

Molecular Genetic Identification of Yeast Strains Isolated from Egyptian Soils for Solubilization of Inorganic Phosphates and Growth Promotion of Corn Plants

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Forty yeast strains isolated from soils taken from different locations in Egypt were tested for their P-solubilizing activities on the basis of analyzing the clear zone around colonies growing on a tricalcium phosphate medium after incubation for 5 days at 25°C, denoted as the solubilization index (SI). Nine isolates that exhibited P-solubilization potential with an SI ranging from 1.19 to 2.76 were genetically characterized as five yeasts belonging to the genus *Saccharomyces cerevisiae* and four non-*Saccharomyces*, based on a PCR analysis of the ITS1-26S region amplified by SC1/SC2 species-specific primers. The highest P-solubilization efficiency was demonstrated by isolate PSY-4, which was identified as *Saccharomyces cerevisiae* by a sequence analysis of the variable D1/D2 domain of the 26S rDNA. The effects of single and mixed inoculations with yeast PSY-4 and *Bacillus polymyxa* on the P-uptake and growth of corn were tested in a greenhouse experiment using different levels of a phosphorus chemical fertilizer (50, 100, and 200 kg/ha super phosphate 15.5% P₂O₅). The results showed that inoculating the corn with yeast PSY-4 or *B. polymyxa* caused significant increases in the shoot and root dry weights and P-uptake in the shoots and roots. The P-fertilization level also had a significant influence on the shoot and root dry weights and P-uptake in the shoots and roots when increasing the P-level from 50 up to 200 kg/ha. Dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 200 kg/ha gave higher values for the shoot and root dry weights and P-uptake in the shoots and roots, yet these increases were nonsignificant when compared with dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 100 kg/ha. The best increases were obtained from

dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 100 kg/ha, which induced the following percentage increases in the shoot and root dry weights, and P-uptake in the shoots and roots; 16.22%, 46.92%, 10.09%, and 31.07%, respectively, when compared with the uninoculated control (fertilized with 100 kg/ha).

Keywords: Genetic identification, 26S rRNA gene, soil yeasts, solubilization, inorganic phosphates

Phosphorus (P) is an essential macroelement for plants, yet the total concentration of P in soils only ranges from 0.02% to 0.5% and averages approximately 0.05%, the variation being largely due to differences in the weathering intensity and parent material composition [34]. Thus, to increase the availability of phosphorus for plants, large amounts of fertilizers are used on a regular basis, yet after application, a large proportion of fertilizer phosphorus is quickly transferred to an insoluble form [27]. Arpana *et al.* [2] previously reported that a great proportion of phosphorus in chemical fertilizers becomes unavailable to plants after its application to soil, as the phosphorus forms strong bonds with calcium and magnesium in alkaline soils and with iron and aluminum in acidic soils. Meanwhile, in calcareous soils, phosphorus fertilizers are fixed by calcium carbonate through adsorption and precipitation, resulting in an efficiency of less than 20% [37].

The solubilization of phosphate-bearing inorganic materials by microorganisms excreting organic acids would seem to be an attractive solution that has been actively studied during the last decade. Phosphate-solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to plants, allowing the sustainable use of phosphate fertilizers. Microorganisms are involved in a range of processes that effect the transformation of soil phosphorus

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(P), making them an integral component of the soil 'P' cycle. Therefore, the application of PSMs to fields has been reported to increase the crop yield. Several mechanisms, such as lowering the pH by acid production, ion chelation, and exchange reactions in the growth environment, have been reported to play a role in P-solubilization by PSMs [9, 11, 39]. Based on the early work of Sperber [33], microbially mediated solubilization has been extensively examined using bacteria and filamentous fungi [12, 20, 23, 28, 29]. Nonetheless, despite the known ability of yeasts to produce organic acids, there have been very few reports on their ability to solubilize inorganic phosphate [6, 19, 38].

Accordingly, the present study isolated and characterized P-solubilizing yeasts using a PCR analysis of the ITS1-26S region amplified using SC1/SC2 species-specific primers. The effects of single and dual inoculations with the best phosphate solubilizer yeast strain PSY-4 and/or *Bacillus polymyxa* on the phosphorus uptake and growth of corn plants under different levels of phosphorus chemical fertilizer in calcareous soil was then evaluated. In addition, sequencing of the D1/D2 domain of the 26S rRNA encoding gene was used to identify the selected strain PSY-4 at the genus and species levels.

MATERIALS AND METHODS

Isolation of Yeasts and Determination of Solubilization Index (SI)

Clay soil samples were collected from different locations in Upper Egypt, and used for the isolation of naturally occurring yeast strains. About 1 g of soil was added to 20 ml of YEPD medium (1% yeast extract, 2% peptone, 2% dextrose, pH 4.5), and incubated at 28°C on a rotary shaker at 150 rpm for 1 day. Aliquots of 100 µl were then spread onto a YEPD agar supplemented with 50 mg/ml ampicillin to inhibit bacterial growth. After incubation for 3 days at 25°C, different colonies were randomly isolated from each soil sample according to the shape, color, and surface feature. The ability of the yeast isolates to solubilize insoluble phosphate was described by the solubilization index (SI): the ratio of the total diameter (colony+halozone) and the colony diameter [5]. Pikovskaya's agar medium was used, modified [35] by having the following composition: Glucose, 10 g; Ca₃(PO₄)₂, 5 g; (NH₄)₂SO₄, 0.5 g; KCl, 0.2 g; MgSO₄·7H₂O, 0.1 g; MnSO₄ trace; FeSO₄ trace (pH 7). The halo zone formation around the yeast growth on Pikovskaya's agar medium was measured after incubation for 5 days at 25°C.

Bacterial Strain

The *Bacillus polymyxa* strain (commercial strain used in the preparation of the biofertilizer called Phosphorien in Egypt) was obtained from the Desert Research Center, Egypt, and maintained on Pikovskaya's agar slant medium (PVK) at 4°C in a refrigerator and as a glycerol stock at -20°C in a deep freezer.

Determination of pH and Soluble P Using Yeast Strains

The ability of the yeast strains to solubilize insoluble tricalcium phosphate was measured in 100-ml aliquots of modified Pikovskaya's

liquid medium. The yeast strains were grown in 100-ml aliquots of the liquid medium for 5 days at 25°C, and the cultures were then filtered and centrifuged at 10,000 rpm for 10 min. The soluble phosphorus and pH in the supernatant and blank sample of the medium were determined using the method described by Jackson [17]. Based on the results of this experiment, the best phosphate solubilizer among the yeast strains was selected for the corn inoculation experiment.

DNA Extraction and Molecular Characterization of Yeast Isolates

The total genomic DNA from the isolated yeast was prepared according to the method described by Harju *et al.* [14]. The isolates were further characterized by amplification with *S. cerevisiae* species-specific primers SC1/SC2. The ITS1-26S region was amplified using the primer pair SC1 (5-AACGGTGAGAGATTCTGTGC-3) and SC2 (5-AGCTGGCAGTATCCCACAG-3) [18]. The PCR was performed in a final volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, each dNTP at a concentration of 0.2 mM, 1.25 IU of *Taq* polymerase, each primer at a concentration of 0.2 mM, and 2 µl of the DNA template. The thermal cycling parameters included an initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 50°C for 30 s, and a primer extension at 72°C for 1 min. Finally, the reaction mixture was heated to 72°C for 10 min and subsequently cooled to 4°C. Five µl of the amplified mixture was then analyzed using 1.5% 0.5×TBE agarose gel electrophoresis. The gel was stained with ethidium bromide, visualized under UV light, and photographed.

Gene Amplification and DNA Sequencing of Strain PSY-4

To identify and determine the correct phylogenetic position of isolate PSY-4, a sequence analysis of the variable D1/D2 domain of the large subunit (26S) ribosomal DNA was performed. Fragments containing about 600–650 bp at the 5' end of the 26S rDNA were amplified using primers: NL-1 (5'-GCA TAT CAA TAA GCG GAG GAAAAG-3') and NL-4 (5'-GGT CCG TGT TTC AAG ACG G-3') [21]. The same conditions as mentioned above were used for this reaction. The PCR was run with an initial step at 95°C for 5 min, followed by 36 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 2 min, an extension at 72°C for 2 min, and a final extension at 72°C for 7 min, and holding at 4°C. The amplified product was then purified and sequenced.

Phylogenetic Analysis

The 26S sequence of isolate PSY-4 was used for a BLAST search in the EMBL/GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>). The 26S sequence of the isolate was further aligned and compared with published 26S rRNA sequences using the taxonomy browser of the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) and GenBank. A phylogenetic tree was constructed with MEGA ver. 4.0 using a neighbor-joining algorithm, plus the Jukes–Cantor distance estimation method with bootstrap analyses for 1,000 replicates was performed.

Greenhouse Experiment

The effects of single and dual inoculations with yeast strain PSY-4 and *B. polymyxa* on the P-uptake and growth of corn (*Zea mays*) was tested in a greenhouse experiment (season, 2008) using

calcareous soil collected from the El-Gorahib Experimental Farm of Assiut University. The chemical properties of the soil were pH (1:1 suspension) 8.2, organic matter 0.25%, total nitrogen 0.003%, available P 5.30 ppm, and calcium carbonate 16.18%. The local cultivar “single hybrid-10” was used in this experiment. Three maize grains of cultivar single hybrid-10 were planted in each plastic pot (25 cm in diameter) containing 5 kg of 2-mm-sieved soil. After germination, the plants were thinned to two plants/pot. The experimental treatments were arranged as split plots on the basis of a randomized Complete Block Design with five replications. The main plot was devoted to different levels of the phosphorus chemical fertilizer (granule super phosphate 15.5% P_2O_5), consisting of P1=50, P2=100, and P3=200 kg/ha, whereas the subplots were assigned for the microbial inoculation: 1, uninoculated; 2, inoculated with yeast strain (Y4); 3, inoculated with *B. polymyxa*; and 4, mixed inoculation with yeast strain and *B. polymyxa*.

Separate cultures of the yeast strain PSY-4 and *Bacillus polymyxa* were respectively grown on 100-ml aliquots of a malt-yeast-glucose-peptone (YM) medium and nutrient broth medium in 250 ml Erlenmeyer flasks. The flasks were incubated at 25 and 30°C for 4 days for the yeast strain and *B. polymyxa*, respectively. Sterilized peat moss was used as the carrier for the inoculant preparations. The pulverized dry peat moss was neutralized to pH 7 with $CaCO_3$ and $Ca(OH)_2$, and distributed in batches of 50 g in polyethylene bags and autoclaved for 30 min at 121°C on three successive days. Aliquots of 25 ml of the yeast or *B. polymyxa* broth culture were used per 50 g of the sterilized carrier material. To inoculate the seeds with the microbial inoculants, the seeds were first moistened with a 40% Arabic gum solution, and after mixing, the peat inoculant was added and thoroughly mixed with the seeds until they were uniformly surface-coated. The inoculant was added to the seeds at a rate of 15 g/100 g of seeds. The counted numbers of viable cells in the peat inoculant at the time of use for inoculation were 7×10^8 and 1.1×10^9 CFU/ml for the yeast strain and *B. polymyxa*, respectively. In the case of the mixed inoculant, an equal volume of the broth culture of the tested strain was mixed in before adding to the sterilized peat just before the seed inoculation.

Plant Analysis

The corn plants were harvested after 40 days of sowing. The shoots and roots were separated and then oven-dried at 70°C to a constant weight to determine the root and shoot weights. The dried shoots were ground and submitted to acid digestion using a 2:1 HNO_3 : $HClO_4$ acid mixture. The digests were then analyzed for P using the chlorostannous phosphomolybdic acid method.

Statistical Analysis

The data reported in this paper are mean values based on three replicates. The differences between the treatments were tested using ANOVA and subsequent *post hoc* comparisons of the means (LSD test, at $P=0.05$). The statistical analysis of the data was performed using a statistical computer program.

Nucleotide Sequence Accession Number

The partial 26S ribosomal DNA sequences of strain PSY-4 reported in this paper have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under Accession No. HM165257.

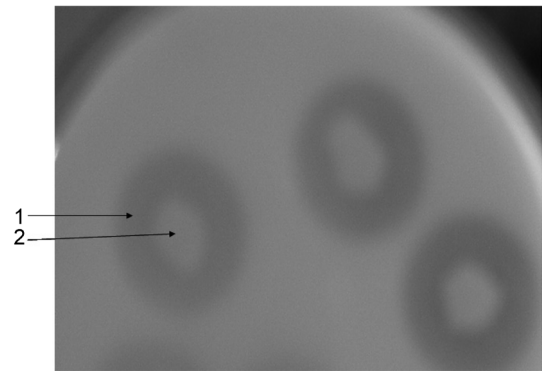


Fig. 1. Colonies of yeast strain PSY-4 grown for 5 days on Pikovskaya's agar medium showing clear zone of phosphate solubilization: arrow 1 indicates a clear zone and arrow 2 indicates a colony.

RESULTS AND DISCUSSION

Isolation and Screening of Phosphate-Solubilizing Yeasts and Estimation of Phosphate Solubilization

Forty yeast strains were isolated from soil samples collected from different locations in Upper Egypt (5 governorates) cultivated with sugarcane plants. The purified isolates were screened for the solubilization of inorganic phosphates on Pikovskaya's agar medium. Nine isolates (22%) showed an ability to solubilize inorganic phosphates based on inducing clear zones around their colonies (Fig. 1). The phosphate solubilization index and amounts of phosphorus solubilized from tricalcium phosphate (TCP) by the nine yeast isolates are shown in Table 1. The highest amount of P solubilized ($2.56 \mu\text{g/ml}$) was recorded for yeast strain PSY-4, which showed a phosphate solubilization index of 2.76 and pH of 4.1 in a 5-day culture (Table 1). The results also showed that the lowest amount of P solubilized was

Table 1. Phosphate solubilization by isolated yeast strains.

Isolated strain No.	Solubilizing index	Solubilized P in liquid culture ($\mu\text{g/ml}$)	pH of cultures
Control (liquid medium)	-	0.009 ± 2.07	6.9 ± 1.43
PSY-1	2.43 ± 0.46	2.20 ± 2.11	4.4 ± 2.56
PSY-2	1.19 ± 0.49	1.32 ± 2.50	5.6 ± 1.39
PSY-3	$1.72 \pm 0.1.02$	1.36 ± 2.36	5.2 ± 2.69
PSY-4	2.76 ± 0.82	2.56 ± 3.45	4.1 ± 3.12
PSY-5	2.02 ± 0.65	1.96 ± 1.96	4.8 ± 2.73
PSY-6	1.96 ± 0.81	1.93 ± 3.012	5.0 ± 2.44
PSY-7	2.53 ± 0.53	2.22 ± 2.29	4.3 ± 3.69
PSY-8	2.13 ± 0.67	2.12 ± 2.16	4.7 ± 1.85
PSY-9	2.49 ± 0.70	2.18 ± 3.06	4.5 ± 3.45
<i>B. polymyxa</i>	2.92 ± 1.08	2.73 ± 3.19	3.8 ± 3.07

All values are the means of three replicates.

recorded by yeast strain PSY-2 (1.32 $\mu\text{g/ml}$). *Bacillus polymyxa* was used as a reference strain to study the phosphate solubilization on an agar and in a liquid culture containing TCP. The phosphate solubilization index of the *B. polymyxa* strain was 2.92 and the solubilization of TCP in Pikovskaya's liquid medium was 2.73 $\mu\text{g/ml}$.

The solubilized amounts of P and determined phosphate solubilization index for the yeast strains were compatible with the acidity produced in their respective cultures (Table 1), indicating that the organic acids produced from fermentation of the sugars in the media by the yeast strains were the main cause of the solubilization.

Some species of bacteria and other microorganisms are well known to produce organic acids from sugar fermentation, and are already used as biofertilizers for solubilizing inorganic phosphate and increasing the phosphorus availability in soil. The solubilization of insoluble P by microorganisms is mainly due to the production of organic acids and chelating substances [24, 36]. Many bacterial, fungal, and actinomycete species capable of solubilizing sparingly soluble phosphorus in a pure culture have already been isolated and studied [11, 13, 39]. Other reports have also shown the ability of certain yeasts to solubilize inorganic phosphates. However, the present study found great variations in this ability among the isolated strains, which may have been due to variations in the kind of organic acid or amounts produced. Kanti and Suidiana [19] reported that 12 isolates out of 23 isolated from Gunung Halimun National Park, West Java, Indonesia, had the ability to dissolve $\text{Ca}_3(\text{PO}_4)_2$. The solubilization of inorganic phosphate simultaneously happens as a result of organic acid production, indicating that yeasts, which are commonly found in soil and the rhizosphere, could play a role in the transformation of organic materials and the mineralization of phosphate in soil. Citric acid, among the three carboxylic groups, seems to be the most active organic acid in solubilizing insoluble phosphates; the amount produced is greatly affected by the pH of the medium and concentration of sugar.

Vassileva *et al.* [38] pointed to the importance of yeasts, as a well-known group of microorganisms in the production of organic acids (especially citric) as well as their high survival rates under extreme soil conditions, in the transformation of rock phosphates and insoluble carbonate, leading to increases in the available phosphorus, Fe, and other micronutrients. They recorded the simultaneous solubilization of rock phosphate and calcium carbonate by free and encapsulated cells of yeast strain *Yarrowia lipolytica* as a result of citric acid production in a repeated-batch shake-flask fermentation medium. They also found that *Yarrowia lipolytica* and other acid-producing yeasts could be successfully applied for rock phosphate solubilization and the preparation of soil inoculants.

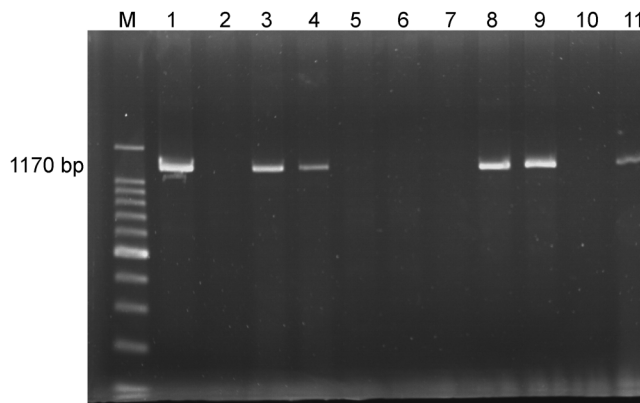


Fig. 2. PCR for isolated yeasts using primers SC1 and SC2. Lane M, DNA ladder (100 bp); lane 1, positive control (*S. cerevisiae*, CGMCC No. 2.614); lane 2, negative control (no DNA); lanes 3–11, isolated PSY strains. Positive bands were identified as *Saccharomyces cerevisiae* and negative bands as non-*Saccharomyces*.

Molecular Characterization of P-Solubilizing Yeast Isolates

Nine isolates that exhibited P-solubilization potential with a SI ranging from 1.19 to 2.76 were further genetically characterized by PCR amplification using *S. cerevisiae* species-specific primers SC1/SC2. These primers permit the amplification of a 1,170 bp DNA fragment located between the ITS-1 region and the LSU gene of *S. cerevisiae* strains. To confirm the specificity of these primers, PCR assays were performed using, as the positive control, commercial *S. cerevisiae* (CGMCC No. 2.614) obtained from the China General Microbiological Culture Collection Center. As shown in Fig. 2, the size of the amplified ITS1-26S region was 1,170 bp for the 5 yeast isolates, which were then considered as *S. cerevisiae*. Meanwhile, 4 of the isolates amplified with these primers did not show any amplification and were characterized as non-*Saccharomyces*. This approach, using SC1/SC2 primers, provides a rapid and sensitive method for discrimination between *S. cerevisiae* and non-*Saccharomyces* [3, 10, 18].

Identification of Strain PSY-4 Using 26S rDNA Sequences and Phylogenetic Analysis

After determining the sequence of the D1/D2 domain of the 26S rRNA encoding gene from strain PSY-4, a database search for similar sequences to the PSY-4 sequence in the GenBank was performed using the BLASTn program. The most similar sequences were found to be sequences of strains belonging to the genus *Saccharomyces*. Moreover, strain PSY-4 showed a 100% 26S ribosomal DNA sequence similarity to *S. cerevisiae*. Phylogenetic trees were constructed with MEGA ver. 4.0, using a neighbor-joining algorithm, plus the Jukes–Cantor distance estimation method with bootstrap analyses for 1,000 replicates was

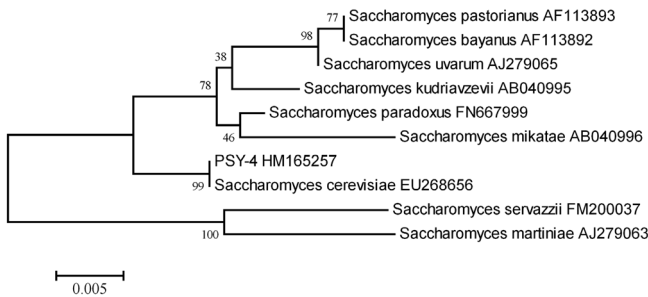


Fig. 3. Phylogenetic tree for *S. cerevisiae* and related species constructed by the neighbor-joining method based on the D1/D2 domain of LSU rRNA gene sequences.

Segments corresponding to an evolutionary distance of 0.005 are shown with bars. Accession numbers for sequences are as shown in the phylogenetic tree.

performed (Fig. 3). As a result, strain PSY-4 was clustered with other *Saccharomyces* species, indicating a phylogenetic position within the genus *Saccharomyces*. Therefore, the strain was designated as *S. cerevisiae* strain PSY-4. The sequencing of the D1/D2 of the large-subunit 26S ribosomal DNA is now widely accepted as a standard procedure for yeast identification. Moreover, a 600 bp length of the D1/D2 domain of the 26S rDNA contains sufficient variation to define individuals at the species level [7, 15, 21, 31]. It has also been found that molecular methods based on the sequences of the 26S rDNA, D1/D2 domain, and ITS region are rapid and precise when compared with physiological methods for the identification and typing of *Saccharomyces* species [16, 22].

Although different yeast strains have already been reported as P-solubilizing microbes [19, 26, 38], the yeast *S. cerevisiae* has never been reported as a P-solubilizing microorganism. Therefore, this is the first time a *S. cerevisiae* strain has been shown to exhibit P-solubilizing ability.

Effects of Inoculation with Yeast *S. cerevisiae* Strain PSY-4 and *B. polymyxa* on P-Uptake and Growth of Corn

The data obtained on the main effects of inoculation with yeast strain PSY-4 or/and *B. polymyxa* and for different levels of P-fertilization are shown in Table 2. The results showed that inoculating the corn with yeast strain PSY-4 or *B. polymyxa* caused a significant increase in the shoot and root dry weights and P-uptake in the shoots and roots when compared with the control (uninoculated plants). The results also showed that co-inoculating yeast strain PSY-4 in a mixture with *B. polymyxa* produced further promotive effects on the shoot and root dry weights and P-uptake in the shoots and roots when compared with a single inoculation treatment with only one strain. As such, the magnified promotion induced by a mixture of the yeast

Table 2. Main effects of P-fertilization levels and inoculation with *S. cerevisiae* strain PSY-4 and *B. polymyxa* on growth and P-uptake of corn plants.

Treatment	Dry weight (g/plant)		P-uptake (mg/plant)	
	Shoots	Roots	Shoots	Roots
Level of P-fertilizer:				
50	25.92	1.84	70.74	2.99
100	27.43	2.16	79.23	3.44
200	30.53	2.47	91.09	3.96
LSD (0.05)	1.25	0.35	7.62	0.58
Inoculation treatment:				
Uninoculated	26.91	1.74	75.98	2.78
PSY-4	27.13	2.11	77.83	3.43
<i>B. polymyxa</i>	28.53	2.29	81.04	3.84
PSY-4+B. <i>polymyxa</i>	29.25	2.58	86.53	3.99
LSD (0.05)	1.76	0.36	12.73	0.62

PSY-4 and *B. polymyxa* strains indicated a synergetic interaction between them. These results are also in accordance with those of other studies and of field trials, showing that dual or multistrain inoculants containing a mixture of bacterial species promote greater beneficial effects than single strain inocula [4]. The increase in the root mass induced by the inoculated strains may also indicate direct hormonal effects. Some reports have shown that yeast is a microorganism that can produce growth-promoting substances, as hormones, amino acid, and vitamins [25, 30]. In addition, the plant growth enhancement by *Bacillus* may have been associated with its ability to produce hormones, especially indole acetic acid [32].

Increasing the P-fertilization level from 50 up to 200 kg/ha had a significant influence on the shoot and root dry weights and P-uptake in the shoots and roots. The optimum P-level was 200 kg/ha, which recorded the highest values for the shoot and root dry weights and P-uptake in the shoots and roots when compared with the other two P-fertilization levels (50 and 100 kg/ha).

The results presented in Table 3 show the interaction effect of inoculation with yeast strain PSY-4 or/and *B. polymyxa* and the P-fertilization levels. The data showed that the promotion effect of inoculating with yeast strain PSY-4 or/and *B. polymyxa* on the plant growth and P-uptake was more obvious at the low fertilization level of 50 kg/ha, whereas increasing the P-fertilization to 200 kg/ha decreased the promotive effect of the inoculation. Furthermore, whereas dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 200 kg/ha gave higher values for the shoot and root dry weights and P-uptake in the shoots and roots, these were nonsignificant increases when compared with dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of

Table 3. Interaction effects of P-fertilization levels and inoculation with *S. cerevisiae* strain PSY-4 and *B. polymyxa* on the growth and P-uptake of corn plants.

Treatment P-fertilizer×Inoculation	Dry weight (g/plant)		P-uptake (mg/plant)		
	Shoots	Roots	Shoots	Roots	
50	Uninoculated	25.24	1.53	61.62	2.40
	PSY-4	25.32	1.79	67.96	2.86
	<i>B. polymyxa</i>	26.11	1.92	75.36	3.13
	PSY-4+ <i>B. polymyxa</i>	26.81	2.11	78.02	3.58
100	Uninoculated	25.46	1.79	75.76	2.80
	PSY-4	26.14	1.96	76.04	3.19
	<i>B. polymyxa</i>	28.53	2.25	76.68	3.25
	PSY-4+ <i>B. polymyxa</i>	29.59	2.63	83.41	3.67
200	Uninoculated	29.64	2.19	84.11	3.14
	PSY-4	29.93	2.28	85.99	3.64
	<i>B. polymyxa</i>	30.16	2.30	86.07	3.85
	PSY-4+ <i>B. polymyxa</i>	30.07	2.65	87.18	3.90
LSD (0.05)	1.11	0.19	12.89	0.65	

100 kg/ha. Dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 100 kg/ha induced the following percentage increases in the shoot and root dry weights, and the P-uptake in the shoots and roots; 16.22%, 46.92%, 10.09%, and 31.07%, respectively, when compared with the uninoculated control (fertilized with 100 kg/ha). Meanwhile, dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 200 kg/ha induced the following percentage increases in the shoot and root dry weights, and P-uptake in the shoots and roots; 1.45%, 21%, 3.64%, and 24.2%, respectively, when compared with the uninoculated control (fertilized with 200 kg/ha). Thus, the best increases were obtained from dual inoculation with yeast strain PSY-4 and *B. polymyxa* at a P-fertilization level of 100 kg/ha. These results also indicate the importance of plant inoculation with P-solubilizing microbes, such as yeast strain PSY-4 and *B. polymyxa*, for decreasing the application of P-fertilizers in agriculture.

In summary, various efficient indigenous phosphate-solubilizing yeasts were isolated from soils taken from different Egyptian geographic regions. One of the isolates, PSY-4, identified as *S. cerevisiae*, efficiently solubilized and released P from an insoluble form and successfully improved the shoot and root growth of corn plants. The isolate PSY-4 could be used as a seed inoculant, especially in alkaline soils. Interestingly, the use of a mixture of *S. cerevisiae* strain PSY-4 and *B. polymyxa* as bioinoculants may also be able to increase the available P in Egyptian agriculture soil, thereby helping to reduce the industrial application of P-fertilizers and alleviate environmental pollution.

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