Possible phosphate solubilisation mechanism and growth promotion of wheat through *Bacillus megaterium* and *Bacillus subtilis*

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Introduction

Phosphorus is the most critical nutrient after nitrogen for crop production. In agricultural soil, P is present in the form of inorganic and organic compounds but most of them are present in fixed or insoluble form. Plant required 30 µM L^{-1} P for their normal growth however, its available concentration in soil is about 1 µM L^{-1}. Consequently, un-availability of P in various soils has been identified as a growth limiting factor in agricultural systems. This issue can be addressed by applying available source of P in the form of chemical fertilizers. Only 10-20% of available P contents are taken up by plants while 70-80% P concentration is lost due to runoff and leaching which is hazardous for environment. Thus; the alternative technology (biofertilizers) is required for sustainable and ecofriendly agriculture. Awareness of the mechanism describe P solubilization is the base to develop P solubilizing biofertilizers which can substitute chemical fertilizers for sustainable production. Although different mechanisms have been described as phosphate solubilizing, but no method was clearly understood. In the present study we assumed that the production of organic acid was a major mechanism of phosphates solubilization. The other mechanisms involved in P solubilization were alkaline phosphatases and soil exoenzyme activities.

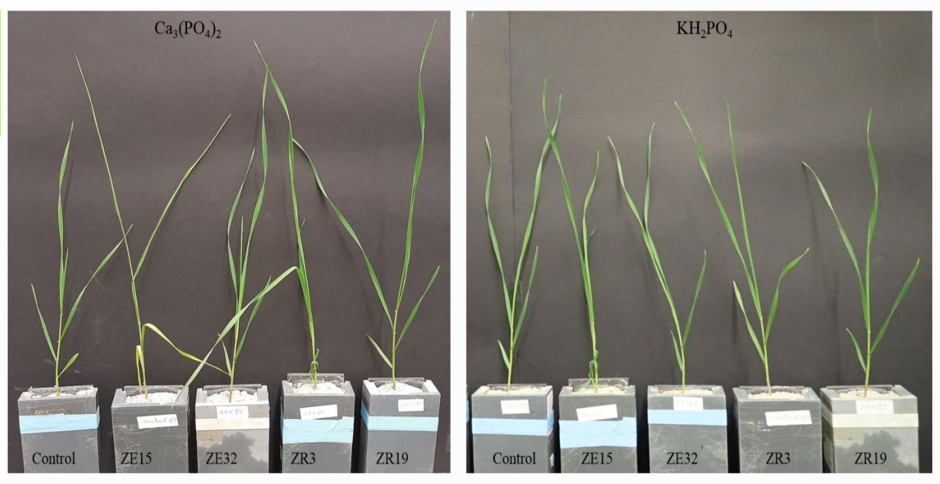
Objectives

The study aimed to find the phosphate solubilizing mechanism through soil exo-enzyme activities, alkaline phosphatase (ALP) gene expression, and production of organic acids in presence of insoluble phosphorous. Furthermore, the potential of phosphate solubilizing strains to improve root development and seedling growth of wheat (*Triticum aestivum* L.) were also determined under controlled conditions.

Materials and Methods

Phosphate solubilizing mechanism and plant growth promotion

- Growth diversity (pH ~3-10)
- Bacterial population
- pH depletion
- Soil Exoenzyme activities
- Secretion of organic acid
- ALP gene expression
- Determine solubilized P
- Root development
- Seedling growth



An overview of seedling growth at 35th day after sowing. Phosphate solubilizing strains *Bacillus subtilis* (ZE15 and ZR3) and *Bacillus magaterium* (ZE32 and ZR19) were tested for plant growth promotion in presence of $Ca_3(PO_4)_2$ (left) and KH_2PO_4 (right).

Results

The tested strains showed different behavior in the presence and absence of tricalcium phosphate (TCP). In the absence, strain's growth reached at stationary phase at 5th day of incubation. However, in the presence of TCP, the strains ZE15, ZE32, ZR3 and ZR19 showed maximum growth at 7th day of incubation which reaches 1.11 × 10^6, 9.64 × 10^5, 1.06 × 10^6 and 9.06 × 10^5 CFU mL^{-1}, respectively. Thus; these strains solubilized considerable amount of phosphorous @ 130, 117, 76 and 42 µg mL^{-1}, respectively.

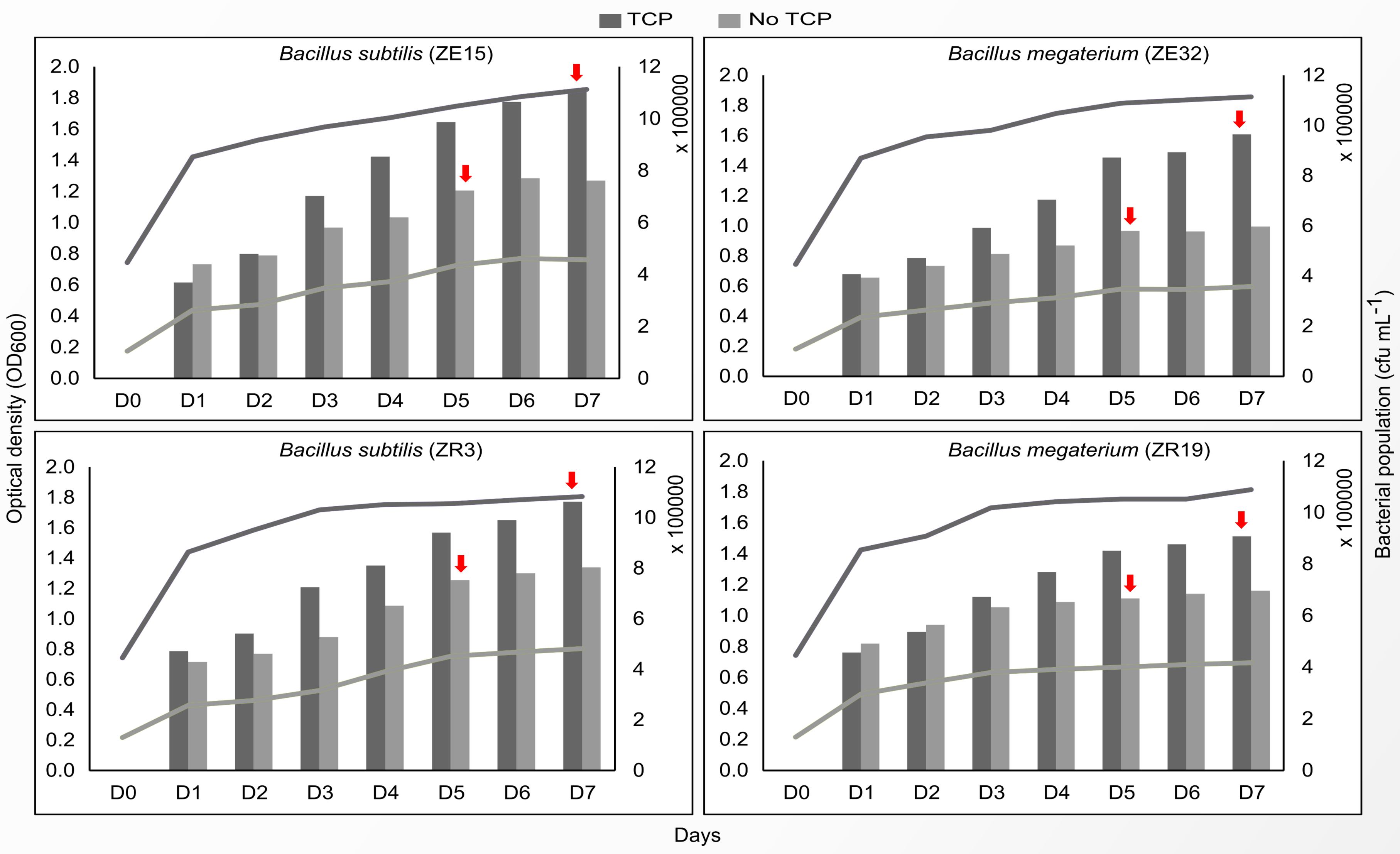


Figure 1: Bacterial growth in term of optical density and population in presence of insoluble P.

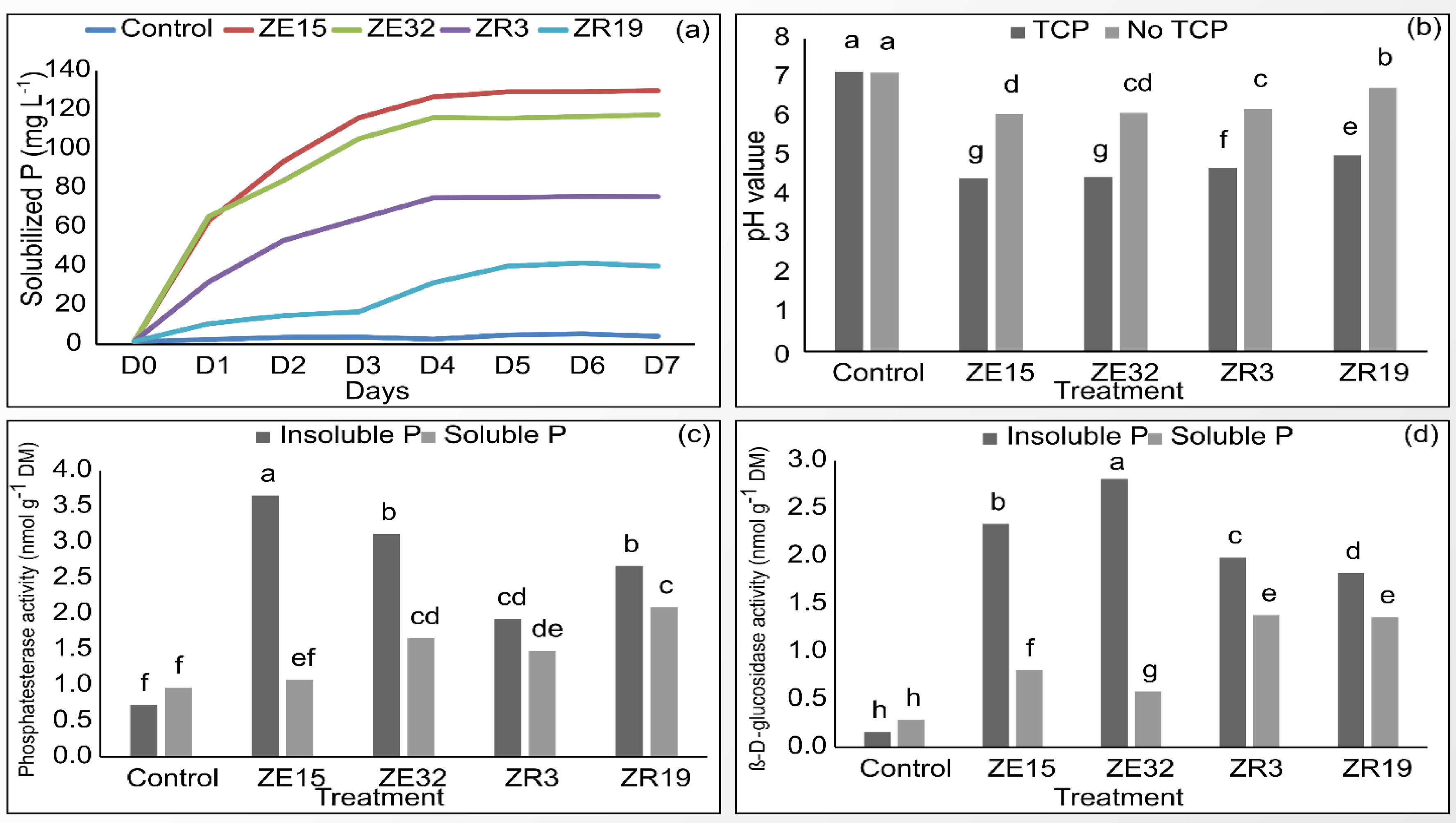


Figure 2: Phosphate solubilizing activity. (a) solubilized P; (b) pH depletion; (c) phosphatase esterase activity; (d) ß-D glucosidase activity

PCR amplification of alkaline phosphatase (ALP) gene from *Bacilli* showing amplicon of 380bp on 1% *w/v* agarose gel. Multidimensional scaling indicates, considerably high amount of organic acid was excrete in the presence of $Ca_3(PO_4)_2$. Separation between treatments indicates that bacterial strains responded differently in the presence and absence of tricalcium phosphate. Difference of strain ZE15 and ZR3 with other strains was notable in presence of tricalcium phosphate.

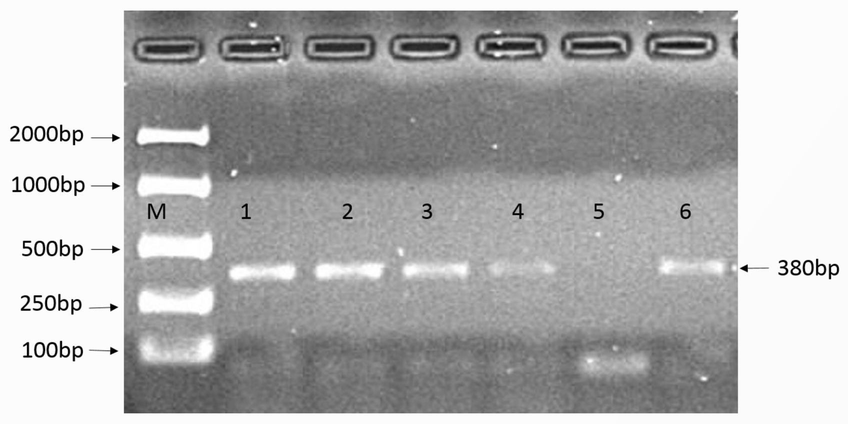
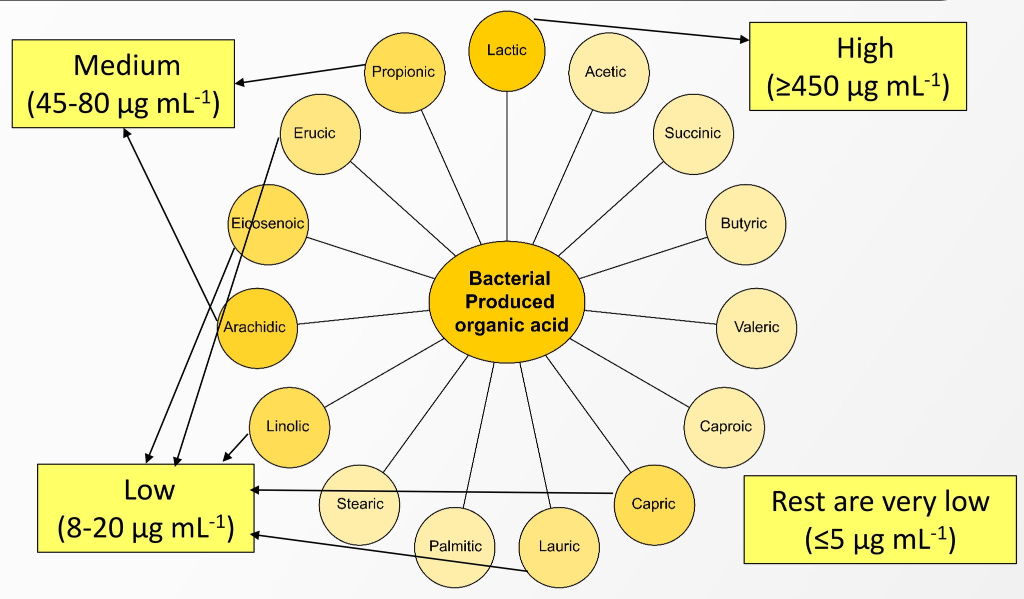


Figure 3: PCR amplification of alkaline phosphatase (ALP) gene from *Bacilli* showing amplicon of 380bp on 1% *w/v* agarose gel. (M) molecular weight marker, (1) ZE15, (2) ZE32, (3) ZR3, (4) ZR19, (5) negative control, (6) positive control.



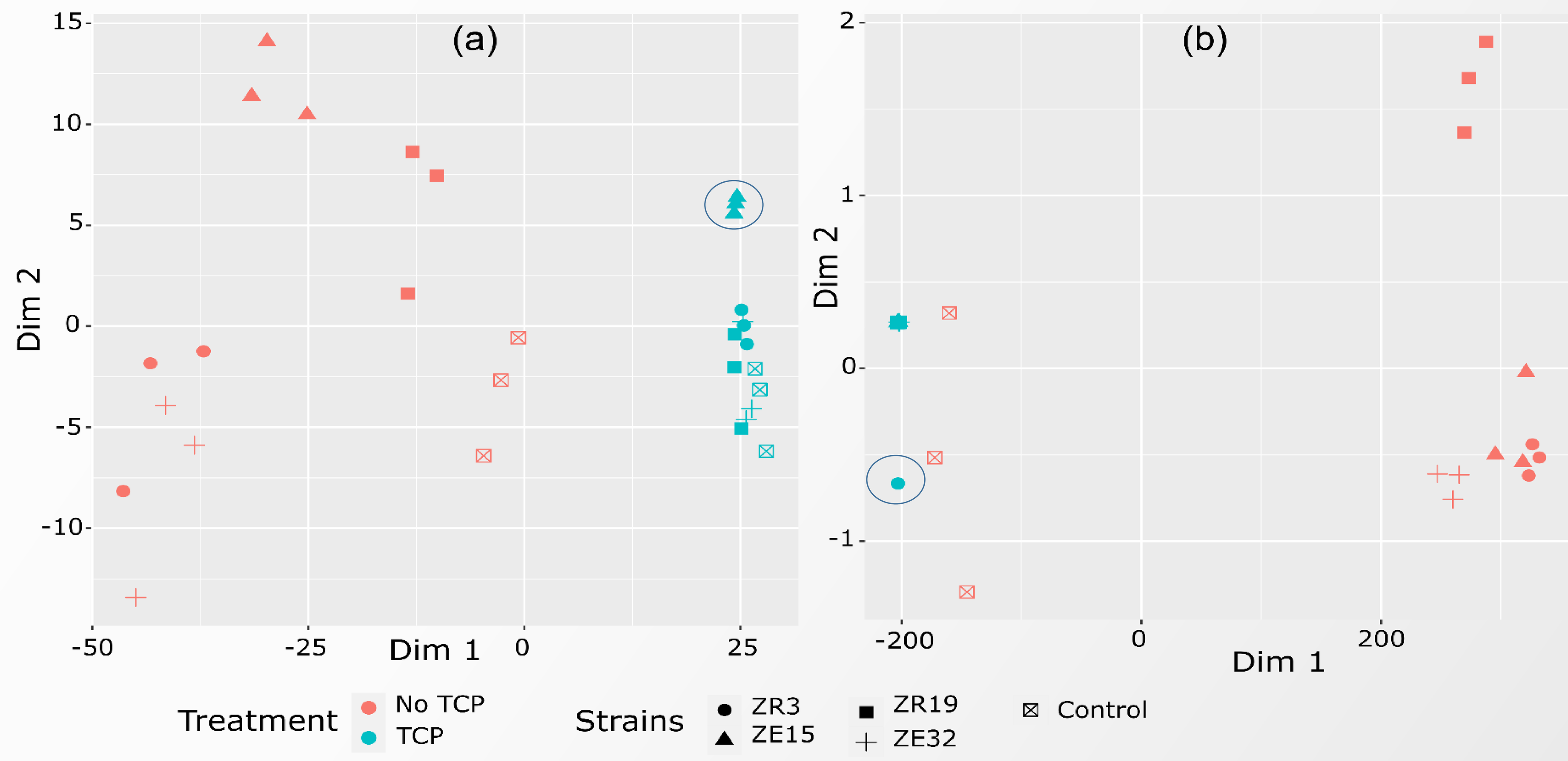


Figure 4: Multidimensional scaling (MDS) plot of bacterial metabolite produced in Pickovskaya's broth culture, colored by identification method; Flame Ionization Detector-GC-FID fame analysis (a, left) and Ion Exclusion Chromatography-GC-IEC (b, right).

In the presence of insoluble P, the strains ZE15, ZE32, ZR3 and ZR19 showed phosphate esterase activity vs ß-D-glucosidase activity, 3.65 vs 2.34, 3.12 vs 2.18, 1.93 vs 1.99 and 2.67 vs 1.82 nmol g^{-1} dry soil, respectively. Whereas, in the presence of soluble P, less exo-enzyme activities were recorded. Plant growth promoting attributes have showed significant results. Maximum root length (23%), root volume (55%) and seedling dry biomass (30%) were increased by strain ZR3. The strain ZE32 showed maximum 45% increase in average root diameter. A positive correlation was found between bacterial population, solubilized P and soil exoenzyme activities. Whereas, a negative correlation was detected between bacterial population and pH of growth media.

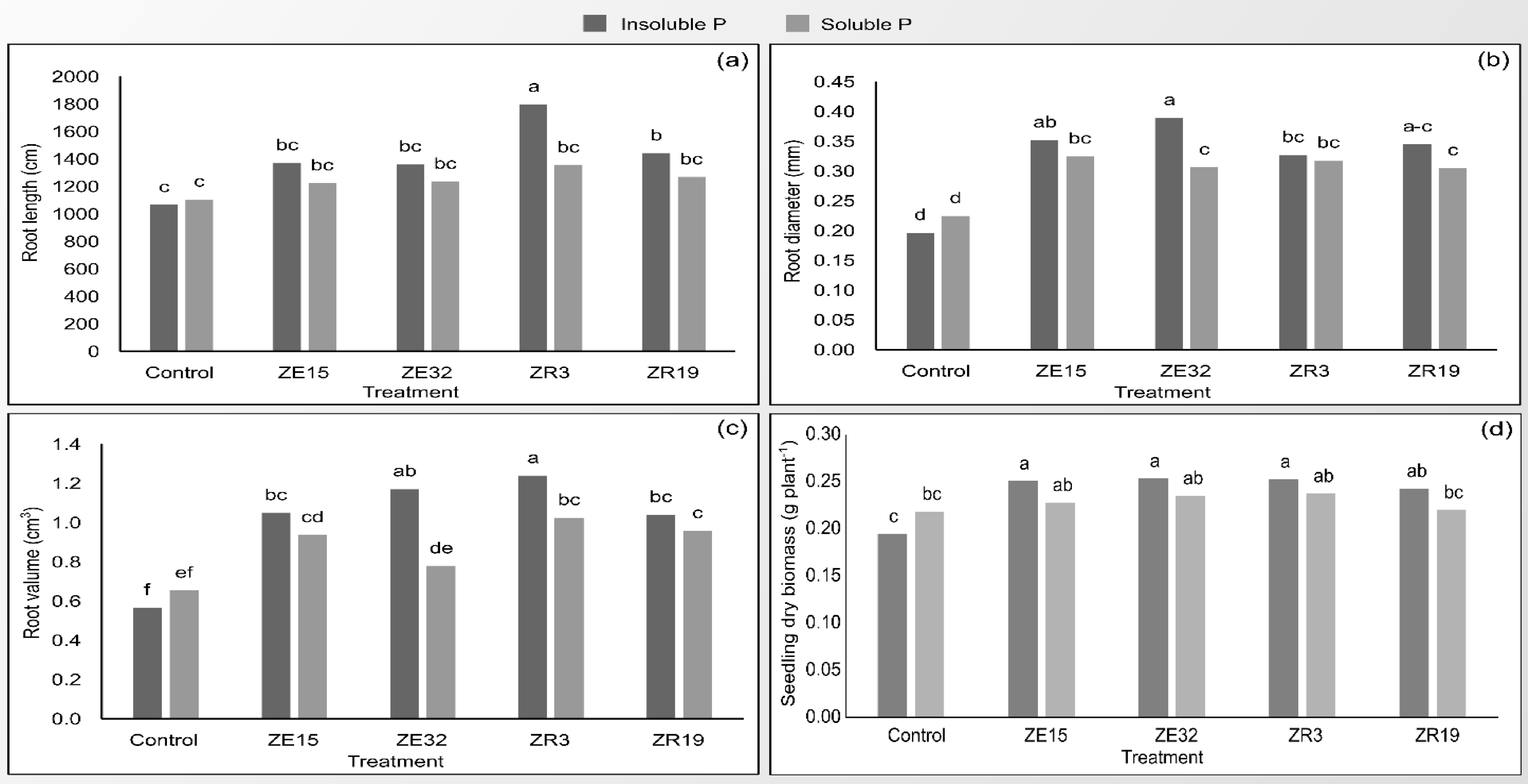


Figure 5: Effect of soil-plant-associate bacteria on root growth and seedling dry biomass in presence of insoluble (tricalcium phosphate) and soluble (potassium dihydrogen phosphate) phosphorous.

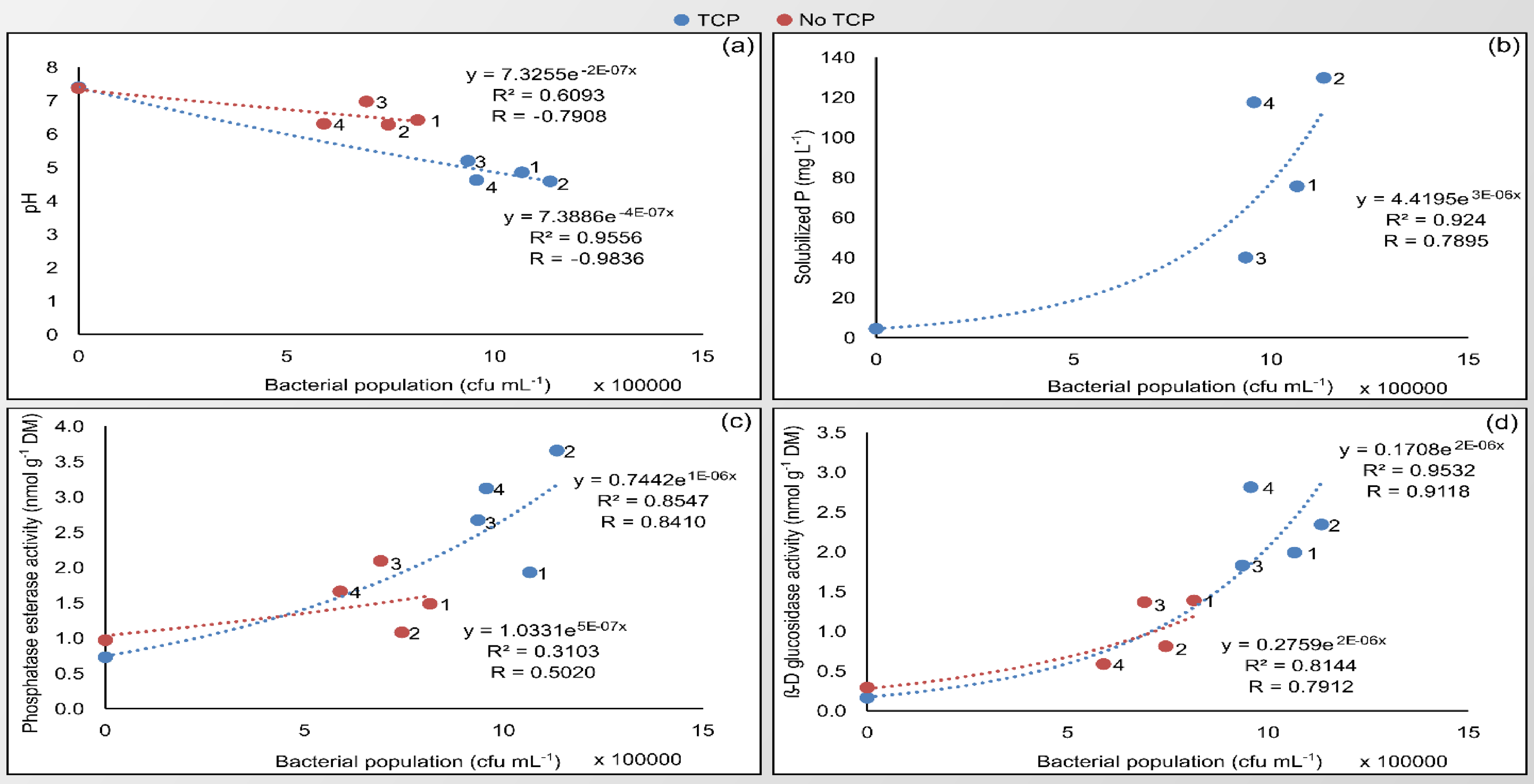


Figure 6: Regression and correlation analyses.

Conclusion

The strain ZE15 was more effective in excretion of organic acids, enzyme activities, P solubilization and seedling growth. Therefore, we can say that excretion of organic acid helps in P solubilization. By viewing such abilities, these strains can be used to develop affordable, effective and ecofriendly P solubilizing multi-strain-biofertilizer in order to reduce the use of chemical fertilizers in alkaline soils.

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