Abstract    Phosphate solubilizing yeast (PSY) were isolated from rhizosphere, non-rhizosphere and fruits from Bhavnagar district. The potential of 25 yeasts were analyzed on the basis of phosphate solubilizing zone to growth on solid medium denoted as solubilization index (SI) which ranged from 1.10 to 1.50. Among 25 yeast isolates, 6 yeast belonging to genus *Saccharomyces* (2), *Hansenula*, *Klokkera*, *Rhodotorula* and *Debaryomyces* exhibited highest SI (1.33–1.50) were further examined for in vitro tricalcium phosphate (TCP) and low grade rock phosphate (RP) solubilization. TCP proved superior to RP with all the yeasts. Within low grade RPs tested, except isolate Y5, all isolates showed maximum solubilization with Hirapur RP (HRP) ranging from 7.24 to 19.30 mg% $P_2O_5$. Among six PSY screened, *Debaryomyces hansenii* showing maximal HRP solubilization was chosen for further physiological studies. Maximum HRP solubilization was expressed in following condition: pH optima 7.0, temperature optima 28°C and optimal period of incubation were 15 days. Acidic pH of the spent media was a constant feature in all the cases. No correlation could be established between final acidity produced by yeasts and the quantity of phosphate liberated.

Keywords    *Debaryomyces hansenii* · Phosphate solubilization · Physiological study · Low grade rock phosphate

Introduction    The agricultural ecosystems of Gujarat vary across regions due to array of soil, environment and soil management related factors. Continuous application of phosphatic fertilizers to agricultural field results in the higher $P$ fixation in soil. Phosphate is required in high amounts to compensate for crop removal and fixation by soils. Despite its wide distribution in nature, it is deficient nutrient in most soils because the concentration of free phosphorus, the form available to plants, even in fertile soils, is generally not higher than 10 $\mu$M even at pH 6.5 where it is most soluble [1]. Hence, there is a need to have a comprehensive approach [2, 3] to $P$ application for sustainable crop production thus enhancing its use efficiency. Phosphate solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to the plants, allowing sustainable use of phosphatic fertilizers. Microbes are involved in a range of process that effect the transformation of soil phosphorus and thus are the integral component of the soil ‘$P$’ cycle. Many bacterial [3–5], fungal [6–8], yeast [9, 10] and actinomycete [11] species capable of solubilizing sparingly soluble phosphorus in pure culture have been isolated and studied. Several mechanisms such as lowering pH by acid production, chelation and exchange reaction in the growth environment have been reported to play a role in $P$ solubilization by phosphate solubilizers. Such microbes not only accumulate $P$ but a large portion of soluble phosphate is released in quantities in excess of their own requirement.
Most of the Indian rock phosphates are not suitable for phosphatic fertilizer production due to low reactivity and impurities present in them and could be utilized as direct application fertilizers with or without modifications. Sustainable P supply in agro ecosystem can be achieved by using rock phosphate in conjunction with PSM [12, 13]. Microbial volatilization of rock phosphate (RP) especially low grade and its use in agriculture is receiving greater attention. Owing to escalating cost of P fertilizers, there is a need to switch over to cheaper source-RP which is indigenously available by using P solubilizers, organic and chemical amendments [14]. The present work thus focuses on isolating phosphate solubilizing yeast (PSY) and examining their RP solubilizing potential and the physiological factors affecting it.

**Materials and methods**

**Isolation:** PSY were isolated from rhizosphere, non-rhizosphere soil and fruits from Bhavnagar district by enrichment culture technique as described by Gaur [11] and identified up to genus level on the basis of morphological, cultural, biochemical and physiological characteristics using standard procedures [15] as described in “The Yeast”.

**Screening:** (i) on solid medium: 25 yeast isolates were spot inoculated on Pikovskaya’s agar [16] and incubated at 28 ±0.2°C for 48 h. The diameter of each colony as well as that of halo zone of P solubilization was noted. Solubilization index (SI) was calculated as ratio of phosphate solubilization zone to growth diameter [17]. (ii) in liquid medium: After primary selection on solid medium, 6 efficient PSY were examined for *in vitro* tricalcium phosphate (TCP) and Indian RPS viz. Udaipur RP (URP), Sonrai RP (RRP) and Hirapur RP(HRP) solubilization individually. The most efficient yeast was identified as *Debaromyces hansenii* by Institute of Microbial Technology, Chandigarh, India.

**Culture media:** Pikovskaya’s medium and modified Pikovskaya’s broth: TCP was replaced individually by URP, RRP and HRP equivalent to 50 mg P$_2$O$_5$. Media containing HRP were prepared with different pH values [3–9]. The sterilization of medium resulted in change of pH which was considered as initial pH.

**Inoculation and growth condition:** Each flask containing sterilized Pikovskaya’s broth was inoculated aseptically with 1.0 ml washed cell suspension (1.0 OD at 640 nm) containing $25 \times 10^5$ cells/ml of *Debaromyces hansenii* grown for 18 h in glucose yeast extract broth at 28 ± 0.2°C. Flasks for pH experiment were incubated in static condition at 28 ± 0.2°C while flasks for study of effect of temperature incubated at 28, 35, 40 and 45°C. The flasks for shake culture condition were incubated at 28°C on rotary shaker (180 rpm). Flasks containing TCP and native RPs were incubated up to 21d while those with HRP for pH, temperature and aeration-agitation were incubated for 15d. Uninoculated medium served as control for each set.

**P-estimation and pH measurement:** Flasks were withdrawn from each set at periodic intervals, the content centrifuged at 20,000 rpm for 20 minutes. The supernatant analyzed for water soluble P content by chlorostannous reduced molybodophosphoric acid blue method [18]. pH of the supernatant was measured by pH meter. All the experiments were conducted in triplicate taking a control (without inoculation) for each experiment. The mean values of each experiment were calculated.

**Statistical analysis:** Statistical analysis [19] was conducted using two-way analysis of variance to determine significant differences of the mean values between treatments. The relationship between soluble P released and spent media pH were analyzed by computing coefficient of correlation (Pearson’s correlation coefficient).

**Results and discussion**

The overall objective of the present investigation was to analyze the process of mineral phosphate solubilization as an inherent function of yeast. Among 25 yeast isolates, 10 were from rhizosphere soils, 2 from non-rhizosphere soils, 1 from curd while remaining 12 isolates from grapes. 5 rhizosphere soil isolates and 1 isolate from fruit showed maximum P solubilization.

The phosphate solubilizing (PS) efficiency of yeast isolates as judged by solubilization index on solid medium ranged between 1.10 and 1.50 (Table 1). The six efficient PSY belonged to the genus *Saccharomyces* (2), *Hansenula*, *Klockera*, *Rhodotorula* and *Debaromyces*. The most efficient among them was further identified to species level as *Debaromyces hansenii*.

All the six yeast readily solubilized TCP rather than RPs tested. As apparent from data in Table 2, more than 80% reduction noted in RP solubilization as compared to TCP solubilization. Thus, TCP always proved better than all three Indian RPs for 6 selected PSY. HRP was maximally solubilized (19.30 mg% P$_2$O$_5$) by *Debaromyces hansenii* on the 6th day. The relative efficiency of various cultures to solubilize different RPs was in the following decreasing order: