

**MICROBIAL FERMENTATION TO IMPROVE  
ELEMENTS AVAILABILITY OF SOME  
ROCKS FOR AGRICULTURAL  
APPLICATION**

By

**ZAHRA HABIB MOHAMED TAYEB**

B.Sc.Agric.Sc. (Agric. Microbiology), Ain Shams University, 2010

**A thesis submitted in partial fulfillment**

**of**

**The requirements for the degree of**

**MASTER OF SCIENCE**

**in**

**Agricultural Science  
(Agricultural Microbiology)**

**Department of Agricultural Microbiology**

**Faculty of Agricultural**

**Ain Shams University**

**2015**

## **Approval Sheet**

# **MICROBIAL FERMENTATION TO IMPROVE ELEMENTS AVAILABILITY OF SOME ROCKS FOR AGRICULTURAL APPLICATION**

**By**

**ZAHRA HABIB MOHAMED TAYEB**

B.Sc.Agric.Sc. (Agric. Microbiology), Ain Shams University, 2010

This thesis for M.Sc. degree has been approved by:

**Dr. Rashed Abdel Fattah Zaghloul** .....

Prof. of Agricultural Microbiology, Faculty of Agriculture, Benha  
University.

**Dr. Elshahat Mohamed Ramadan** .....

Prof. Emeritus of Agricultural Microbiology, Faculty of Agriculture,  
Ain Shams University.

**Dr. Hemmat M. Abdelhady** .....

Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain  
Shams University.

**Date of Examination: 27/7/ 2015**

# **MICROBIAL FERMENTATION TO IMPROVE ELEMENTS AVAILABILITY OF SOME ROCKS FOR AGRICULTURAL APPLICATION**

By

**ZAHRA HABIB MOHAMED TAYEB**

B.Sc.Agric.Sc. (Agric. Microbiology), Ain Shams University, 2010

**Under the supervision of:**

**Dr. Hemmat M. M. Abdelhady**

Prof. of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

**Dr. Khadiga A. A. Abou-Taleb**

Associate Prof. of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

**Dr. Shimaa A. Amin**

Lecturer of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

## ABSTRACT

**Zahra Habib Mohamed Tayeb. "Microbial Fermentation to Improve Elements Availability of Some Rocks for Agricultural Application". Unpublished M.Sc. Thesis, Department of Agric. Microbiology. Faculty of Agriculture, Ain shams University, 2015.**

Inorganic phosphate (P) and potassium (K) are more essential macronutrient for the growth and development of plants. Only a small amount of the applied soluble P fertilizer is used by plants and the remainder fraction is hold in soil as insoluble forms. The integration of microbial inoculation with P and K solubilizing microorganisms, and rock P and K materials amendent can improve crop mineral nutrients in nutrient-deficient soils. Therefore, the investigation was designed to study the production fermented solutions of soluble P by local microbial strains in order to use in some agriculture applications.

In the present study, a number of 108 phosphate solubilizing isolates and 20 potassium solubilizing isolates were isolated from different soil samples. Only four isolates were selected as the most efficient phosphate solubilizing isolates on both solid and liquid cultures. Some nutritional and environmental factors affecting the RP solubilization by selected isolates using shake flasks as batch cultures. In a series of experiments on phosphate solubilization, the ingredients of basal med.3 were modified to give the highest phosphate solubilization after 10 or 12 days at 30°C by bacterial and fungal isolates, respectively.

Different treatments of bagasse, corn cobs, black sugar cane molasses, olive cake wastes, rice straw, sugar beet waste and whey were used as a sole carbon source at different treatments for highest phosphate solubilization by tested isolates. The maximum RPs activity was obtained by bacterial and fungal isolates in sugar beet waste and whey as whole media supplemented with 7.0 and 10.0 gL<sup>-1</sup> RP at pH 7.0 and 5.5 after 10 and 12 days at 30°C which increased RPs content about 1.15 and 3.29 fold comparing to med.3, respectively.

The most efficient RP solubilization isolate was identified with 18S rDNA gene sequencing to close the gene sequencing of *Aspergillus tubingensis* USMI03. The fermented solution of this strain contains the highest concentration of citric acid, indole acetic acid (IAA) and elements as well as phosphatase activity. This solution was applied as mineral source for microbial growth and a phosphate fertilized of bean plants cultivation. Using the fermented solution as mineral source (supplemented with sucrose) increased the growth of *Aspergillus* sp., *Pencillium* sp. and *Tricoderma* sp. about 1.94, 1.53 & 1.33 fold comparing with that obtained by Czapek's Dox media after 4 day incubation period at 30°C using shake flasks as a batch culture. Foliar spray by un-filtrated fermented solution at 197.01 µgP ml<sup>-1</sup> increased the plant hight, yield and P content about 2.53, 1.97 and 2.87 fold after 45 days harvest period comparing to control treatment irrigated with Hoagland solution only, suggesting its potential use a P fertilizer.

**Keywords:** Phosphate solubilizing microorganisms (PSMs), potassium solubilizing microorganisms (KSMs), *Serratia* sp, *Aspergillus tubingensis*, batch culture, Rock phosphate, bean plant, growth improvement, fermented solutions.

## ACKNOMLEDGMENT

Praise and thanks be to **ALLAH**, the most merciful for assisting and directing me to the right way.

The author to express her deepest gratitude to principal supervisor **Prof. Dr. Hemmat Mohamed Mohamed Abdelhady**, Prof. of Agric. Microbiology, Dept. of Microbiology, Faculty of Agric., Ain Shams University, for constractive criticism of the manuscript, close guidance and keen interest.

Thanks also due to **Dr. Khadiga A. A. Abou-Taleb**, Associate Prof. of Agric. Microbiology, Dept. of Microbiology, Fac. of Agriculture, Ain Shams University for her invaluable advices, precious assistance and helpful comments on the manuscript.

Special thanks are due to **Dr. Shimaa Abd El-Rauof Amin Ali** Lecturer of Agric. Microbiology, Dept. of Microbiology, Fac. of Agriculture, Ain Shams University for her invaluable assistance so necessary to the accomplishment of this study.

I am indebted to my family and particularly very thankful to my parents for their precious understanding, patience, encouragement and support.

## CONTENTS

|                                                                       | Page     |
|-----------------------------------------------------------------------|----------|
| <b>1. INTRODUCTION.....</b>                                           | <b>1</b> |
| <b>2. REVIEW OF LITERATURE.....</b>                                   | <b>4</b> |
| 2.1. Important of phosphate and potassium elements.....               | 4        |
| 2.1.1. Phosphorus.....                                                | 4        |
| 2.1.2. Potassium.....                                                 | 6        |
| 2.2. Microorganisms dissolution of insoluble soil minerals P and K... | 8        |
| 2.2.1. Phosphate solubilizing microorganisms (PSMs).....              | 8        |
| 2.2.2. Potassium solubilizing microorganisms.....                     | 11       |
| 2.3. Mechanism of microbial mineralization.....                       | 12       |
| 2.3.1. Production of organic acids.....                               | 13       |
| 2.3.2. Enzymes.....                                                   | 16       |
| 2.3.3. Growth promoting.....                                          | 17       |
| 2.3.4. Siderophores.....                                              | 18       |
| 2.3.5. Exopolysaccharides (EPSs).....                                 | 18       |
| 2.4. Factors affecting rock phosphate solubilization.....             | 18       |
| 2.4.1. Nutritional factors.....                                       | 18       |
| 2.4.1.1. Carbon sources.....                                          | 18       |
| 2.4.1.2. Effect of nitrogen source.....                               | 20       |
| 2.4.1.3. Agro-industrial residues.....                                | 22       |
| 2.4.1.3.1. Olive cake waste.....                                      | 22       |
| 2.4.1.3.2. Sugarcane bagasse.....                                     | 23       |
| 2.4.1.3.3. Sugar beet waste.....                                      | 24       |
| 2.4.1.3.4. Rice straw.....                                            | 24       |
| 2.4.1.3.5. Corn cobs.....                                             | 25       |
| 2.4.1.3.6. Molasses.....                                              | 25       |
| 2.4.1.4. Effect of rock concentration in liquid media.....            | 25       |
| 2.4.2. Environmental factors.....                                     | 26       |
| 2.4.2.1. Initial pH.....                                              | 26       |
| 2.4.2.2. Incubation temperature.....                                  | 26       |

|                                                                                          |           |
|------------------------------------------------------------------------------------------|-----------|
| 2.4.2.3. Inoculum size.....                                                              | 27        |
| 2.4.2.5. Incubation time.....                                                            | 27        |
| 2.5. Effect of inoculation of P and K solubilizers on plant growth and yield.....        | 28        |
| <b>3. MATERIALS AND METHODS.....</b>                                                     | <b>30</b> |
| 3.1. Samples.....                                                                        | 30        |
| 3.2. Microorganisms used.....                                                            | 31        |
| 3.3. Media used.....                                                                     | 31        |
| 3.4. Nutrient (Hoagland) solution.....                                                   | 36        |
| 3.5. Seeds.....                                                                          | 36        |
| 3.6. Soils.....                                                                          | 38        |
| 3.7. Agro-industrial wastes and by-products.....                                         | 38        |
| 3.8. Isolation of phosphate and potassium solubilizing microorganisms.....               | 39        |
| 3.9. Maintenance of cultures.....                                                        | 40        |
| 3.10. Standard inoculum.....                                                             | 40        |
| 3.11. Screenings of the most efficient phosphate solubilizing microorganisms (PSMs)..... | 40        |
| 3.12. Identification of phosphate solubilizing microorganisms (PSMs).....                | 41        |
| 3.13. Factors affecting the rock phosphate solubilization.....                           | 41        |
| 3.13.1. Carbon sources.....                                                              | 42        |
| 3.13.2. Glucose concentrations.....                                                      | 42        |
| 3.13.3. Nitrogen sources.....                                                            | 42        |
| 3.13.4. Nitrogen concentrations.....                                                     | 42        |
| 3.13.5. Agro-industrial wastes and by-products uses for RP solubilization.....           | 43        |
| 3.13.6. Rock phosphate concentrations.....                                               | 43        |
| 3.13.7. Initial pH.....                                                                  | 43        |
| 3.13.8. Inoculum size.....                                                               | 43        |
| 3.13.9. Incubation temperature.....                                                      | 43        |

|                                                                                                                             |           |
|-----------------------------------------------------------------------------------------------------------------------------|-----------|
| 3.14. Molecular identification of the most efficient tested isolate by<br>18S rDNA sequence analysis.....                   | 44        |
| 3.14.1. DNA extraction and partial sequencing of 18S rDNA.....                                                              | 44        |
| 3.13.2. Phylogenetic analysis.....                                                                                          | 44        |
| 3.15. Some applications of fermented solution produced.....                                                                 | 45        |
| 3.15.1. As mineral source for microbial growth.....                                                                         | 45        |
| 3.15.2. As a P fertilizer in green house pot experiment.....                                                                | 45        |
| 3.16. Analytical methods.....                                                                                               | 47        |
| 3.16.1. Microbial count.....                                                                                                | 47        |
| 3.16.2. Phosphate concentration.....                                                                                        | 47        |
| 3.16.3. Potassium concentration.....                                                                                        | 47        |
| 3.16.4. pH.....                                                                                                             | 47        |
| 3.16.5. Indole acetic acid (IAA) assay.....                                                                                 | 47        |
| 3.16.6. Citric acid.....                                                                                                    | 48        |
| 3.16.7. Acid phosphatase activity.....                                                                                      | 48        |
| 3.17. Calculation.....                                                                                                      | 48        |
| 3.17.1. Phosphate and potassium solubilization Parameters.....                                                              | 48        |
| 3.17.2. Growth and biological activity parameters.....                                                                      | 49        |
| 3.18. Statistical analysis.....                                                                                             | 50        |
| <b>4. RESULTS AND DISCUSSION</b>                                                                                            | <b>51</b> |
| 4.1. Isolation of phosphate and potassium solubilizing<br>microorganisms (PSM & KSM) .....                                  | 51        |
| 4.2. Quantitative and qualitative determination of the most efficient<br>phosphate solubilizing microorganisms (PSMs) ..... | 57        |
| 4.3. Identification of the most efficient phosphate solubilizing<br>isolates.....                                           | 64        |
| 4.4. Factors affecting rock phosphate (RP) solubiliaztion.....                                                              | 66        |
| 4.4.1. Effect of nutritional requirements.....                                                                              | 66        |
| 4.4.1.1. Carbon sources.....                                                                                                | 67        |
| 4.4.1.2. Glucose concentrations.....                                                                                        | 72        |
| 4.4.1.3. Nitrogen sources.....                                                                                              | 78        |
| 4.4.1.4. Nitrogen concentrations.....                                                                                       | 85        |

|                                                                                                                                   |            |
|-----------------------------------------------------------------------------------------------------------------------------------|------------|
| 4.4.1.5. Use of some agro-industrial wastes and by-products for rock phosphate solubilization.....                                | 92         |
| 4.4.1.6. Rock phosphate concentrations.....                                                                                       | 112        |
| 4.4.2. Effect of environmental factors.....                                                                                       | 119        |
| 4.4.2.1. Initial pH.....                                                                                                          | 119        |
| 4.4.2.2. Incubation temperature.....                                                                                              | 122        |
| 4.4.2.3. Inoculum size.....                                                                                                       | 127        |
| 4.5. Molecular identification of the most efficient fungal isolate of <i>Aspergillus</i> sp. RPf10 and the phylogenetic tree..... | 134        |
| 4.6. Some properties of produced fermented solutions.....                                                                         | 136        |
| 4.7. Some application of <i>Aspergillus tubingensis</i> RPf10 fermented solution.....                                             | 138        |
| 4.7.1. As a mineral source for microbial growth.....                                                                              | 138        |
| 4.7.2. As nutrient solution for bean plant growth improvement.....                                                                | 152        |
| <b>5. SUMMARY.....</b>                                                                                                            | <b>158</b> |
| <b>6. REFERENCE.....</b>                                                                                                          | <b>164</b> |
| <b>ARABIC SUMMARY.....</b>                                                                                                        |            |



## LIST OF FIGURES

| Fig<br>(No) |                                                                                                                                                                                                                                                           | Page |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1           | The percentages of phosphate solubilizing microorganisms (PSM <sub>5</sub> ) obtained from different sources.....                                                                                                                                         | 54   |
| 2           | Soluble rock phosphate content and phosphate solubilization efficiency of bacterial isolates after 10 days incubation period on med.3 using shake flasks as a batch culture.....                                                                          | 62   |
| 3           | Soluble rock phosphate content and phosphate solubilization efficiency of fungal isolates after 10 days incubation period med.3 using shake flasks as a batch culture.....                                                                                | 63   |
| 4           | Cultural characteristics of <i>Aspergillus</i> sp. Bf6 and RPf10 isolates.....                                                                                                                                                                            | 66   |
| 5           | Effect of different carbon sources on RP solubilization activity <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 during 10 days of incubation periods at 30°C on med.3 using shake flasks as a batch culture.....                                    | 70   |
| 6           | Effect of different carbon sources on RP solubilization activity by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. RPf10 at during 12 days of incubation periods at 30°C on med.3 using shake flasks as a batch culture.....                       | 71   |
| 7           | Effect different glucose concentrations (gL <sup>-1</sup> ) on RP solubilization activity by <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 during 10 days of incubation periods at 30°C on med.3 using shake flasks as a batch culture.....        | 75   |
| 8           | Effect different glucose concentrations (gL <sup>-1</sup> ) on RP solubilization activity by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. RPf10 during 12 days of incubation periods at 30°C on med.3 using shake flasks as a batch culture..... | 76   |
| 9           | Effect of different nitrogen sources on RP solubization activity by <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 after 8 days on med.3 at 30°C using shake flasks as a batch culture.....                                                         | 83   |

|    |                                                                                                                                                                                                                                                                        |     |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 10 | Effect of different nitrogen sources on RP solubization activity by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. Rpf10 after 10 days on med.3 at 30°C using shake flasks as a batch culture                                                                   | 84  |
| 11 | Effect of different yeast extract concentrations ( $\text{gL}^{-1}$ ) on RP solubilization activity by isolates of <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. Rpf10 during 12 days of incubation periods at 30°C using shake flasks as a batch culture..... | 87  |
| 12 | Effect of different tryptone concentrations ( $\text{gL}^{-1}$ ) on RP solubilization activity by isolates of <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 during 10 days of incubation periods at 30°C using shake flasks as a batch culture.....             | 90  |
| 13 | pH and RP solubilization content of <i>Serratia</i> sp. Rs7 isolate as affected by different treatments of some agro-industrial on modified Pikovskaya's medium No.3 after 12 days at 30°C using shake flasks as a batch culture.....                                  | 100 |
| 14 | pH and RP solubilization content of <i>Serratia</i> sp. Rs22 isolate as affected by different treatments of some agro-industrial on modified Pikovskaya's medium No.3 after 12 days at 30°C using shake flasks as a batch culture.....                                 | 101 |
| 15 | pH and RP solubilization content of <i>Aspergillus</i> sp. Bf6 isolate as affected by different treatments of some agro-industrial modified Pikovskaya's medium No.1 after 12 days at 30°C using shake flasks as a batch culture.....                                  | 109 |
| 16 | pH and RP solubilization content of <i>Aspergillus</i> sp. Rpf10 isolate as affected by different treatments of some agro-industrial on modified Pikovskaya's medium No.3 after 12 days at 30°C using shake flasks as a batch culture.....                             | 110 |
| 17 | Effect of different concentrations of RP on the solubilization activity of RP by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. Rpf10 isolates after 12 days incubation period at 30°C on sugar beet waste medium using shake flasks as a batch culture.....    | 114 |

|    |                                                                                                                                                                                                                                                         |     |
|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 18 | Effect of different concentrations of RP on the solubilization activity of RP by <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 isolates after 10 days incubation period at 30°C on whey medium using shake flasks as a batch culture.....        | 117 |
| 19 | Effect of different temperatures on RP solubization activity by <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 isolates during 10 days on whey medium using shake flasks as a batch culture.                                                      | 125 |
| 20 | Effect of different temperatures on RP solubization activity by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. RPf10 isolates after 12 days on sugar beet waste medium using shake flasks as a batch culture.....                                | 126 |
| 21 | Effect of different inoculum sizes on RP solubization activity by <i>Serratia</i> sp. Rs7 and <i>Serratia</i> sp. Rs22 isolates of incubation period at 30°C on whey medium using shake flasks as a batch culture.....                                  | 130 |
| 22 | Effect of different inoculum sizes on RP solubization activity by <i>Aspergillus</i> sp. Bf6 and <i>Aspergillus</i> sp. RPf10 isolates after 12 days of incubation period at 30°C on sugar beet waste medium using shake flasks as a batch culture..... | 132 |
| 23 | Agarose gel analysis of PCR amplification product using 18S rDNA primers for <i>Aspergillus</i> sp. RPf10 isolate.....                                                                                                                                  | 134 |
| 24 | Phylogenetic tree showing the relationship between the fungal isolate <i>Aspergillus</i> sp. RPf10 (unknown) and related ascomycetes from NCBI database upon neighbor joining analysis of partial 18S rDNA sequences.....                               | 135 |
| 25 | Phosphatase activity, citric acid and IAA production by <i>Aspergillus tubingensis</i> RPf10, <i>Aspergillus</i> sp. Bf6, <i>Serratia</i> sp. Rs22 and <i>Serratia</i> sp. Rs7.....                                                                     | 137 |
| 26 | Growth curves of tested bacterial culture grown on nutrient broth and nutrient glucose broth as well as <i>Aspergillus tubingensis</i> RPf10 fermented solution as a whole medium or supplemented with glucose during 36 hours at 30°C using            | 142 |

|    |                                                                                                                                                                                                                                                                                                                          |     |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
|    | shake flasks as a batch culture.....                                                                                                                                                                                                                                                                                     |     |
| 27 | Growth curves of tested yeast culture grown on yeast malt broth and yeast extract malt extract broth as well as <i>Aspergillus tubingensis</i> RPf10 fermented solution as a whole medium or supplemented with glucose during 72 hours at 30°C using shake flasks as a batch culture.....                                | 143 |
| 28 | Specific growth rate ( $h^{-1}$ ) and doubling time ( $t_d$ ) of tested bacterial and yeast isolates grown on different media and fermented solution treatments using shake flasks as a batch culture.....                                                                                                               | 144 |
| 29 | Growth curves of tested fungal culture grown on malt and Czapek's Dox media broth as well as <i>Aspergillus tubingensis</i> RPf10 fermented solution as a whole medium or supplemented with glucose during 36 hours at 30°C using shake flasks as a batch culture.....                                                   | 148 |
| 30 | Specific growth rate ( $h^{-1}$ ) and doubling time ( $t_d$ ) of tested fungal isolates grown on different media and fermented solution treatments using shake flasks as a batch culture.....                                                                                                                            | 149 |
| 31 | The parameters of bean plant growth as affected by different irrigation treatments with water (T1), Hoagland solution (T5), Hoagland free P (T2), filtrated and non-filtrated solution at 197.01 + Hoagland free P (T3) & (T4) or complete Hoagland solution (T6) & (T7), respectively after 45 days harvest period..... | 156 |