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Fuzzy-genetic approaches for estimation of microbial rock phosphate solubilization in sandy clay loam textured soil

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ABSTRACT

Soils are often rich in insoluble mineral and organic phosphates solubilized by specific bacterial species. The present study investigates the factors affecting microbial solubilization of phosphate in the sandy clay loam textured soil. Phosphate solubilization was measured using pot soil experiment spiked with rock phosphate and inoculated with phosphate solubilizing microorganism. The *Mogul* and *Thrift* fuzzy-genetic algorithm (GA) approaches have been implemented in order to estimate the solubilization of phosphate using the experimental parameters, including incubation period, temperature, pH of the soil, and concentration of glucose, ammonium sulfate, malic acid and single super phosphate (SSP). The estimation performance of fuzzy-GA approaches has been evaluated on the basis of root mean square error (RMSE), the coefficient of determination (R^2) and correlation (ρ). The Mogul based fuzzy-GA approach results in the minimum value of RMSE 7.66, and the maximum values of correlation coefficient 0.72 and coefficient of determination 0.51 in between the predicted and original values of phosphate solubilization.

1. Introduction

Phosphorus (P) is the most critical element for plant growth (Marschner, 2011; Perez et al., 2007), building 0.2% of plants on dry weight basis (Raghothama and Karthikeyan, 2005). It plays an essential role in photosynthesis, respiration, energy storage and transfer, cell division, and enlargement etc. (Shen et al., 2011; Kalaji et al., 2014, 2017; Wiwart et al., 2009). Besides, P also contributes to root elongation, seed formation, winter shield, and disease resistance of plants. The quality of fruits, vegetables and grain crops is also affected by the P absorption (Kalaji et al., 2014, 2017; Wiwart et al., 2009). Soils have often higher concentration of total P but deficient in available orthophosphate (Pi) (Sharma et al., 2013). The concentration of soluble P in soil (grassland and forest) ranges from 0.05 to 10 mg/L (Turner and Haygarth, 2001) as more than 80% of P is immobile. The principal characteristic of primary minerals (oxyapatite, hydroxyapatite, and apatite) is their poor solubility. The soluble inorganic phosphate used in agricultural soil is quickly immobilized and becomes inaccessible to plants (Khan et al., 2013) due to adsorption, precipitation or converting to organic form (Young, 2012).

The organic fraction of P is found in the form of phosphomonoesterase, phosphodiesters, phosphotriesters, phospholipids, and nucleic acids. Phosphorus is least accessible and immobile nutrient for plants in soil (Takahashi and Anwar, 2007). Consequently, for enhancing agriculture production, external soluble phosphatic fertilizers are being used in agriculture (Shen et al., 2011; Chien et al., 2011). After application, it is quickly precipitated to an insoluble form and results in complexes with Ca^{+2}, Fe^{+3}, Al^{+3}, Co^{+2}, or Zn^{+2} or adsorbed to the surfaces of calcium carbonate, aluminum oxide, iron oxide, and aluminum silicate depending upon characteristics of soil (Chien et al., 2011). Consequently, availability and efficiency of such phosphatic fertilizers decrease which results in increased use of additional fertilizers to agricultural fields. The uncontrolled use of phosphate fertilizers has increased agricultural costs and originated a variety of environmental harms (Chen et al., 2007). The concentration of soluble P is usually very low in soils due to the phenomenon of chemical fixation of phosphate. The latter depends on soil type and its pH value. In acidic soils, free oxides and hydroxides of aluminium and iron fix P while in alkaline soils Ca fixes it (Walpola and Yoon, 2012; Marra et al., 2012).

The increasing demand and high cost of soluble phosphate fertilizer require the development of some alternate source. Natural rock phosphates (RP) can serve a valuable source of P fertilizer by partial acidulation of RP (Kumari and Phogat, 2008; Goenadi and Sugiarto, 2000). Though, all of the RP resources are not freely available to plants as RP

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are not agronomically reactive when applied directly to the soil (Shen et al., 2011; Sharma et al., 2013). The capability of soil microorganisms to solubilize insoluble organic and mineral phosphates into soluble inorganic phosphates is recognized in several past studies (Walpola and Yoon, 2012; Marra et al., 2012; Kumari and Phogat, 2008; Goenadi and Sugiarto, 2000). Microorganisms are an integral part of the soil P cycle, which participate in processes that affect the transformation of soil P. Mostly soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through the process of solubilization and mineralization (Hameed et al., 2008). Numerous microbes, including the bacteria, fungi, and yeast, etc. are capable of solubilizing insoluble phosphates by their metabolites (inorganic or organic acids) in the soil (Bojinova et al., 2008; Delvasto et al., 2006).

The microbial solubilization of P from insoluble phosphates is one of the vital mechanisms involved in the rapid release of P in the soil. The capability of a microorganism to solubilize the inorganic rock phosphate is severely affected by various environmental and nutritional parameters. Their appropriate combination is important in the overall estimation of the bioprocess. In some recent studies, mathematical and neural network approaches were used (Leitner et al., 2010; Zafar et al., 2012; Johnson et al., 2014; Asadi-Eydivand et al., 2014; Keshavarzi et al., 2015), including a dynamical mathematical model to compute the phosphate uptake by root hairs of plants (Leitner et al., 2010), a hybrid artificial neural network (ANN)-genetic algorithm (GA) method in the optimization of polyhydroxybutyrate production (prediction error of 2.52%) (Zafar et al., 2012) (ANN-GA method performs better than the response surface methodology), ANN methods for the protein solubility prediction modeling (Johnson et al., 2014) in the prediction of the composition of calcium phosphate (Asadi-Eydivand et al., 2014) (mean square error (MSE) of 1.78×10^{-3}), and soil phosphorous content estimation ($R^2 = 0.423$) (Keshavarzi et al., 2015).

Though, in most of the studies, ANN method is used in the prediction of P content and related parameter estimation. The outcomes of ANN method are not stable and fluctuate in each run due to weight and bias updates. The hybrid artificial intelligence (AI) methods can result in a better prediction of P content. With this motivation the present study targets to search the optimum conditions for the solubilization of P using two combined fuzzy-GA approaches based on the Mogul (Herrera et al., 1998) and the Thrift (Thrift, 1991) approaches. The input parameters used in the analysis include the number of days, temperature, pH, and concentration of glucose, ammonium sulfate, maleic acid and single super phosphate (SSP). The fuzzy-GA method has the proficiency to adjust the initial population to avoid local minima with the objective to obtain higher prediction accuracy which cannot be obtained by using a neural network or fuzzy logic method independently. The GA is used for the parameter optimization of the fuzzy method using an initial population. The fuzzy method controls the estimation of the output variable during the optimization process and revises the boundaries of each of the input variables. The set of new input variables was used in the next iteration to find the optimal target values. It minimizes the time consumed in the optimization process and maximizes the stability and prediction efficiency.

2. Materials and methods

2.1. Isolation of phosphorus solubilizing microorganism, media and growth conditions

The rhizospheric soil was collected from the wheat plants and stored in polythene bags. The non-rhizospheric soil was dislodged by agitating the roots intensely and the soil firmly adhering to the roots was used for isolation. Rhizobacteria were isolated by employing a serial dilution plate technique (Paul, 2014) using trypticase soy agar (TSA) medium (Atlas, 2010). Additional purification and multiplication of isolates were done by streaking on fresh plates using the NBRIP (Glucose 10 g, Rock phosphate 5 g, $MgCl_2 \cdot 6H_2O$ 5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.1 g) media. The phosphorus solubilizing potential of the bacterial isolates was determined qualitatively by using plate assay using National Botanical Research Institute phosphate (NBRIP) growth medium(Kumar et al., 2012). Bacterial isolates were cultured for 2 days on agar plate media. After that, a full loop containing cultured bacteria was placed on separate plates, individually using toothpicks and incubated at 30 °C for 7 days. Formation of halo zone indicates the capability of producing organic acids and rock phosphate solubilization. The experiment was performed in triplicate. Quantitative screening was done to obtain efficient bacterial strains which can solubilize the rock phosphate using the NBRIP medium. The liquid NBRIP medium was prepared by processing rock phosphate as a source of P. The medium was inoculated with the respective inocula of eighteen isolates at 30 °C for 72 h. Subsequently, the medium was centrifuged at 10,000 rpm for 10 min. Then solubilizing activity was measured by measuring P content using spectrophotometer at 492 nm (Kirkham, 2014). Percent solubilization was determined and most efficient isolate with the maximum solubilization ability was selected for further analysis. The sterilized NBRIP broth (40 ml) containing rock phosphate was added to the autoclaved test tubes. The medium was inoculated with the bacterial strain of uniform cell density at 30 °C under static conditions (Bashan et al., 2014).

2.2. Phosphate solubilizing activity measurement

Out of eighteen, one bacterial strain having maximum phosphate solubilizing activity was selected for the phosphate solubilizing measurement. The inocula of the selected isolate (MZ10) were prepared by inoculating the sterilized broth with individual isolates in trypticase soy broth (TSB) at 28 ± 1 °C for 48 h at 100 rpm. An optical density of 0.6 at λ = 492 nm of broth containing P solubilizing microorganism (PSM) was achieved at a uniform cell density (108–109 cfu ml^{-1}).

2.3. Phosphate solubilizing factors optimization

A lab study was conducted for the optimization of rock phosphate solubilization related parameters. For this purpose, 250 g sandy clay loam textured soil was spiked with 100 g RP in plastic beakers. An inoculum of P solubilizing bacteria (10 ml) was applied in each plastic beaker except for control. A constant water holding capacity (50%) was maintained throughout the experiment. Beakers were incubated at 30 ± 1 °C. Moist soil samples were drawn from each beaker and analyzed by 0.5 M $NaHCO_3$ (pH 8.5). All the experiments were conducted in triplicate. Several environmental and nutritional parameters were assessed on rock phosphate solubilization by the selected bacterial isolate (Table 1) using Taguchi design. Phosphorus in the extract was determined by spectrophotometer (Smith and Read, 2010). Measured values of P solubilization with their dependent variables are summarized in Table 2.

2.4. Physico-chemical properties of soil

The physical (soil texture, saturation percentage) and chemical

Table 1
Values of environmental and nutritional parameters.

Independent variables	Units	Values			
Incubation period	Days	3	6	9	12
Temperature	°C	25	30	35	40
pH	Moles of H^+/L	6.0	7.0	8.0	9.0
Glucose	%	0.0	2.5	5.0	10
Ammonium sulfate	%	0.1	0.25	0.5	1.0
Malic acid	%	0.1	0.25	0.5	1.0
SSP	%	0.1	0.25	0.5	1.0