

Phosphate Solubilization in Vitro By Some Soil Yeasts

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إذابة الفوسفور في المختبر بوساطة بعض خمائر التربة

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جمعت عينات التربة من المملكة العربية السعودية وعزلت الخمائر التالية:

Candida tropicalis, *Geotrichum capitates*, *Geotrichum candidum*,
Rhodotorula minuta and *Rhodotorula rubra*.

أجريت تجربة معملية لدراسة دور خمائر التربة في إذابة فوسفات الكالسيوم غير الذائبة. ووجد ان أكبر كمية فوسفور (45 ميكروجرام / مل) قد تكونت بوساطة الخميرة *Geotrichum capitatum*. بينما ظهر أقل تركيز للفوسفور عند استخدام كل من *Geotrichum candidum* و *Rhodotorula minuta* والتي بلغت 2 ميكروجرام / مل ، 4 ميكروجرام / مل على التوالي في نهاية فترة التحضين. أدت إذابة الفوسفور الى انخفاض في درجة حموضة الوسط البيئي.

Keywords: soil, yeasts, biomass, phosphate solubilization.

ABSTRACT

Soil yeasts including, *Candida tropicalis*, *Geotrichum capitatum*, *Geotrichum candidum*, *Rhodotorula minuta* and *Rhodotorula rubra* were isolated from soils of Saudi Arabia. The ability of these soil yeasts to solubilize insoluble calcium phosphate

$\text{Ca}_3(\text{PO}_4)_2$ *in vitro* was investigated. An incubation study was conducted to determine the role of selected soil yeasts on the solubilization of insoluble calcium phosphate. The largest amount of phosphate (45 $\mu\text{g/ml}$) was formed by the yeast of *G. capitatum*. But the soil yeasts *G. candidum* and *R. minuta* produced the lowest amount of phosphate with 2 $\mu\text{g/ml}$ and 4 $\mu\text{g/ml}$ respectively at the end of the incubation period. Solubilization of calcium phosphate led to a marked reduction in the pH of the medium.

Introduction

Phosphorus is an essential element in all living systems, and is also limiting nutrient in nutrition and production of most plants. Microbial activity in the soil is important in the cycling of phosphorus.

A wide variety of heterotrophic fungi and bacteria have been shown to be capable of solubilizing insoluble phosphate [1,2]. In fact, the ability of fungi and heterotrophic bacteria to solubilize insoluble phosphate is well documented [1, 2, 3, 4, 5].

Although soils are known to contain yeasts, little is known about their ecology and the role which they play in mineral cycling. This lack of interest probably reflects the low population density and relatively small biomass of yeasts in most soils [6, 7]. Data on phosphate solubilization *in vitro* by soil yeasts are scarce or lacking, although such results would be useful as an essential prerequisite to determining the role of soil yeasts in mediating this transformation in soil.

It has been found that the soil yeast *Williopsis californica* is able to oxidize ammonium sulphate to nitrate via nitrite and it could also solubilize insoluble phosphate [8].

The present investigation was undertaken to study the role of selected soil yeasts, isolated from soils of Saudi Arabia, on the solubilization of insoluble calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$. The biomass of these soil yeasts and changes in medium pH were also investigate

Materials and Methods

Isolation of soil yeasts

Soil yeasts were isolated from a sandy soil (total C, 0.3 %; total N, 0.1 %; pH, 7.2, obtained from Riyadh, central region, Saudi Arabia). The yeast strains included, *Candida tropicalis* (Cast.) Berkhout, *Geotrichum candidum* Link, *Geotrichum capitatum* (Diddens & Lodder) V. Arx, *Rhodotorula minuta* (Saito) Harrison var. *texensis* and *Rhodotorula rubra* (Demme), which were isolated and identified according to the method described by Van der Walt [9].

Media and Culture

The basal medium used in this study was Czapek-Dox liquid medium for the cultivation of the soil yeasts. Suspensions (1 ml) containing 1.4×10^5 yeast cells were used to inoculate liquid Czapek Dox medium (100 ml in 250 ml capacity Erlenmeyer flasks), adjusted to pH 6.0 with 2N NaOH. The medium was then amended with calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$ (final suspension weight 1.0 % w/v).

The flasks were placed in shaking incubator (100 rpm) at 25°C for 4 weeks. After each week three flasks were removed and the contents were filtered through pre-dried and pre-weighed Whatman No.1 filter papers. Yeast biomass was determined in the medium after filtration. The weight of yeast cells retained by the filter papers was then determined (after drying to constant weight at 80°C for 24 h) as a measure of yeast cell biomass. This value was subtracted from an uninoculated control flask containing media plus calcium phosphate. Flasks were set up in triplicate and uninoculated flasks were included to account for any non-biological phosphate solubilization.

Phosphate concentration was determined colorimetrically according to Hesse [10]. The pH of the medium was measured with a glass electrode.

Statistical analysis

A Minitab-for-Windows program was used in statistical analysis for all results obtained in this investigation. Both t-test and correlation coefficients (r) were calculated to evaluate the variance effects of all treatments (Table 1). The t-test was used for determining if significance exists between biomass of the soil yeasts and phosphate solubilization or medium pH. The primary purpose of using correlation coefficients was to establish whether the data sets were correlated or uncorrelated with each other. Analysis of variance (ANOVA Oneway Unstacked) was used to calculate t-tests; basic statistics (correlation) was used for correlation coefficients.

Table 1: Correlation coefficients (r) and t-test values (t) between biomass, pH and soluble phosphate (r_{xy}), t ; * Significant, ** Highly significant $P < 0.01$.

| Yeasts biomass | Soluble phosphate | | pH | |
|----------------------|-------------------|------|---------|------|
| | r | t | r | t |
| <i>C. tropicalis</i> | 0.72** | 2.65 | -0.45 | 3.10 |
| <i>G. candidum</i> | 0.36 | 1.19 | -0.21 | 1.04 |
| <i>G. capitatum</i> | 0.95** | 5.67 | -0.92** | 6.07 |
| <i>R. minuta</i> | 0.43 | 1.87 | -0.23 | 1.11 |
| <i>R. rubra</i> | 0.90** | 5.54 | -0.83** | 5.78 |

Theoretical procedures of statistical analysis were done according to Snedecor and Cochran [11].

Results and Discussion

The soil yeasts isolated from Saudi Arabian soil were identified as *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum*, *Rhodotorula minuta* and *Rhodotorula rubra*.

Figure 1 shows that *C. tropicalis*, *G. capitatum* and *R. rubra* solubilized insoluble calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) liberating phosphate HPO_4^{2-} into the medium. The largest amount of phosphate (45 $\mu\text{g/ml}$) was formed by the yeast of *G. capitatum* followed by *R. rubra* yeast with 38 $\mu\text{g/ml}$. While the soil yeasts *G. candidum* and *R. minuta* reached the lowest amount of phosphate with 2 $\mu\text{g/ml}$ and 4 $\mu\text{g/ml}$ respectively at the end of the incubation period.

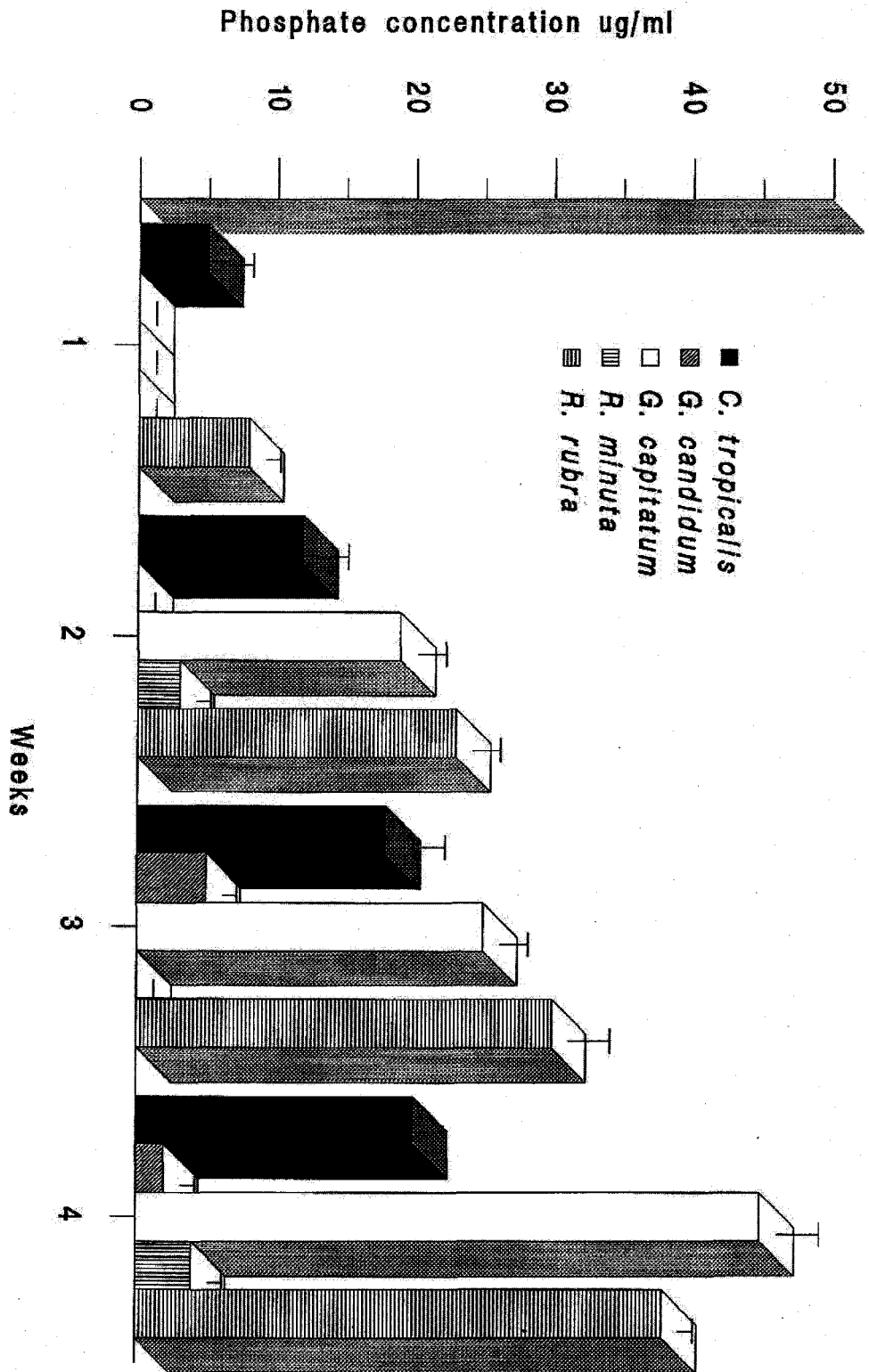


Fig. 1. Solubilization of calcium phosphate (1% w/v) by the soil yeasts, all values are means of triplicates \pm SD.

The *C. tropicalis* yeast showed an average of phosphate solubilization (20 µg/ml). Moderate biomass of soil yeasts was observed following phosphate supplementation (Figure 2). The yeasts biomass in a medium amended with phosphate was ranged between 0.2 to 0.6 g. The highest amount of biomass was produced by *G. capitatum* followed by *R. rubra* yeast with 0.55 g. Moderate biomass was exhibited by *C. tropicalis* and *G. candidum* with 0.3 g and 0.25 g respectively at the end of the incubation period.

The phosphate solubilization process was associated with a reduction in the pH of the medium (Figure.3). The lowest pH value was observed in the case of *G. capitatum* (pH 2.0) after four weeks of an incubation period which showed the largest amount of biomass and phosphate solubilization. On the other hand, both of *G. Candidum* and *R. minuta* exhibited the highest pH values (pH 4.8 and 5.0 respectively) and they reached the lowest amount of phosphate and biomass production. However, at a low pH values other elements may become unavailable for plant nutrition.

Yeasts biomass was increased during phosphate solubilization process. Correlation coefficients (r) and t-test values (t) between biomass, pH and soluble phosphate are presented in Table 1. Overall, with exception of *G. candidum*, yeasts biomass was found to be correlated with soluble phosphate production. Phosphate solubilization was highly correlated with the biomass of *G. capitatum*, *R. rubra* and *C. tropicalis*. On the other hand, phosphate solubilization was negatively correlated with the medium pH. Hence the soluble phosphate HPO_4^{2-} production increased with decreasing pH values.

The soil yeast isolated solubilized insoluble phosphate *in vitro* leading to the formation of large amounts of soluble phosphate. All yeasts, failed to form soluble phosphate until the 2nd week, after which time the concentration of the ion increased as yeast biomass increased.

Many heterotrophic bacteria and filamentous fungi liberate phosphate from insoluble phosphates *in vitro* and are also thought to play a role in this process in soils; again the potential role of soil yeasts in this process has been largely overlooked, although Katznelson *et al.*, [12] isolated from soil a species of *Candida* capable of phosphate solubilization. Phosphate solubilization by heterotrophs is generally thought to result from the production of organic acids, including citrate and 2-ketogluconate, although chelating agents may also play a role [5]. Some soil yeasts, including *Geotrichum candidum*, *Geotrichum capitatum* and *Williopsis californica* are able to produce extracellular enzymes [13]. This study clearly shows that at least three soil yeasts can achieve substantial rates of phosphate solubilization *in vitro*.

It is difficult, using the currently available literature, to make meaningful comparisons of the relative activity of fungi in the processes studied here. In fact, these reports were based on studies employing various growth media and incubation conditions. As a generalization however, it appears that the five yeasts used here are less effective at solubilizing insoluble phosphates [12, 14] *in vitro* than are filamentous fungi such as species of *Aspergillus*, *Fusarium* and *Penicillium*.

In conclusion, the soil yeasts *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum*, *Rhodotorula minuta* and *Rhodotorula rubra* solubilized insoluble phosphates. While such *in vitro* studies do not provide direct evidence that soil yeasts can mediate these processes when growing in soil, they indicate that yeasts have the potential to participate in such nutrient transformations. This process appears to involve the production of organic acids and/or chelating agents [3, 15, 16, 17].

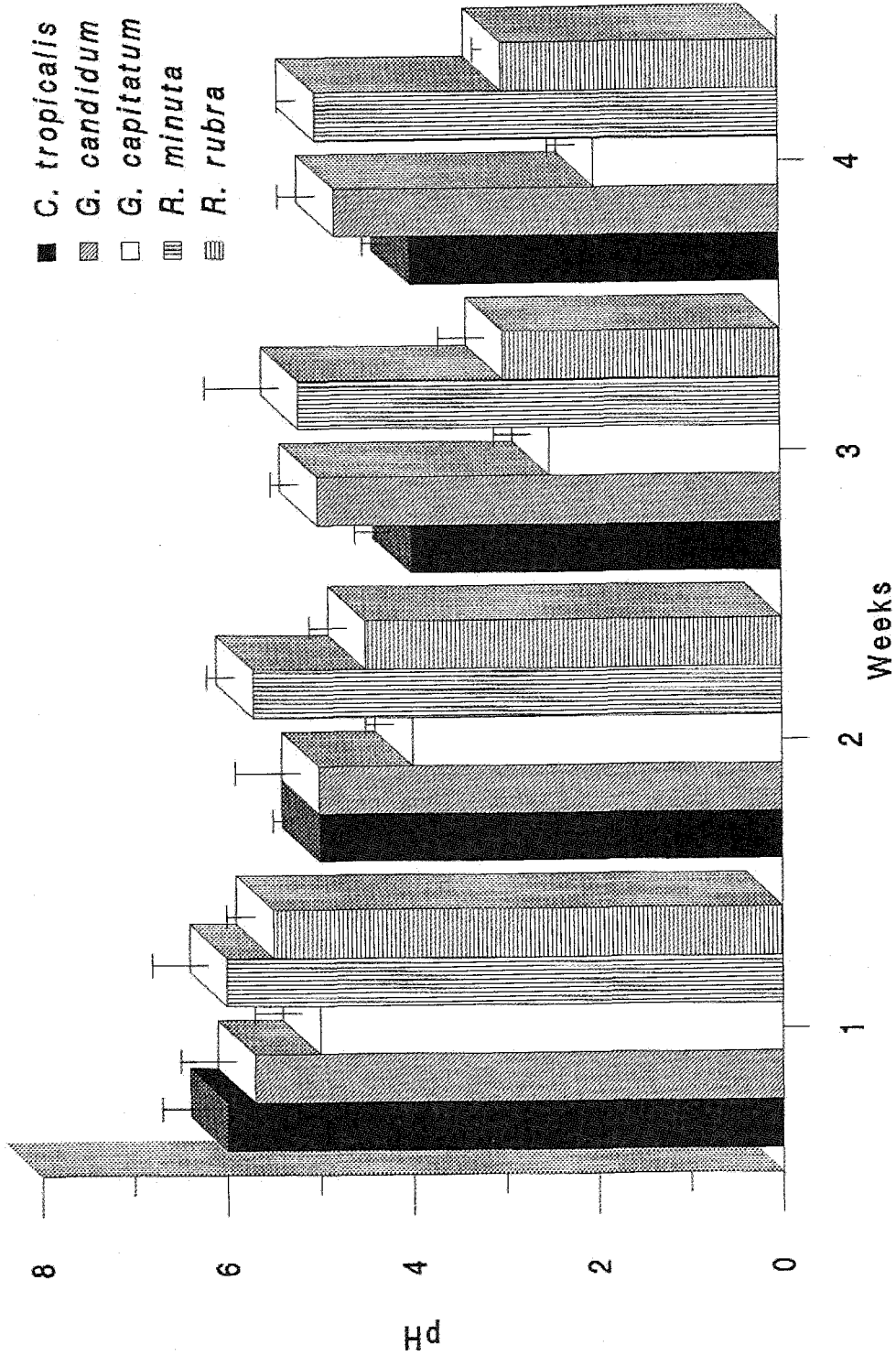


Fig. 3. Medium pH during phosphate solubilization, all values are means of triplicates \pm SD.

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