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Effect Of Carbon And Nitrogen Sources On Phosphate Solubilization By Some Local Isolates From Egyptian Rock Phosphate Deposit.

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ABSTRACT

One hundred and eight phosphate solubilizing (PS) isolates were isolated from different rhizosphere plants, rock phosphate and soil with the percentage of 36.11%, 45.33% and 18.51%, respectively. Four out of 108 isolates were selected as the most efficient Ps isolates which gave the highest values of phosphate solubilization index (PSI) and phosphate solubilization efficient (PSE%) in Pikovskaye's agar medium containing tri-calcium phosphate as a source of insoluble phosphate (qualitative estimation). These isolates were Rs22, Rs7, Bf6&RPf10which recorded PSE of 320%, 300%, 126% and 125%, respectively. These isolates also recorded the maximum rock phosphate solubilization efficiency (RPSE%) being 4.3%, 3.4%, 1.1% and 0.8% by RPf10, Bf6, Rs22 and Rs7, respectively in Pikovskaye's medium supplemented with 5% RP after 10 days of 30°C using shake flasks as a batch culture. The most efficient bacterial and fungal isolates were phenotypic identified as *Serratia* and *Aspergillus* genus. Maximum rock phosphate solubilization(RPS)was recorded in the presence of glucoseas the best carbon source at concentration of 10 gl^{-1}or 12.5-15gl^{-1}andtryptoneor yeast extract as the most favorable nitrogen source with 1.25gl^{-1} or 1.5-2gl^{-1}concs.after 8 or 10 days of incubation for tested bacterial or fungal isolates, respectively. This modification of Pikovskaye's medium led to increase the RPS about 10%, 19%, 49%&59 % by *Serratia* sp.Rs22, *Serratia* sp.Rs7, *Aspergillus* sp. RPf10 &*Aspergillus* sp. Bf6, respectively comparing to control. Also, whey and sugar beet waste were the best agro-industrial residues for highest RP solubility by tested bacterial and fungal isolates, respectively when used as a sole carbon sources,.

Keywords: phosphate solubilization, rock phosphate, *Serratia*, *Aspergillus*, agro-industrial residues, carbon and nitrogen sources.

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INTRODUCTION

Phosphorus (P) normally occurs as phosphates in both inorganic and organic compounds **[1] and [2].** It is an essential constituent of nucleic acid, phospholipids, the coenzymes of deoxy-ribonucleic acid (DNA) and nicotinamide adenine nucleotide phosphate (NADP), and most importantly adenosine triphosphate (ATP) that supplies energy utilized throughout the plant **[3].** Thus, phosphorus often known as "energizer" since it helps store and transfer energy. Besides, phosphorus can activates coenzymes for amino acid production used in protein synthesis and decomposes carbohydrates produced during photosynthesis. Furthermore, phosphorus play important role in many other metabolic processes which are required for normal growth for example glycolysis, respiration and fatty acid synthesis **[3].** Also, Phosphorus is one of the major nutrient elements limiting agricultural production in the world. It is added to the soil in the form of phosphate fertilizers, a part of which is utilized by plants and the rest is rapidly converted into insoluble complexes in the soil **[4].** This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable **[5]** The insoluble forms of P such as tricalcium phosphate ($Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4), etc. may be converted to soluble P by P-solubilizing organisms inhabiting different soil ecosystems **[6] and [7].** Microorganisms play an important role in all three major components of the soil P cycle namely, dissolution – precipitation, sorption – desorption, and mineralization – immobilization **[8].** A large number of microorganisms are capable of solubilizing rocks such as the genera of *Aspergillus ,Penicillium, Thrichoderma,Pseudomonas , Agrobacterium, Bacillus, Rhizobium, Micrococcus,Aereobacter,Erwinia,Flavobacterium* and *Actinomycetes* **[9]; [10] and [11].** The solubilization effect is generally due to the production of organic acids by these microorganisms 12].. These acids lower the pH and bring about the dissolution of bound forms of phosphate. Bioconversion of the agro-industrial residues using microbial methods is a safe and economic way to produce various fermented and value added products **[13]**. Moreover, bioconversion of waste is probably the most cost effective & environment friendly procedure for waste utilization.

The objective of this study was designed to isolate the phosphate solubilizing microorganisms from different plants rhizosphere, soils and rock phosphate in Egypt and screenfor potentialsolubilizers rock phosphate.Some nutritional conditions and some agro-industrial residues were tested to optimizethe RP solubilization.

MATERIALS AND METHODS

Sampling

Four plant rhizosphere soil samples were collected as usual manner from the fertile fields planted with broad bean (*Vicia faba*), Egyptian clover (*Medicago sativa*), wheat (*Triticumae stivum*) and sugar cane (*Saccharum officinarum*) in Menoufia governorate. Whereas soil sample was obtained from Fac.of Agric., Ain Shams Univ., Qalubiya governorate. Rock phosphate soil and RP mine samples were collected from El-Nasr Mining Company, Cairo, Egypt. These samples were used as a source for isolation of phosphate solubilizing microorganisms (PSMs). Physico-chemical characteristics analysis of rock phosphate sample were determined, the size analysis was as follows (%):chemical analysis consisted of P_2O_5,29.50 - 30.00%; CaO, 47.00 - 49.00%; MgO, 0.35-0.45%; Fe_2O_3, 2.00-2.50%; Al_2O_3, 0.35 - 0.55%; K_2O, 0.05 - 0.065%; Na_2O, 0.35 -0.50%; SiO_2, 7.00-9.00%; SO_3,1.50-2.50%; F, 2.50-3.50%; Cl,0.04 - 0.07%; CO2, 5.00-5.50%; ORG.MATT., 0.15-0.25% and H_2O, 4.00-6.00%.

Media used

Medium (1): Nutrient agar medium **[14]** was used for maintenance and preservation of bacteria. It has the following composition (gl^{-1}): beef extract, 3.0; peptone, 5.0; agar, 20 and adjusted to pH 7.0.
Medium (2): Malt agar medium **[15]** was used for maintenance and preservation of fungi. It has the following composition (gl^{-1}): malt extract, 30.0; agar, 20 and adjusted to pH 5.0.
Medium (3): Pikovskaya's agar (PVK) medium **[16]** was used for isolation of phosphate solubilizing microorganisms and qualitative estimation of phosphate solubilizing. Its composition was as follows (gl^{-1}): glucose, 10.0; $Ca_3(PO_4)_2$, 5.0; $(NH_4)_2SO_4$, 0.5; NaCl, 0.2; $MgSO_4.7H_2O$, 0.1; KCl, 0.2; $MnSO_4.H_2O$, 0.002; $FeSO_4.7H_2O$, 0.002; yeast extract, 0.5; agar, 18.0 and adjusted to pH 7.0. Pikovskaya's broth medium containing insoluble rock phosphate was used for quantitative estimation of P solubilizing.

Isolation and screening of phosphate solubilizing microorganisms

Ten gram representative samples were suspended in 90 ml of sterile tap water and shaken thoroughly for 10 min. Phosphate solubilizing microorganisms were isolated from collected samples by streak plate method for bacteria and spread plate techniques for fungi using Pikovskaya's agar medium supplemented with 0.003% wv^{-1} rose bengal, which is a selective medium for isolation of phosphate solubilizers. The plates were incubated at 30°C for 24 - 48 h and the colonies show clear halo-zone around growth colonies were measured to calculate phosphate solubilizing index (PSI) and phosphate solubilizing efficient (PSE) according to **[17] and [18]**, respectively. The selected isolates were preserved at 5°C on agar slant for further studies.

Identification of phosphate solubilizing microorganisms (PSMs)

The most efficient phosphate solubilizing bacterial isolates were selected and identified using their morphological, cultural, physiological and characteristics according to [**19].**The most efficient P solubilizing fungal isolates were identified based on the microscopic shape and color of conidia according to **[20].**

Submerged fermentation process

It was carried out in 250 ml plugged Erlenmeyer flasks, each containing 100 ml sterile Pikovskaya's broth medium supplemented with rock phosphate (as insoluble phosphate) and inoculated with 2% of bacterial standard inoculums (1.63 - 3.8×10^{8} CFU ml^{-1}) or spore suspensions (1.03-3.18×10^{8} spores ml^{-1}) for the tested bacterial or fungal isolates which incubated at 30°C on rotary shaker at 150 rpm for 8 or 10 days, respectively. The fermented medium was centrifuged (Sigma 2 - 16PK) at 10,000 rpm for 10 min or filtrated for bacterial or fungal culture, respectively, then pH value was measured using pH meter and phosphate concentration was determined in the supernatant. The amount of solubilized phosphate was also detected and RPSE% was calculated. All the experiments were carried out at least in triplicate **[21].**

Factors affecting solubilization of rock phosphate

Effect of fermentation period

This experiment was designed to detect the proper time for maximum RP solubilization on Pikovaskay's broth medium using shake flask. The previous procedures of propagation were used. During the fermentation period, samples (10 ml) were taken every day, then the phosphate concentration and pH value were determined, and the amount of phosphate content and RPSE% were calculated.

Effect of medium components on rock phosphate solubilization

Carbon sources

In order to evaluate the efficiency of tested isolates for RP solubilization as affected by different carbon sources, the bacterial and fungal isolates were cultivated Pikovskaya's medium supplemented with RP and containing the tested carbon sources. Therefore, the carbon source of Pikovaskay's broth medium supplemented with RP was replaced by seven sources (glycerol, sucrose, fructose, lactose, starch, maltose and mannitol) of carbon in equal amount to that present in the original medium in order to avoid the error which may resulted from the differences on carbon concentration in each source. The previous procedures of propagation and chemical analyses were used.

Glucose concentrations

Different concentrations of glucose were tested as a carbon source in Pikovskaya's medium in order to select best concentration that gives the highest rock phosphate solubility.Therefore, seven trails of glucose concentrations (2.5, 5, 7.5, 10, 12.5, 15 and17.5) were used

Nitrogen sources

The effect of different organic and inorganic nitrogen sources onRP solubilization were studied. The nitrogen sources applied were beef extract, yeast extract, tryptone, peptone, protose peptone, casein, urea, ammonium sulfate, ammonium chloride, sodium nitrate and potassium nitrate. The amount of nitrogen compounds added was calculated to give the same nitrogen concentration in Pikovaskay's medium.

Nitrogen sources concentrations

Different tryptone or yeast extract concentrations ranging between 0.5 to 2.5 gl^{-1}were added to in modified Pikovaskay's medium in order to select the best treatment for RP solubilization by tested isolates.

Use of some agro-industrial wastes and by-products

Some agro-industrial wastes such as bagasse, black strap cane molasses, corn cobs, olivecake, rice straw, sugar beet waste and whey were used to study their effects on RP solubilization by tested isolates. Some agro-industrial residues were replaced by equivalently to the original carbon amounts in modified Pikovaskay's medium. The previous procedures of propagation were used and phosphorus parameters were calculated at the end of fermentation period.

Analytical procedures

Viable counts of bacteria were determined by using plate count technique **[22]** and number of fungal spores was counted in the filtrate using Haemocytometer slide **[23]**Phosphorus content in fermented liqueur was determined by using the colorimetric molybdenum blue method suggested by **[21].**pH of the medium was recorded with a pH-meter model (Hanna211) equipped with glass electrode. Total sugar was determined by phenol–sulfuric acid method **[24].**

Calculations

Phosphate solubilization index (PSI) was determined by measuring the clear halo-zone diameter and the colony diameter, using the following formula according to **[17].** Phosphate solubilization efficient (PSE %) or rock phosphate solubilization efficiency (RPSE %) was calculated using the following formula according to equations according to **[18]**, respectively**.** The following formulas were used to calculate these parameters: PSI = (Colony diameter + Halo-zone diameter)/ (Colony diameter), PSE (%) on agar medium (plates) = (solubilization diameter (halo-zone))/ (Growth diameter) ×100 or/and RPSE (%) on broth medium= (soluble phosphate concentration)/ (total phosphate concentration) ×100.

Statistical analysis

The correlation coefficient was analyzed with Microsoft Office Excel 2013.

RESULTS AND DISCUSSION

Isolation and screening of phosphate solubilizing microorganisms (PSM)

A Total of 108 phosphate solubilizing microbial colonies were isolated on the Pikovskaya's agar medium, containing insoluble tri-calcium phosphate (TCP) from different sources,data illustrated by **Fig. (1a)** indicated that the highest figure of isolates number and percentage were obtained from rock phosphate soil (36 isolates) with 33.33% followed by isolates taken from soil (20 isolates with 18.51%), Egyptian clover (16 isolates with 14.82%) and rock phosphate mine (13 isolates with 12%). The lowest number of isolates was collected from broad bean, wheat and sugar cane being 8, 8 and 7 isolates with the isolate percentage of 7.41%, 7.41% and 6.51%, respectively. These isolates were classified into 3 categories (high, moderate and weak) according to their activity for phosphate solubilization, which exhibiting zone of phosphate solubilization (clear hole-zone around the microbial growth) ranged from 21 to 37 mm, from 8 to 20 mm and from 1 to 7 mm, respectively **(Fig. 1b)**. The highest number of these isolates was presented in second category (55 bacterial isolates with 50.9%) followed by that found in third and first categories being 41 isolates with 38.0%

and 12 isolates with 11.1%, respectively. These results are in agreement with those obtained by **[25], [26] and [27].** They stated that the production of clear hole-zone around the microbial growth on Pikovskaya's agar plates were considered as phosphate solubilization. **[28]** found that the maximum amount of P solubilization was obtained by *A. niger* followed by *Pencillium* sp. and *B. subtilis*. While, the lowest P solubilization was noted *with Micrococcus* sp.

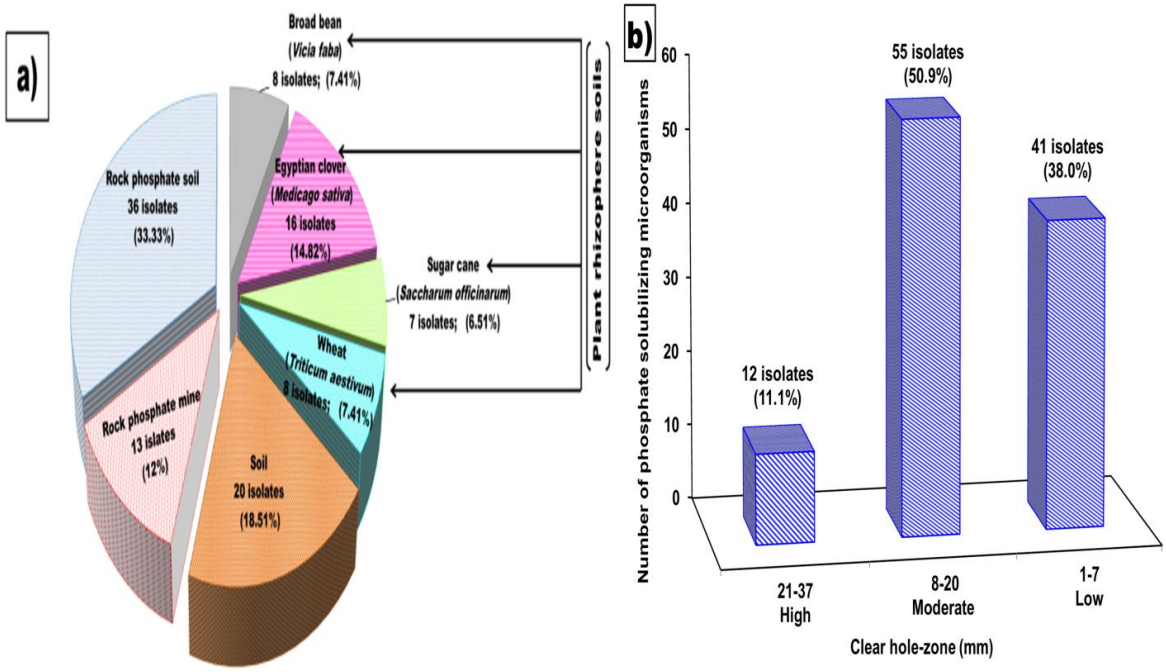

Fig 1: The numbers and percentage distribution of phosphate solubilizing microorganisms

a) according to their activity for P solubilizationon Pikovskaya's agar medium supplemented with 0.003% rose bengal at 30°C after 24 h.
b) different categories ofclear hole-zone (mm) of solubilized phosphate, produced by tested isolates.

Also, **[29]** stated that bacterial isolates from the rhizosphere soil exhibiting high phosphate solubilization ability in vitro.

Data clearly recorded in **Tables (1 & 2)**show that the range of phosphate solubilization index (PSI) and phosphate solubilization efficient (PSE%)after 24h of bacterial isolates varied from one source and other. The greatest range of PSI and PSE% ranged from 2.1 to 4.2 mm and from 110 to 320 % were recorded by bacterial isolates from rock phosphate soil (RS) followed by that obtained from rock phosphate mine (RM) (2.2 - 3.5 mm & 120 - 250%) and Egyptian clover rhizosphere soil (C) (2.1-3.2 mm & 112.5 - 216.7%). Results also, indicated that the highest phosphate solubilization index were obtained by Rs22 followed by Rs7 bacterial isolates which isolated from rock phosphate soil being 4.2 and 4.0 mm with efficiency of 320 and 300%, respectively. With respect to PSI and PSE of fungal isolates, data presented in **Table (2)** showed that the maximum phosphate solubilization index and efficiency were obtained by Bf6 followed by RPf10 fungal isolates being 2.3 mm & 126% and 2.3 mm &125%, respectively. These results are in accordance with **[27]** they noticed that the highest phosphate solubilization index value (PSI) which selected as qualitative activity ranged from 1.13 to 3.0 for 8 microbial isolates.

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Table 1: Qualitative and quantitative estimations of rock phosphate solubilization by different bacterial isolates on Pikovskaya's medium after 8 days at 30°C using shake flasks as a batch.

Bacterial isolates code	PSI (mm)	PSE (%)	RPs content (µgP ml^{-1})	RPSE (%)	Bacterial isolates code	PSI (mm)	PSE (%)	RPs content (µgP ml^{-1})	RPSE (%)	Bacterial isolates code	PSI (mm)	PSE (%)	RPs content (µgP ml^{-1})	RPSE (%)
Rs1	2.6	160.0	5.2	0.4	Rs33	2.5	150.0	5.2	0.4	W4	2.1	111.1	0.0	0.0
Rs2	2.2	116.7	0.1	0.0	Rs34	2.3	125.0	3.2	0.2	W5	2.2	116.7	0.0	0.0
Rs3	2.1	114.3	0.0	0.0	Rs35	2.8	175.0	6.8	0.5	W6	2.4	140.0	4.4	0.3
Rs4	2.2	120.0	2.4	0.2	Rs36	2.7	166.7	8.9	0.6	W7	2.3	125.0	3.9	0.3
Rs5	2.7	166.7	6.5	0.4	S1	2.1	111.1	0.0	0.0	W8	2.2	120.0	2.0	0.1
Rs6	2.8	180.0	9.1	0.6	S2	2.1	108.3	0.0	0.0	Su1	2.2	116.7	0.7	0.0
Rs7	4.0	300.0	12.4	0.8	S3	2.2	120.0	0.2	0.0	Su2	2.1	114.3	0.0	0.0
Rs8	2.1	110.0	0.0	0.0	S4	2.2	116.7	0.0	0.0	Su3	2.1	110.0	0.1	0.0
Rs9	2.1	111.1	0.0	0.0	S5	2.1	111.1	0.0	0.0	Su4	2.1	111.1	0.0	0.0
Rs10	2.6	157.1	7.7	0.5	S6	2.1	112.5	0.0	0.0	Su5	2.3	128.6	3.2	0.2
Rs11	2.1	114.3	0.0	0.0	S7	2.1	109.1	0.0	0.0	Su6	2.6	157.1	5.2	0.3
Rs12	3.6	260.0	10.0	0.7	S8	2.2	116.7	0.0	0.0	Su7	2.4	140.0	4.7	0.3
Rs13	2.6	160.0	7.8	0.5	S9	2.3	133.3	2.7	0.2	B1	2.2	120.0	3.1	0.2
Rs14	3.3	225.0	10.6	0.7	S10	2.2	120.0	1.0	0.1	B2	2.8	183.3	9.4	0.6
Rs15	2.2	116.7	0.0	0.0	S11	2.3	133.3	3.0	0.2	B3	2.7	171.4	8.2	0.6
Rs16	2.2	120.0	2.2	0.2	S12	2.8	175.0	7.4	0.5	B4	3	200.0	9.2	0.6
Rs17	2.3	133.3	4.2	0.3	S13	2.2	120.0	2.1	0.1	C1	2.7	160.0	6.4	0.4
Rs18	2.3	125.0	3.2	0.2	S14	2.8	175.0	8.4	0.6	C2	2.6	166.7	5.3	0.4
Rs19	2.6	160.0	8.0	0.5	S15	2.8	175.0	6.1	0.4	C3	2.2	120.0	2.4	0.2
Rs20	2.6	160.0	6.3	0.4	RM1	2.6	157.1	8.1	0.5	C4	2.3	160.0	4.6	0.3
Rs21	2.4	140.0	4.5	0.3	RM2	2.7	171.4	8.8	0.6	C5	3.2	216.7	10.7	0.7
Rs22	4.2	320.0	16.1	1.1	RM3	2.5	150.0	5.3	0.4	C6	2.5	150.0	5.9	0.4
Rs23	2.7	166.7	8.2	0.6	RM4	2.4	142.9	4.5	0.3	C7	2.2	120.0	1.3	0.1
Rs24	2.2	116.7	0.7	0.0	RM5	3.5	250.0	10.9	0.7	C8	2.6	160.0	7.5	0.5
Rs25	2.8	180.0	6.8	0.5	RM6	2.4	142.0	10.6	0.7	C9	2.2	116.7	0.0	0.0
Rs26	3.0	200.0	10.0	0.7	RM7	2.2	120.0	2.7	0.2	C10	2.8	175.0	7.5	0.5
Rs27	2.1	114.3	0.0	0.0	RM8	2.2	122.1	3.2	0.2	C11	2.7	171.4	6.1	0.4
Rs28	2.4	140.0	3.2	0.2	RM9	2.4	140.0	5.8	0.4	C12	2.3	125.0	3.3	0.2
Rs29	2.3	125.0	0.9	0.1	RM10	2.7	188.7	7.0	0.5	C13	2.1	112.5	1.2	0.1
Rs30	2.3	133.3	7.5	0.5	W1	2.3	125.0	0.6	0.0	C14	2.4	140.0	2.7	0.2
Rs31	3.0	200.0	10.3	0.7	W2	2.2	116.7	0.2	0.0	C15	2.2	120.0	0.9	0.1
Rs32	2.2	120.0	0.4	0.0	W3	2.1	114.3	0.0	0.0	C16	2.2	116.7	0.0	0.0

PSI = Phosphate solubilization index (mm)**, PSE =**Phosphate solubilization efficiency (%)**, RPSE**=Rock phosphate solubilization efficiency (%), **Rs** = isolates collected from rock phosphate soil, **S**= isolates isolated from soil, **RM**= bacteria isolates collected from from rock phosphate mine, isolates isolated from wheat, **B** = isolates isolated from broad bean. **C**= isolates collected from Egyptian clover, **Su**=isolates isolated from sugar cane.

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Table 2: Qualitative and quantitative estimations of rock phosphate solubilization by different fungal isolates on Pikovskaya's medium after 10 days at 30°C using shake flasks as a batch culture.

Fungal isolates code	PSI (mm)	PSE (%)	RPs content (µgP ml^{-1})	RPSE (%)
Sf1	2.2	117	43.7	2.9
Sf2	2.2	119	47.4	3.2
Sf3	2.2	121	50.7	3.4
Sf4	2.1	109	30.9	2.1
Sf5	2.0	104	43.8	2.9
Bf6	2.3	126	51.0	3.4
Bf7	2.2	116	23.9	1.6
Bf8	2.1	113	38.9	2.6
Bf9	2.1	104	29.4	2.0
RPf10	2.3	125	64.7	4.3
RPf11	2.1	113	22.8	1.5
RPf12	2.2	122	19.3	1.3

PSI = Phosphate solubilization index (mm), **PSE =** Phosphate solubilization efficiency (%), **Sf =** Fungal isolates isolated from soil, **Bf =** Fungal isolates isolated from broad bean,**RPf =** Fungal isolates collected from rock phosphate.

Whereas **[26]** observed that the most efficient phosphate solubilizer on medium supplemented with TCP showed a solubilization index ranged from 3.76 to 4.14.

Data also clearly show that the highest value of rock phosphate solubilization (RPs) content in liquid medium was obtained by bacterial isolates Rs22 followed by Rs7, RM5 and C5 being 16.1, 12.4, 10.9 and 10.7 µgP $ml^{-1}$with the rock phosphate solubilization efficiency (RPSE%) were 1.1, 0.8, 0.7 and 0.7 %, respectively **(Table 1)**. Also, it could be noticed that all bacterial cultures isolated from rock phosphate mine had the ability for rock phosphate solubilization in liquid culture whereas 20.8% of bacterial culture isolated from other sources failed to give RPs in shake flasks as a batch culture. The solubilization RP content in the liquid medium (Pikovskaya's medium) by different fungal isolates ranged between 19.3 to 64.7 µgP ml^{-1} after 10 days and RPSE % ranged from 1.3 to 4.3%, respectively. The highest figures of soluble RP content was recorded by RPf10 isolate followed by Bf6 being 64.7 and 51.0 µgP ml^{-1} with RPSE % of 4.31% and 3.44%, respectively. These results are in line with those obtained by **[30]** demonstrated that five Actinobacteria strains were different abilities to release soluble phosphate from rock phosphate (RP) ranged from 4.38 to 25.87 µgP ml^{-1}. **[28]** they found that liquid media inoculated with *A.niger* released more available phosphate than *B. subtilis*. In similar studies, four fungal isolates out of 145 isolates were selected by **[31]** as most efficient P solubilizing isolates showed the highest available phosphate in liquid medium (ranged from 2.75 to 3.01 mg P_2O_3 ml^{-1}) without significant different.

From the previous results, it could be noticed that the bacterial isolates were more efficiency for phosphate solubilization on solid culture than fungal isolates whereas the vice versa was true for submerged culture. Where the most efficient RP solubilizing isolates were bacteria Rs22 & Rs7 and fungiBf6 & RPf10.Therefore, these isolates were selected for further studies as RP solubilizing isolates.

Identification of the most efficient phosphate solubilizing isolates

The most efficient bacterial isolates Rs22 and Rs7 were subjected to identification using their morphological, cultural and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology **(Brenner*et al,* 2005).** Both isolates were rod-shaped cells, occurring singly, Gram negative, non-acid fast, non-spore forming, motile and facultatively anaerobic. Colonies were irregular, white or creamy, no hydrolysis of blood agar. Optimum pH.7 and pH range for growth were 6 to 8.Growth is optimal at NaCl up to 7%. Both isolates were fermented D-glucose, D-mannitol, sorbitol, lactose, sucrose and fructose. Positive results were attained for catalase, methyl red, Vogas-Proskauer, citrate utilization and gelatin hydrolysis whereas isolate Rs22 hydrolyzed of gelatin higher than isolate Rs7. Negative results were observed for nitrate reduction and urea hydrolysis. Both isolates gave the characteristics similar to genus *Serratia*. With respect to the most efficient phosphate solubilizing fungal isolates Bf6 and RPf10, their morphological properties were

subject to the preliminary classification to be *Aspergillus* genus according to **[20].** Both isolates showed granular colonies on Czapek' sDox agar. The colonies were flat, with radial grooves. Microscopic observation of a fungal isolates, indicated erect conidiophores with globose vesicles bearing chains of conidia. In this respect, the most efficient phosphate solubilizing fungal isolates (4 isolates) selected by **[31]** were also characterized as *Aspergillus*sp**.**

Some factors affecting on RP solubilization

Fermentation time

Data illustrated by **Fig.(2)** revealed that the RP solubilization activity as content and efficiency of the most efficient tested bacterial (*Serratia* Rs7 & Rs22) and fungal isolates (*Aspergillus*sp. Bf6 & RPf10) were increased gradually during the fermentation periods and reached to maximum peak after 8 and 10 days, respectively, then decreased with fermentation period increased.At this periods,the content of RP solubilization was 12.42, 16.02, 51.60 & 64.40 µgP $ml^{-1}$with efficient (RPSE) 0.78, 1.00, 3.44 &4.45 % and drop in pH to 4.58, 4.45, 3.84 & 3.74 for *Serratia* sp.Rs7, *Serratia* sp.Rs22, *Aspergillus*sp. Bf6 and *Aspergillus*sp.

RPf10, respectively. There was significant correlation ($P \leq 0.05$) between fermentation period and each of RP solubilization, RPSE and decrease in pH of tested bacteria and fungi, where r values ranged between 0.63 &0.93, 0.65& 0.95 and -0.64 &- 0.99, respectively. These results are in line with those obtained by **[32]** who found that *A. niger* gave the highest amount of RP solubilization after 9 days of incubation period..Moreover, **[33]** observed that the highest content of P solubilization was reached up to 7 days of incubation by *Pseudomonas lurida*. From the foregoing results, it could be noticed that, 8 or/and 10 days fermentation period resulted to the highest RP solubilization for tested *Serratia* or/and *Aspergillus*, so this period will be applied in further experiments.

Different carbon sources

Data in **Fig. (3a,b)** clearly show that the highest values of RPs and RPSE obtained by *Serratia* sp. Rs7 and *Aspergillus* sp. RPf10 were noticed on glucose medium followed on sucrose and glycerol being 12.42µgP ml^{-1} & 0.78 %, 10.79 µgP ml^{-1}& 0.72% and 10.09 µgP ml^{-1} &0.67% for first isolate and being 64.40 µgP ml^{-1} & 4.45%, 60.91 µgP ml^{-1}& 4.06% and 58.8 µgP ml^{-1} and 3.92% for second isolate, respectively. Whereas the highest figures of these parameters recorded on glucose medium by both *Serratia* sp. Rs22 and *Aspergillus* sp. Bf6, followed by sucrose and mannitol. At different carbon source treatments, *Serratia* sp. Rs22 gave higher RP content than *Serratia* sp. Rs7. Also, *Aspergillus* sp. RPf10 gave the same trend and recorded RPSE% higher than *Aspergillus* sp. Bf6. Moreover, higher phosphate content and RPES% were obtained by fungal isolates than bacterial isolates.On the other hand, the lowest figures of rock phosphate solubilization content and efficiency of tested isolates were observed on starch followed by lactose.The acidification of the fermented cultures was varied from tested organisms to other at different carbon sources. The pH dropped from 7.0 or 6.0 to record the lowest values at the end of fermentation period which ranged from 4.45 to 6.14 and from 3.74 to 4.74 for bacterial and fungal isolates at different carbon sources, whereas the lowest value was observed on glucose medium by all tested isolates. The solubilization activity of microorganism is related to its organic acid production important which is dependent on the carbon source supplied **[34] and [35]** have reported such bacterial isolates which solubilize only in presence of glucose. In similarstudies, glucose was the best carbon source followed by sucrose for P solubilization by *A.tubingensis***[36]**, *P. purpurogenum* **[37]** and *Ps.lurida* **[33].** The pH dropped from neutral to acidic at all carbon sources in the presence of RP from 7.5 to 4.3 was observed by **[36]** when glucose was used as a carbon source. Whereas the lowest pH value of 5.9 was reported by **[33].** They also added that sugar could act as energy source.

Results of the effect of different glucose concentration on RP solubiziation were illustrated in **Fig.** (**4 a,b)**. A gradual increase in RPs content and RPSE% was attained by all tested isolates with increase of glucose concentration reaching a maximum at 10.0 gl^{-1} for bacterial isolate after 8 days being 12.40 µgP ml^{-1} & 0.83% and 16.22 µgP ml^{-1} & 1.08 % for *Serratia* sp. Rs7 and Rs22 which recorded the lowest pH being 4.75 and 4.81, respectively **(Fig. 4 a)**. With respect to fungal isolates, *Aspergillus*sp. Bf6 and *Aspergillus*sp. RPf10 gave the maximum RP solubilization after 10 days at 12.5 and 15 gl^{-1} glucose, repectively**(Fig 4 b).** Moreover, the second fungi recorded the higher RPs content and RPSE% (72.07 µgP ml^{-1}& 4.71%) than the first fungi (66.14 µgP ml^{-}

[1]&4.21%) which recorded the lowest pH values being 3.75 and 3.83, respectively. Increasing the glucose concentration higher than the previous concentrations led to decrease in RP solubiziation activity and increase the final pH values by tested isolates. Similar inverse relationship between pH and soluble phosphate was reported earlier by **[38]** The statically analysis of the pervious data revealed that the correlation coefficient (r) between glucose concentrations and each of RPs content & RPSE was a high positive (r ranged from 0.71 to 0.93) and pH was a high negative (r ranged from -0.72 to -0.85) for all tested isolates, excepted *Serratia* sp. Rs22 was low negative correlation coefficient (r) for RPs content and RPSE being -0.23 and -0.25, respectively. The aforementioned results are in line with those obtained by **[33] and [39].** They reported that the content of phosphate solubiziationby *Ps. lurida, B. subtilis, Enterobacter* spp. and *Bacterium* sp. was detected in medium containing 10 gl^{-1} glucose as a sole carbon source. Also, **[40]** found that highest solution Pi concentration by *Mortierella* sp. was obtained with 10 gl^{-1} glucose (118.26 mgl^{-1}),glucose concentration below above this level produced a solution Pi concentration significantly lower.

Different nitrogen sources

Generally, it could be stated that the organic nitrogen sources (8 sources) gave the higher rock phosphate solubilization by all tested isolates than inorganic sources (4 sources) as illustrated by **Fig. (5 a, b)**. The highest figures of RPs content and RPSE% being 14.86 µgP ml^{-1}& 0.99 % and 17.84 µgP ml^{-1}& 1.19 % were obtained by *Serratia* sp. Rs7 andRs22, respectively after 8 days on Pikovskaya's medium containing tryptone as sole nitrogen source.

Also, it could be noticed that tryptone was better than mixture of yeast extract and ammonium sulfate (control) for RP solublization by tested both bacteria *Serratia* sp. Rs22 and Rs7 (by increasing about 12 and 27 %more than control).Whereas yeast extract only led to increase the RP content to 76.43 µgP ml^{-1} and 87.95 µgP ml^{-1} by *Aspergillus* sp. Bf6 and RPf10 (16 & 23 % more than control), respectively after 10 days incubation periods at 30°C. At all these treatments of yeast extract and tryptone the lowest pH values being 3.59, 3.69, 4.66 and 4.74 were recorded by *Aspergillus* sp. RPf10, *Aspergillus* sp. Bf6,*Serratia* sp. Rs22 and *Serratia* sp. Rs7, respectively. Whereas the lowest RPs activity was attained by all tested isolate in medium supplemented with potassium nitrate as a nitrogen source.

This result has been reported previous by **[41].** The favorable effect of yeast extract or tryptone in increasing RP solublization by tested isolates could be interpreted on the basis that yeast extract or tryptone serves not only as a nitrogen sources but also as a source of growth factors which play an important role in enhancement the microbial growth and solubilization of phosphate. Therefore, the tryptone and yeast extract were chosen for the further studies. Obtained results are generally in agreement with those obtained by **[42]** who used yeast extract as a nitrogen source for phosphate solubilization by *Pseudomonas* sp. while disagree with the results of **[43],** they reported that $(NH_4)_2SO_4$ followed casein were the best nitrogen sources for P solubilization byphosphate-solubilizing bacteria (PSB). In addition, **[33]** noticed that inorganic nitrogen sources proved better than organic one, which found that ammonium sulphate was the best nitrogen source for P solubilization by *Pseudomonas lurida.*Different concentrations of tryptone or yeast extract ranged from 0.5 to 2.5 gl^{-1} were tested for maximize RPSE% by the tested bacterial and fungal isolates. Data presented in **Fig (6 a)** show that optimum concentration of tryptone was 1.25 gl^{-1} which gave the highest content of RP solubilization (14.83 and 17.70 µgP ml^{-1}) and RPSE (0.99 and 1.18%)after 8 days of fermentation leading to pH drop to 4.72 and 4.63,respectively. Also, data in **Fig. (6 b)**indicated that 1.5 gl^{-1} yeast extract gave the highest content of P solubilization by *Aspergillus* sp. Bf6 isolate was 82.30 µgP ml^{-1} with 5.49% of RPSE and the lowest value of pH was 3.52. While, 2 gl^{-1} yeast extract was the best concentration for *Aspergillus*sp. RPf10 followed by 1.5 gl^{-1} yeast extract giving 96.12 and 93.12 µgP ml^{-1} with 6.41 and 6.21% RPSE after 10 days as well as the pH value reduction to 3.03 and 3.31, respectively. Also, it could be noticed that the content of RP solubilization by *Aspergillus* sp. Bf6 and RPf10increasedby 6.16 and 7.94% in medium supplemented with 1.5 and 2.0 gl^{-1}yeast extract compared to control (1.25gl^{-1} yeast extract).These results are in disagreement with those of **[42]**found that increasing the Concentration of yeast extract to more than 0.5 gL^{-1} resulted the reduction of phosphate solubilization whereas the addition of yeast extract at 0.1 gl^{-1} led to increase the solubilization of phosphate by *Pseudomonas* sp.

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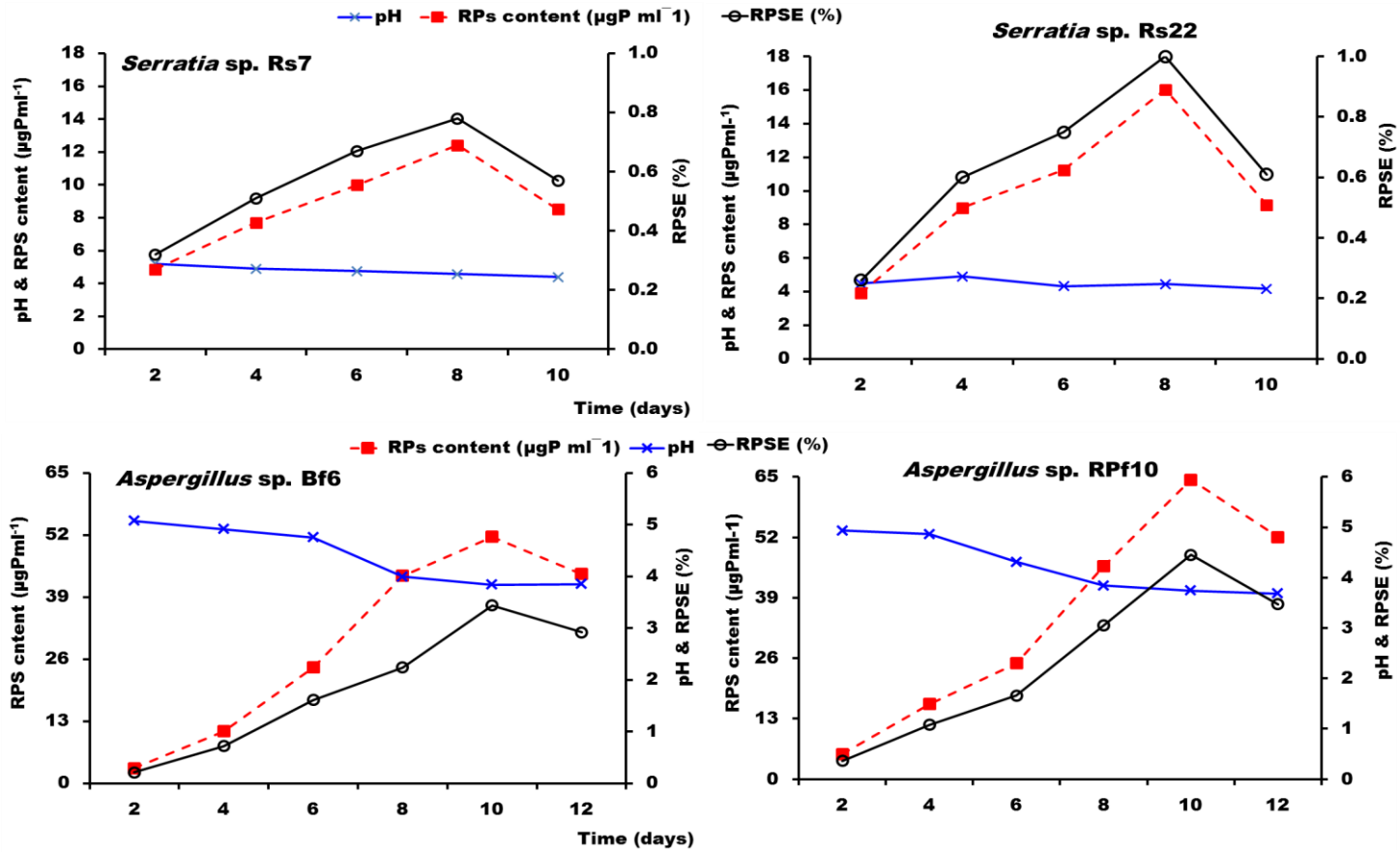

Fig 2: RP solubilization activity (content and efficiency) of the most efficient isolates on broth Pikovskaya's medium during 10: 12 days of incubation periods at 30°C using shake flasks as a batch culture.

RPs content = Rock phosphate solubilization content, **RPSE %** = Rock phosphate solubilization efficiency %.

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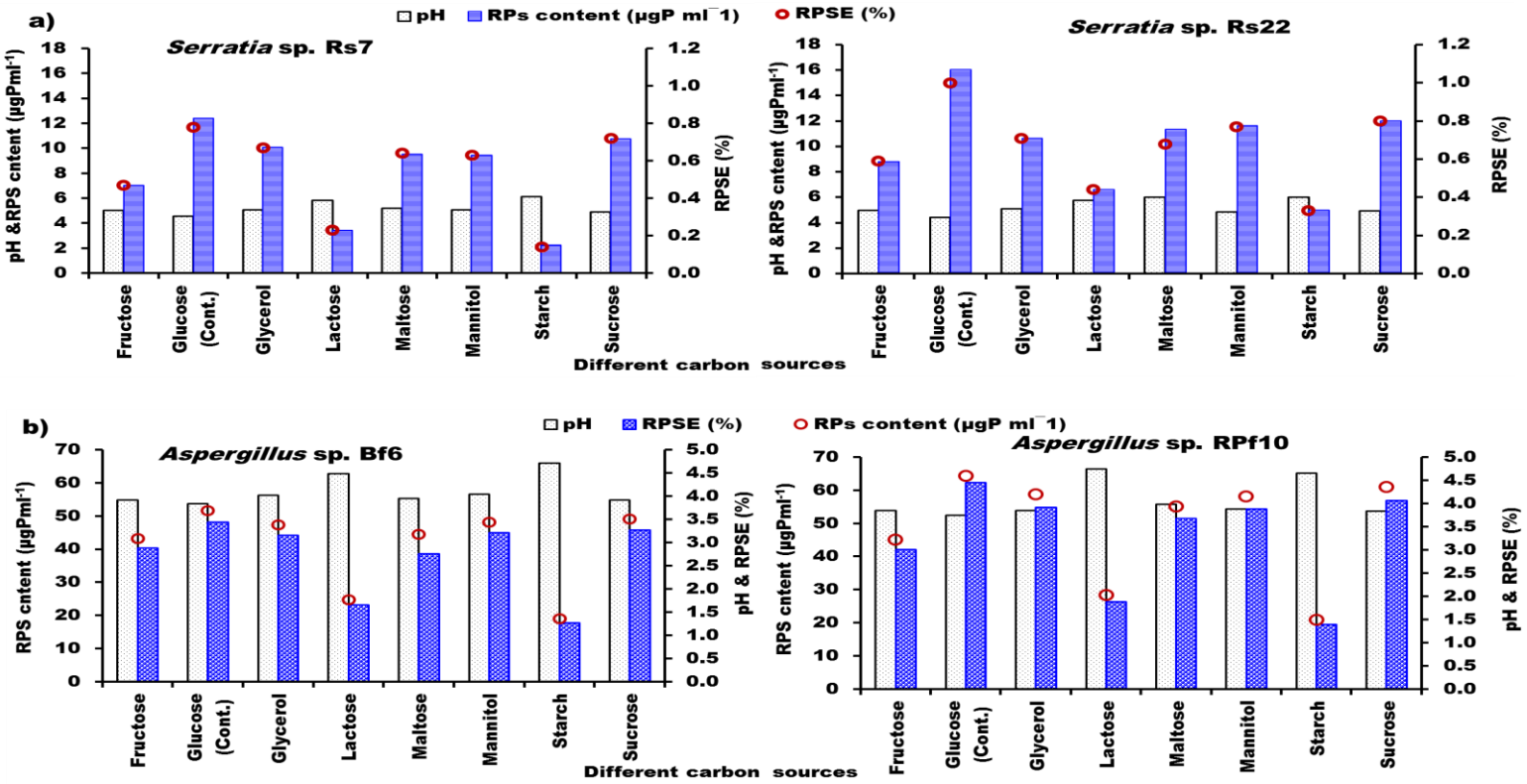

Fig 3: Effect of different carbon sources on RP solubilization activity (content and efficiency) by tested isolates at 30°C on Pikovskaya'sbroth medium using shake flasks as a batch culture.

a) Bacterial isolates*Serratia* sp. Rs7 and Rs22 after 8 days. b) Fungal isolates*Aspergillus*sp. Bf6 and RPf10 after 10 days.
RPs content = Rock phosphate solubilization content, **RPSE =** Rock phosphate solubilization efficiency, Cont= Control.

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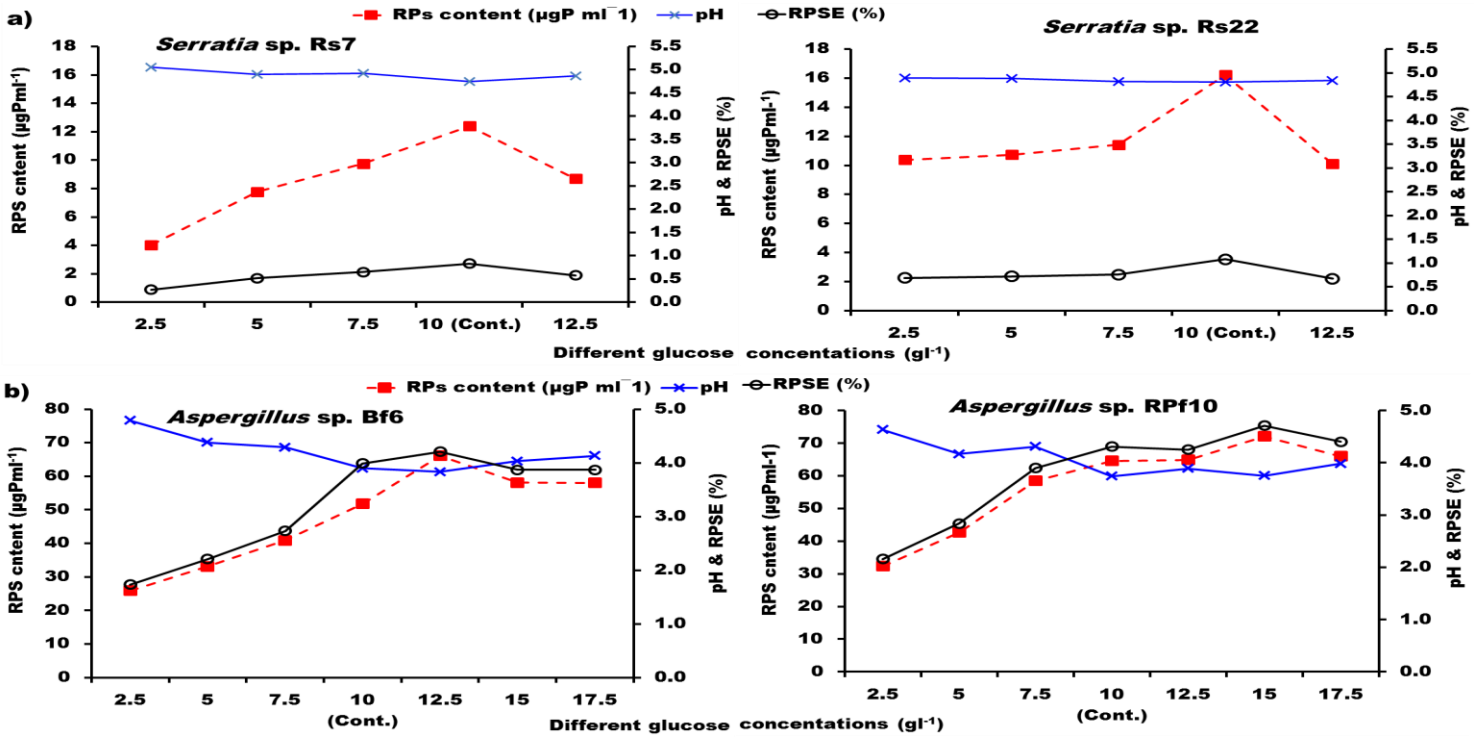

Fig 4: Effect of different glucose concentrations (gl^{-1}) on RP solubilization activity (content & efficiency) by tested isolates at 30°C on Pikovskaya's medium using shake flasks as a batch culture.

a) Bacterial isolates *Serratia* sp. Rs7 and Rs22 after 8 days. b) Fungal isolates *Aspergillus* sp. Bf6 and RPf10 after 10 days.
RPs content = Rock phosphate solubilization content, **RPSE =** Rock phosphate solubilization efficiency, Cont= Control.

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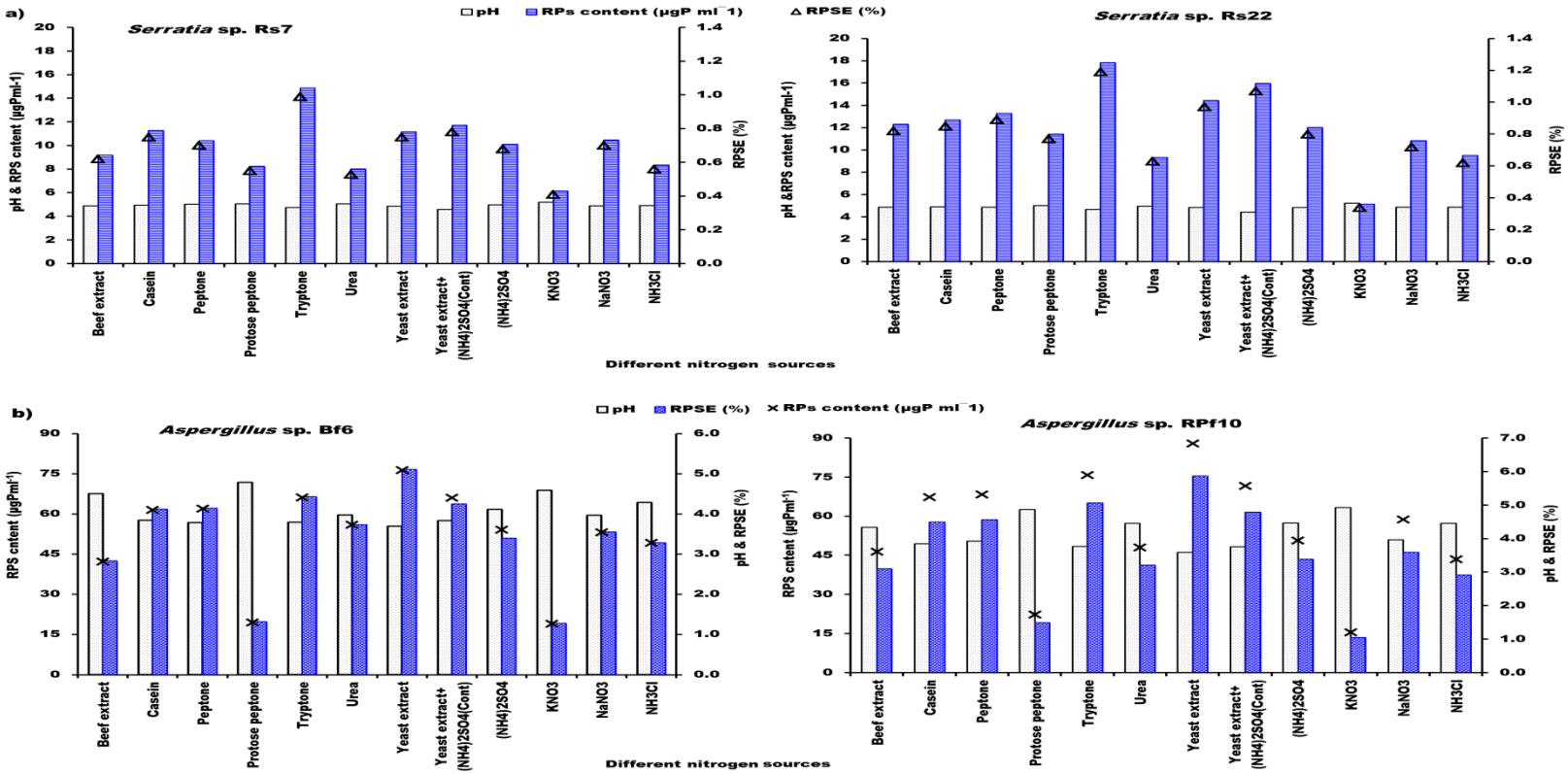

Fig 5: Effect of different nitrogen sources on RP solubilization activity (content & efficiency) by tested isolates at 30°C on Pikovskaya'smedium using shake flasks as a batch culture.

a) *Bacterial isolates Serratia* sp. Rs7 and Rs22 after 8 days. b) Fungalisolates *Aspergillus*sp. Bf6 and RPf10 after 10 days.
RPs content = Rock phosphate solubilization content, **RPSE =** Rock phosphate solubilization efficiency, Cont= Control.

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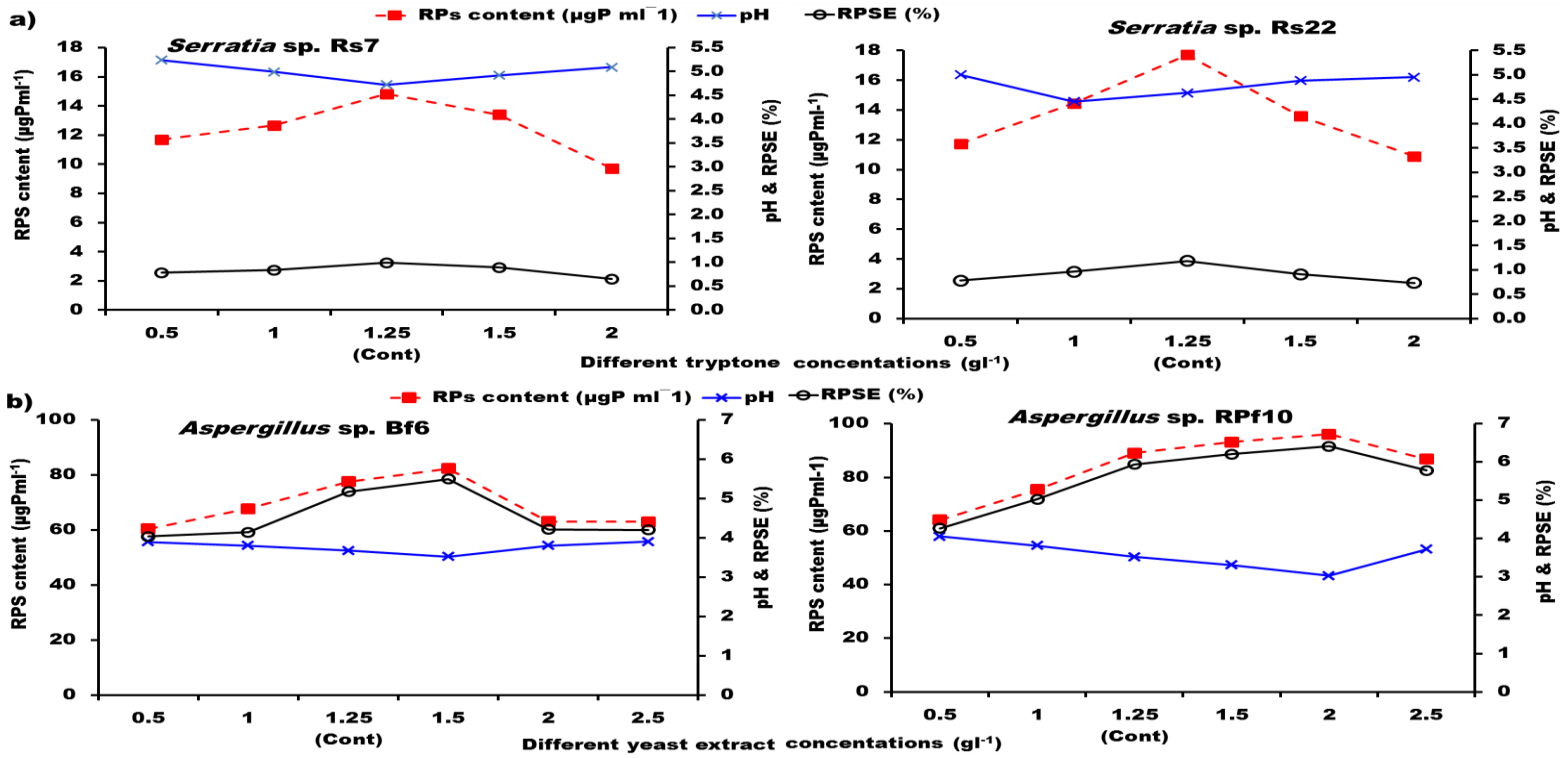

Fig 6: Effect of different concentrations ofthe proper nitrogen sources (gl^{-1}) on RP solubilization activity (content & efficiency) by tested isolates at 30°C on Pikovskaya's medium using shake flasks as a batch culture.

a) Bacterial isolates*Serratia* sp. Rs7 and Rs22 after 8 days. b) Fungal isolates *Aspergillus*sp. Bf6 and RPf10 after 10 days.
RPs content = Rock phosphate solubilization content, **RPSE =** Rock phosphate solubilization efficiency, Cont= Control.

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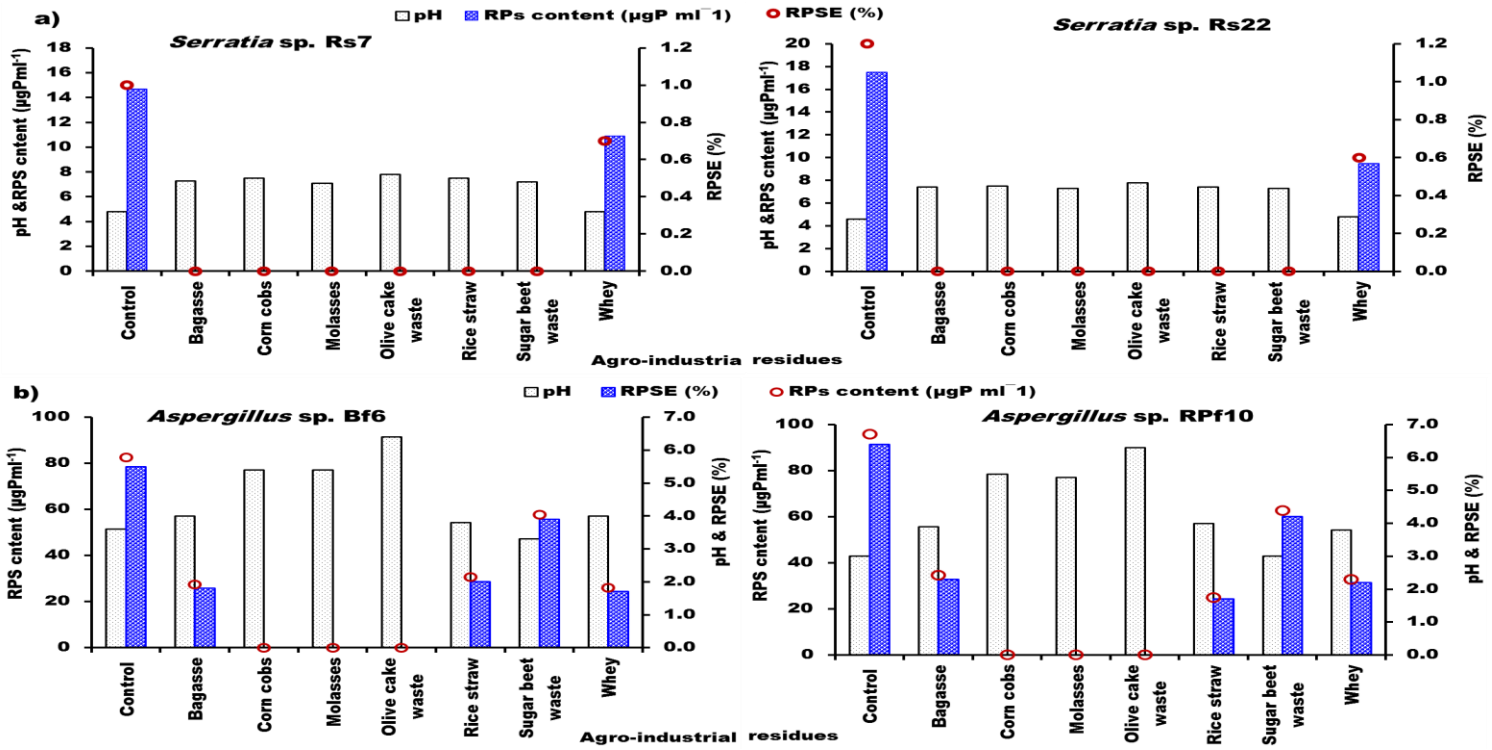

Fig 7: pH and RP solubilization activity (content & efficiency) of tested isolates as influenced by some agro-industrial residues at 30°C using shake flasks as a batch culture.

a) Bacterial isolates *Serratia* sp. Rs7 and Rs22 after 8 days. b) Fungal isolates *Aspergillus*sp. Bf6 and RPf10 after 10 days.
RPs content = Rock phosphate solubilization content, **RPSE =** Rock phosphate solubilization efficiency.

From the previous data, it could be concluded that, using the Pikovskaya's medium after replacing the mixture yeast extract and ammonium sulphate with yeast extract at 1.5 gl^{-1} and 2.0 gl^{-1} as well as containing glucose concentration at 12.5 and 15 gl^{-1}led to increase the RPScontent about 59% and 49% by *Aspergillus* sp. Bf6 and RPf10, respectively. Whereas the modified Pikovskaya's medium containing 10 gl^{-1} glucose and 1.25 gl^{-1} tryptoneled to increase P solubilization about 19% and 10% by *Serratia* sp. Rs7 and *Serratia* sp. Rs22, respectivelycomparing to control (10 gl^{-1} glucose+ 0.5 gl^{-1} yeast extract+ 0.5 gl^{-1} ammonium sulphate). So, it could be stated that these modification Pikovskaya's media could be more favorable than basal medium for RP solubilization by all the tested microorganisms.

Use of some agro-industrial residues

seven wastes i.e. bagasse, corn cobs, black sugar cane molasses, olive cake wastes, rice straw, sugar beet waste and whey were containing total sugar being 19.20, 48.30, 39.60, 47.55, 42.20, 50.0 and 4.60 % (not show)and tested for RP solubilization by the most efficient P solubilization isolates *Serratia* sp. Rs7, *Serratia* sp. Rs22,*Aspergillus*sp. Bf6 and *Aspergillus*s p. RPf10as a sole carbon sources on modified Pikovskaya's medium using shake flasks as a batch culture. Using all tested wastes as carbon source of modified Pikovskaya's medium led to frustration of RP solubilization by *Serratia* sp. Rs7 and Rs22 except whey as shown in **Fig. (7a)**. Whey showed positive impact on RP solubilization content by both *Serratia* sp. Rs7 and Rs22 being 10.9µgP $ml^{-1}$with 0.7 % of RPSE and 9.5 µgP $ml^{-1}$with 0.6 % ofRPSE.Whereas the highestfigure of fungal RP solubilization activity as influenced by agro-industrial wastes can be arranged in the following order: sugar beet waste >rice straw> bagasse > whey for *Aspergillus* sp. Bf6 and sugar beet waste > bagasse > whey > rice straw for *Aspergillus*sp. RPf10**(Fig. 7 b)**. Whereas the frustration of RP solubilization was noticed in presence of corn cobs, black strap sugar cane molasses and olive cake waste. The failure of the some wastes used to support RP solubilization may due to the insufficient nutrients or to the presence of some inhibitors such as hydroxyl methyl furfural in molasses **[44].** These previous results are in line with those obtained by **[45], [46[& [47]**and **[48]** they found that *A. niger* and *A. fumigates* had the ability to solubilization of rock phosphate when grown on media supplemented with sugar beet wastes and olive cake wastes. Also, **[49]** found that *A. niger* was capable of solubilization part of the P present in the RP using sugar cane bagasse.

CONCLUSION

Several bacterial and fungal isolates were isolated from different rhizosphere plants, rock phosphate and soil and tested for their ability for RP solubilization. All fungal isolates were more efficient for phosphate solubilization than bacterial isolates and recorded the highest degree of solubilization. Among these isolates 4 isolates namely,Rs22, Rs7, RPf10 & Bf6 were found to given the highest values of P solubilization in solid and in liquid Pikovskaye's medium after 8:10 days at 30°C using shake flasks as a batch culture. Some nutritional conditions (carbon and nitrogen sources) were done to optimize RP solubilization by tested *Serratia* and *Aspergillus*. The modified Pikovskaye's medium supplemented with glucose (as cabon source) and tryptone or yeast extract (as nitrogen sources) were more favorable than basal medium for RP solubilization by tested isolates, as it increased about 1.1- 1.5 fold than that solubilized in basal medium (control).Also, these isolates were capable to produce the fermented solution with highest P solubilization as rock phosphate in medium containing the whey or sugar beet waste (agro-industrial carbon residues)as sole carbon source using shake flasks as a batch culture.

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