344	0,5846	1,9085
Acclimated-19	2,7699	4,3415	7,914	0,6241	0,8601
Acclimated-20	2,846	4,538	7,0527	1,1238	0,778
Mean	2,277685	3,492895	9,13734	0,774445	1,141365
STD	0,422281	0,753994	2,725230386	0,3208401	0,4919926
p-value	0.285	0.156	0.013*	0.0099**	0.0193*

Table 12. Band area ratio of certain bands for control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them

Experimental Groups	CH_3 symmetric stretching/ CH_3 asymmetric stretching	Amides I + II/ester C=O stretching	Ester C=O stretching/ CH_3 asymmetric	RNA/ Amides I + II	Amide I/Amide I+Amide II
Control-1	0,34	32,15	0,325	0,025	0,535
Control-2	0,41	26,91	0,383	0,011	0,531
Control-3	0,39	36,87	0,332	0,01	0,513
Control-4	0,42	23,42	0,431	0,01	0,532
Control-5	0,42	23,64	0,474	0,014	0,524
Control-6	0,40	24,25	0,466	0,017	0,526
Control-7	0,47	35,04	0,392	0,025	0,507
Control-8	0,58	27,18	0,374	0,028	0,561
Control-9	0,39	24,37	0,407	0,024	0,549
Control-10	0,28	28,73	0,265	0,03	0,548
Control-11	0,42	26,63	0,374	0,025	0,506
Control-12	0,41	28,90	0,348	0,024	0,5
Control-13	0,43	29,35	0,341	0,028	0,5
Control-14	0,43	30,94	0,331	0,026	0,499
Control-15	0,42	33,35	0,307	0,028	0,507
Control-16	0,42	31,16	0,334	0,027	0,506
Control-17	0,42	31,81	0,328	0,026	0,506
Control-18	0,42	31,18	0,329	0,025	0,506
Control-19	0,40	37,71	0,266	0,024	0,508
Control-20	0,41	27,84	0,35	0,025	0,5
Mean	0,4137373	29,57209	0,36	0,0226	0,5182
STD	0,0537984	4,206707	0,05625157	0,00639407	0,01883334

Table 12 (continued)

Experimental Groups	CH_3 symmetric stretching/CH_3 asymmetric stretching	Amides I + II/ester C=O stretching	Ester C=O stretching/CH_3 asymmetric	RNA/ Amides I + II	Amide I/Amide I+Amide II
Acclimated-1	0,38	18,44	0,542	0,012	0,571
Acclimated-2	0,39	21,73	0,485	0,012	0,553
Acclimated-3	0,39	19,23	0,531	0,015	0,554
Acclimated-4	0,40	20,37	0,501	0,014	0,55
Acclimated-5	0,40	20,62	0,486	0,014	0,55
Acclimated-6	0,40	20,29	0,512	0,015	0,547
Acclimated-7	0,40	16,09	0,66	0,018	0,566
Acclimated-8	0,40	18,88	0,547	0,018	0,551
Acclimated-9	0,40	28,47	0,363	0,018	0,535
Acclimated-10	0,37	16,16	0,569	0,01	0,584
Acclimated-11	0,44	42,71	0,238	0,012	0,547
Acclimated-12	0,33	43,02	0,242	0,011	0,503
Acclimated-13	0,48	36,99	0,261	0,032	0,537
Acclimated-14	0,43	27,65	0,361	0,029	0,544
Acclimated-15	0,34	47,05	0,217	0,026	0,5
Acclimated-16	0,42	45,48	0,232	0,023	0,501
Acclimated-17	0,42	28,88	0,356	0,013	0,508
Acclimated-18	0,40	32,76	0,32	0,011	0,504
Acclimated-19	0,45	19,99	0,518	0,013	0,541
Acclimated-20	0,40	21,96	0,414	0,026	0,535
Mean	0,4015116	27,33848	0,41775	0,0171	0,53905
STD	0,0337981	10,39230	0,13450645	0,0065846	0,0243061
p-value	0.177	0.101	0.13362	0.0434*	0.0434*

Table 13. Bandwidth data of control and cobalt acclimated *Bacillus* sp. with statistical significance (p-value) of difference between them

Experimental Groups	CH_2 asymmetric stretching of lipids	CH_2 symmetric stretching of lipids	Amide I	Amide II
Control-1	14,36	3,9	25,37	25,79
Control-2	13,64	3,81	25,48	27,62
Control-3	13,94	4,31	26,87	28,04
Control-4	13,44	4,12	25,54	28,22
Control-5	13,87	4,12	26,09	27,29
Control-6	12,98	5,12	26,52	26,18
Control-7	12,83	5,02	25,75	26,48
Control-8	12,68	5,03	26,45	26,24
Control-9	12,52	5,04	27,04	24,88
Control-10	12,96	4,27	24,66	27,6
Control-11	13,1	4,45	24,49	27,97
Control-12	13,1	4,5	24,51	27,92
Control-13	12,83	4,37	23,74	27,45
Control-14	13,02	4,41	24,33	28,16
Control-15	13	4,31	24,54	27,63
Control-16	12,66	4,51	25	27,74
Control-17	12,7	4,34	24,93	27,39
Control-18	12,45	4,34	24,87	27,32
Control-19	12,46	4,28	25,07	26,6
Control-20	12,53	4,14	24,66	26,98
Mean	13,0535	4,4195	25,2955	27,175
STD	0,536541312	0,370241386	0,91019648	0,887328931

Table 13 (continued)

Experimental Groups	CH_2 asymmetric stretching of lipids	CH_2 symmetric stretching of lipids	Amide I	Amide II
Acclimated-1	15,08	3,83	24,79	23,85
Acclimated-2	13,3	3,67	25,14	25,12
Acclimated-3	14,3	3,79	24,07	24,5
Acclimated-4	13,96	3,85	24,46	22,82
Acclimated-5	13,88	3,79	24,36	23,67
Acclimated-6	13,54	4,94	25,79	24,94
Acclimated-7	13,57	4,78	24,49	23,76
Acclimated-8	13,74	4,81	26,17	23,67
Acclimated-9	12,07	5,27	26,79	21,37
Acclimated-10	13,15	4,53	24,69	25,02
Acclimated-11	12,88	4,67	26,12	25,06
Acclimated-12	12,32	4,8	26,91	24,47
Acclimated-13	12,35	4,37	24,93	26,01
Acclimated-14	12,62	4,46	23,75	25,44
Acclimated-15	12,25	4,65	25,11	21,94
Acclimated-16	12	4,61	25,72	25,28
Acclimated-17	14,39	4,1	24,53	25,99
Acclimated-18	14,79	4,3	24,06	25,92
Acclimated-19	13,53	4,1	24,1	25,84
Acclimated-20	13,96	4,1	24,05	25,68
Mean	13,384	4,371	25,0015	24,5175
STD	0,913342041	0,452454127	0,945917961	1,335234555
p-value	0.25	0.764	0.263	0.000001***

APPENDIX C

FTIR DATA FOR *E. COLI* (n = 10) WITH MEAN, STANDARD DEVIATION (STD) AND STATISTICAL SIGNIFICANCE (p)

Table 14. Frequency data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	3271,62	2958,36	2925,71	2873,15
Control-2	3273,08	2958,32	2925,71	2873,04
Control-3	3277,37	2958,19	2925,75	2873,2
Control-4	3275,34	2958,19	2925,72	2873,15
Control-5	3274,08	2958,15	2925,54	2873,17
Control-6	3275,67	2958,36	2925,83	2872,95
Control-7	3274,92	2958,36	2925,83	2872,95
Control-8	3274,22	2958,36	2925,75	2873,01
Control-9	3276,71	2958,22	2925,75	2872,78
Control-10	3275	2958,36	2925,85	2873,04
Mean	3274,801	2958,287	2925,744	2873,044
STD	1,676023402	0,08807194	0,088593955	0,129631958
Acclimated-1	3265,51	2958,3	2924,86	2873,22
Acclimated-2	3255,16	2958,15	2925	2873,36
Acclimated-3	3270	2958,07	2924,67	2873,22
Acclimated-4	3272,37	2958,07	2924,54	2873,29
Acclimated-5	3269,42	2957,96	2924,67	2873,26
Acclimated-6	3278	2958,37	2925,33	2872,9
Acclimated-7	3278	2958,37	2925,33	2873,03
Acclimated-8	3277,27	2958,26	2925,26	2872,98
Acclimated-9	3277,27	2958,3	2925,33	2872,76
Acclimated-10	3277,6	2958,27	2925,21	2872,96
Mean	3272,06	2958,212	2925,02	2873,098
STD	7,418960694	0,140933396	0,31329787	0,197922544
p-value	0.9681	0.32708	0.00018***	0.42952

Table 14 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Control-1	2852,41	1741,99	1651,77	1637,9
Control-2	2852,3	1742,3	1651,86	1638
Control-3	2852,36	1742,71	1651,91	1638,54
Control-4	2852,28	1742,84	1652	1638,33
Control-5	2852,23	1743,52	1652,58	1638,4
Control-6	2852,36	1742,39	1651,66	1637,94
Control-7	2852,33	1741,38	1651,82	1638,05
Control-8	2852,38	1742,21	1651,6	1638,23
Control-9	2852,38	1740,3	1651,64	1638,23
Control-10	2852,47	1740,27	1651,63	1638,15
Mean	2852,35	1741,991	1651,847	1638,177
STD	0,068475462	1,057927219	0,290097685	0,208595835
Acclimated-1	2852,02	1741,8	1651,6	1638,8
Acclimated-2	2852,16	1741,86	1651,88	1639,11
Acclimated-3	2852,16	1743,34	1651,85	1638,57
Acclimated-4	2851,89	1741,8	1651,91	1638,28
Acclimated-5	2852,14	1742,75	1651,84	1639,78
Acclimated-6	2852,35	1742,8	1651,63	1638,84
Acclimated-7	2852,35	1740,33	1651,59	1638,09
Acclimated-8	2852,37	1742,41	1651,62	1638,47
Acclimated-9	2852,35	1742,03	1651,61	1638,27
Acclimated-10	2852,35	1740,55	1651,57	1638,59
Mean	2852,214	1741,967	1651,71	1638,68
STD	0,167411668	0,951443932	0,13984118	0,491641242
p-value	0.03752*	0.9681	0.14986	0.00578**

Table 14 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Control-1	1534,83	1452,82	1393,95	1230,13
Control-2	1534,51	1451,93	1393,71	1230,25
Control-3	1534,38	1451,56	1393,49	1230,5
Control-4	1534,13	1451,62	1393,31	1230,33
Control-5	1534,5	1451,12	1392,92	1229,66
Control-6	1533,21	1450,85	1393,16	1230,66
Control-7	1534,2	1451,09	1393,34	1231,09
Control-8	1534,13	1451,09	1393,41	1231,09
Control-9	1533	1451,05	1393,25	1231,37
Control-10	1532,63	1450,85	1392,97	1230,89
Mean	1533,952	1451,398	1393,351	1230,597
STD	0,737500282	0,611860551	0,314446604	0,52393914
Acclimated-1	1538,79	1452,08	1397,08	1231,94
Acclimated-2	1539,16	1452,58	1398,2	1232,95
Acclimated-3	1535,45	1451,47	1393,79	1229,43
Acclimated-4	1537,33	1452,55	1394,63	1231,73
Acclimated-5	1536,38	1452,47	1394,88	1231,03
Acclimated-6	1533,11	1451,34	1393,18	1230,33
Acclimated-7	1535,17	1451,72	1393,62	1231,39
Acclimated-8	1533,93	1451,73	1393,31	1231,24
Acclimated-9	1533,07	1451,39	1393,26	1230,56
Acclimated-10	1534,06	1451,49	1393,26	1230,93
Mean	1535,645	1451,882	1394,521	1231,153
STD	2,221102079	0,497433413	1,764322029	0,960775728
p-value	0.14156	0.0536*	0.09692	0.11184

Table 14 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Control-1	1117,25	1083	1056,61	993,7	966,4
Control-2	1117,5	1083	1057	993,34	966,32
Control-3	1117,29	1083	1057,13	993,56	966,18
Control-4	1117,5	1083	1056,65	993	965,94
Control-5	1117,5	1082,88	1056	992,89	965,72
Control-6	1117,8	1081,36	1056,65	993,35	965,89
Control-7	1117,9	1081,45	1055,85	993,56	965,66
Control-8	1118,14	1081,61	1055,83	993,56	965,92
Control-9	1117,9	1081,32	1056,14	993,85	965,64
Control-10	1117,8	1081,41	1056,11	993,53	965,8
Mean	1117,658	1082,203	1056,397	993,434	965,947
STD	0,291844	0,819010	0,471523535	0,2982988	0,2692396
Acclimated-1	1117,8	1083,65	1057,39	993,56	967,07
Acclimated-2	1117,5	1083,71	1057,15	993,63	966,51
Acclimated-3	1117,37	1082,92	1057,1	993	966,23
Acclimated-4	1117,5	1083,31	1057,63	993,44	966,42
Acclimated-5	1117,6	1082,9	1056,83	993	966,13
Acclimated-6	1117,66	1083	1057,03	992,73	966,06
Acclimated-7	1117,83	1083,14	1057,11	992,9	966
Acclimated-8	1117,81	1083	1057,88	992,69	965,9
Acclimated-9	1117,65	1083	1057,14	992,97	965,9
Acclimated-10	1117,4	1082,9	1057,62	993,04	966,08
Mean	1117,612	1083,153	1057,288	993,096	966,23
STD	0,168179	0,304523	0,328694387	0,3322382	0,3578019
p-value	0.79486	0.01732*	0.00078***	0.0703	0.0536*

Table 15. Band area data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them

Experimental Groups	Amide A	CH_3 asymmetric stretching of fatty acids	CH_2 asymmetric stretching of lipids	CH_3 symmetric stretching of proteins
Control-1	55,2595	4,7855	6,4367	1,82
Control-2	63,1007	4,7602	6,3961	1,8088
Control-3	64,7056	4,7192	6,3212	1,7864
Control-4	67,815	4,4422	5,9426	1,681
Control-5	69,9294	4,8225	6,4343	1,8176
Control-6	64,0922	4,0282	5,4316	1,5371
Control-7	63,9725	4,6809	6,2869	1,7777
Control-8	64,2839	4,6666	6,2561	1,7542
Control-9	59,9686	4,6888	6,2503	1,7421
Control-10	65,697	4,707	6,245	1,7391
Mean	63,88244	4,63011	6,20008	1,7464
STD	4,034553661	0,234969017	0,304779555	0,085098714
Acclimated-1	63,1688	4,7069	6,4005	1,7803
Acclimated-2	62,7182	4,704	6,4088	1,7849
Acclimated-3	62,8736	4,7059	6,3766	1,7724
Acclimated-4	64,3309	4,7081	6,3498	1,7662
Acclimated-5	63,9092	4,7498	6,5057	1,8257
Acclimated-6	62,1244	4,4025	6,0643	1,6649
Acclimated-7	66,7602	4,6469	6,3778	1,7679
Acclimated-8	62,2221	5,0172	6,6703	1,9291
Acclimated-9	69,687	4,8424	6,4636	1,7988
Acclimated-10	61,9403	4,6863	6,403	1,778
Mean	63,97347	4,717	6,40204	1,78682
STD	2,46295269	0,153536539	0,150768234	0,064874525
p-value	0.42952	0.72786	0.0455*	0.42952

Table 15 (continued)

Experimental Groups	CH_2 symmetric stretching of lipids	Ester C=O stretching of triglycerides	Amide I A	Amide I B
Control-1	2,2376	0,3173	3,2343	31,1866
Control-2	2,2215	0,3184	3,2084	30,9559
Control-3	2,2074	0,3135	2,6709	29,9622
Control-4	2,0674	0,296	3,1842	28,1545
Control-5	2,2293	0,3018	3,238	30,9173
Control-6	1,9192	0,2588	3,1506	25,6156
Control-7	2,2076	0,3013	2,9414	30,3916
Control-8	2,1678	0,3176	3,2561	30,7327
Control-9	2,1486	0,3256	3,2874	30,9463
Control-10	2,1424	0,3073	3,2608	30,6952
Mean	2,15488	0,30576	3,14321	29,95579
STD	0,097705246	0,01893	0,192767387	1,758879298
Acclimated-1	2,2102	0,3758	3,3237	25,3609
Acclimated-2	2,2176	0,3737	3,3233	25,2965
Acclimated-3	2,202	0,378	3,3043	24,9983
Acclimated-4	2,1862	0,355	3,2846	25,6194
Acclimated-5	2,3074	0,3793	3,3052	23,2161
Acclimated-6	2,105	0,4003	3,1008	20,7071
Acclimated-7	2,2276	0,3616	3,018	22,911
Acclimated-8	2,4509	0,3536	3,13	22,6982
Acclimated-9	2,2598	0,3888	2,8254	22,634
Acclimated-10	2,2149	0,3888	3,073	24,9286
Mean	2,23816	0,37549	3,16883	23,83701
STD	0,090747981	0,015223918	0,168064267	1,631390366
p-value	0.12114	0.00018***	0.56868	0.52218

Table 15 (continued)

Experimental Groups	Amide II	CH_2 bending of lipids	COO^- symmetric stretching of amino acid side chains and fatty acids	PO_2 asymmetric stretching of nucleic acids
Control-1	25,763	6,4935	9,4438	10,8803
Control-2	25,4788	6,4236	9,3835	10,703
Control-3	24,6568	6,2788	9,0835	10,2682
Control-4	23,3018	5,9576	8,708	9,7418
Control-5	25,6709	6,4683	9,4012	10,6974
Control-6	20,3017	5,0872	7,2686	9,1367
Control-7	24,4881	6,0508	8,6825	10,7471
Control-8	24,1698	5,7349	8,2274	10,6521
Control-9	24,2227	5,602	8,0564	10,7112
Control-10	24,0712	5,6208	8,067	10,5051
Mean	24,21248	5,97175	8,63219	10,40429
STD	1,583016758	0,462131511	0,722704746	0,552691339
Acclimated-1	6,1129	8,7944	4,3386	11,178
Acclimated-2	6,1272	8,9145	4,395	11,0006
Acclimated-3	6,0134	8,7784	4,2573	10,6719
Acclimated-4	6,2106	8,976	4,3667	11,1746
Acclimated-5	5,6663	8,5151	3,9832	11,0698
Acclimated-6	4,9139	7,3028	3,622	10,1666
Acclimated-7	5,3853	7,9027	3,7721	11,3149
Acclimated-8	5,5896	9,0947	3,9431	9,6855
Acclimated-9	5,3196	8,3102	3,9355	10,359
Acclimated-10	6,0457	8,6886	4,2906	10,9978
Mean	5,73845	8,52774	4,09041	10,76187
STD	0,433546095	0,554818722	0,274177219	0,531261168
p-value	0.242	0.79486	0.72786	0.12114

Table 15 (continued)

Experimental Groups	CC sym. stretching of ribose	PO_2 sym. stretching of nucleic acids and phospho-lipids	C–O–C, P–O–C symmetric stretching of poly-saccharides on pepti-doglycan	Ribose skeleton	Backbone CC stretching of nucleic acids
Control-1	3,0839	4,9527	3,8079	1,4093	0,6928
Control-2	3,0916	4,9191	3,7613	1,393	0,6803
Control-3	2,899	4,702	3,5851	1,3173	0,7731
Control-4	2,759	4,4188	3,3963	1,294	0,8133
Control-5	3,0103	4,9079	3,7917	1,4515	0,7819
Control-6	2,5368	4,3521	3,3185	1,1294	0,697
Control-7	3,0067	5,0678	3,7456	1,1147	0,6721
Control-8	3,0226	5,0004	3,6334	1,0257	0,7423
Control-9	3,0791	5,1357	3,6702	0,9849	0,7412
Control-10	3,0142	5,0664	3,7287	1,1295	0,7015
Mean	2,95032	4,85229	3,64387	1,22493	0,72955
STD	0,176605	0,273429	0,167168518	0,1681821	0,0481576
Acclimated-1	2,9872	4,893	3,6249	0,9346	0,694
Acclimated-2	2,9744	4,804	3,5207	0,9258	0,662
Acclimated-3	2,8611	4,6985	3,4538	0,9333	0,6962
Acclimated-4	2,949	4,9096	3,6871	0,9741	0,7176
Acclimated-5	3,2853	5,5721	4,0594	1,2173	0,8952
Acclimated-6	3,0444	4,9255	3,4164	0,9311	0,6989
Acclimated-7	3,2781	5,7896	4,3588	1,3085	0,9661
Acclimated-8	3,0826	4,7292	3,1907	0,8824	0,634
Acclimated-9	3,2017	5,0956	3,4662	0,9547	0,6945
Acclimated-10	2,9504	4,7992	3,5307	0,9136	0,6879
Mean	3,06142	5,02163	3,63087	0,99754	0,73464
STD	0,147445	0,368686	0,339461332	0,1434989	0,1071089
p-value	0.52218	0.9124	0.3843	0.00362**	0.47152

Table 16. Band area ratio of certain bands for control and cobalt acclimated *E.coli* with statistical significance (p-value) of difference between them

Experimental Groups	CH_3 symmetric stretching/CH_3 asymmetric stretching	Amides I + II/ester C=O stretching	Ester C=O stretching/CH_3 asymmetric	RNA/Amides I + II
Control-1	0,38	179,48	0,066	0,025
Control-2	0,38	177,24	0,067	0,025
Control-3	0,38	174,22	0,066	0,024
Control-4	0,38	173,84	0,067	0,025
Control-5	0,38	187,50	0,063	0,026
Control-6	0,38	177,42	0,064	0,025
Control-7	0,38	182,14	0,064	0,02
Control-8	0,38	172,87	0,068	0,019
Control-9	0,37	169,44	0,069	0,018
Control-10	0,37	178,22	0,065	0,021
Mean	0,37724379	177,2380724	0,0659	0,0228
STD	0,003944927	5,121158468	0,001911951	0,002973961
Acclimated-1	0,38	151,40	0,08	0,016
Acclimated-2	0,38	151,66	0,079	0,016
Acclimated-3	0,38	148,55	0,08	0,017
Acclimated-4	0,38	160,71	0,075	0,017
Acclimated-5	0,38	140,61	0,08	0,023
Acclimated-6	0,38	123,23	0,091	0,019
Acclimated-7	0,38	147,05	0,078	0,025
Acclimated-8	0,38	144,25	0,07	0,017
Acclimated-9	0,37	134,96	0,08	0,018
Acclimated-10	0,38	144,87	0,083	0,016
Mean	0,378781186	144,7303149	0,0796	0,0184
STD	0,003937951	10,23558284	0,005358275	0,003134042
p-value	0.72786	0.00018***	0.00018***	0.00578**

Table 17. Bandwidth data of control and cobalt acclimated *E. coli* with statistical significance (p-value) of difference between them

Experimental Groups	CH_2 asymmetric stretching of lipids	CH_2 symmetric stretching of lipids	Amide I	Amide II
Control-1	6,39	4,33	27,6	28,9
Control-2	6,64	4,38	27,4	28,75
Control-3	6,64	4,33	27,7	28,68
Control-4	6,65	4,32	27,23	28,82
Control-5	6,48	4,43	27,37	29,07
Control-6	6,48	4,15	27,24	30,76
Control-7	6,48	4,2	28,02	28,86
Control-8	6,48	4,24	27,77	29,02
Control-9	6,48	4,19	27,38	30,42
Control-10	6,48	4,13	27,54	31,23
Mean	6,52	4,27	27,525	29,451
STD	0,089566859	0,10198039	0,251760468	0,959461076
Acclimated-1	6,8	4,58	27,18	24,8
Acclimated-2	6,49	4,47	26	24,05
Acclimated-3	6,49	4,7	27,7	27,85
Acclimated-4	6,5	4,75	27,5	25,6
Acclimated-5	6,5	4,7	27,4	26,54
Acclimated-6	6,63	4,51	27,82	30,37
Acclimated-7	6,67	4,53	27,6	27,22
Acclimated-8	6,5	4,63	27,6	29
Acclimated-9	6,47	4,63	27,7	30,32
Acclimated-10	6,37	4,7	27,7	28,48
Mean	6,542	4,62	27,42	27,423
STD	0,122999548	0,094633797	0,531329569	2,192548238
p-value	0.34722	0.00018***	0.79486	0.020880