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RESEARCH ARTICLE

Evaluation of media for isolation and screening of silicate solubilising bacteria

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ABSTRACT

Silicate solubilising bacteria (SSB) are used as biofertilizer to crops as it solubilises silica and potassium from soil silicate minerals. The investigators use different types of media containing an insoluble silicate mineral of their choice to determine the solubilisation potential of the isolates. However no specific medium is recommended either for isolation or for enumeration and screening. In the present study nutrient agar, Bunt and Rovira medium, Soil extract agar and Glucose agar medium containing 0.25% magnesium trisilicate were compared for their suitability for isolation, enumeration and screening. Soil extract agar medium containing 0.25% magnesium trisilicate is more ideal for enumeration based on the growth and clarity of dissolution zone while for screening the isolates plain glucose medium with 0.25% magnesium trisilicate is ideal as there is rapid solubilisation and clearing larger zone.

INTRODUCTION

Silicate solubilising bacteria (SSB) or silicate bacteria are of great interest in recent times because of their role in solubilisation of silicate minerals rendering silica and potassium available for crop uptake thus reducing the potash fertilizer requirement (Sheng, 2005) and also due to their role in desilication of ores like bauxite (Zhout *et al*., 2006). Studies have shown that these bacteria solubilised silica besides releasing phosphate, potassium, iron and calcium from the soil silicate minerals. Therefore these micro organisms attracted the attention of scientists to advocate these organisms as potassium mobilising biofertilizers. In the absence of any specific recommended media, the investigators selected a medium of their choice and supplemented with an insoluble silicate minerals like magnesium trisilicate (Purushothaman *et al*., 1974; Muralikannan and Anthoni Raj, 1998) illite (Zhou *et al*., 2006) muscovite (Archana, 2007) and feldspar (Sheng *et al*., 2008) for isolation. Invariably either Bunt and Rovira, Aleksandrov or mineral medium supplemented with these insoluble silicates have been used. Isolation of SSB from soil and water in Bunt and Rovira medium containing 0.25% magnesium trisilicate was made earlier (Muralikannan and Anthoni Raj, 1998). However the clearing zones produced required a sharp observation for their visibility and evaluation. An attempt was made to test alternative media containing magnesium trisilicate for effective enumeration and screening of SSB and the results are reported hereunder.

MATERIALS AND METHODS

Soil samples collected from rice field and commercially available talc were serially diluted and appropriate dilutions were plated to enumerate the total bacteria in Nutrient agar medium (glucose 5.0g; peptone 5.0g; beef extract 3.0g; NaCl 5.0g; agar 20.0g; tap water 1000 ml; pH 7.0) and Bunt and Rovira (glucose 20.0g; peptone 1.0g; yeast extract 1.0g; $(NH_4)_2 SO_4$ 0.5g; K_2HPO_4 0.4g; $MgCl_2$ 0.1g; $FeCl_3$ 0.01g; soil extract 250 ml; agar 20.0g; tap water 750 ml; pH 6.6–7.0), Soil extract agar (glucose 1.0g; K_2HPO_4 0.5g; soil extract 100 ml, agar 20.0g; tap water 900 ml; pH 7.0–7.2) and Glucose agar (glucose 10.0g; agar 20.0g; distilled water 1000 ml; pH 7.0). These media supplemented with 0.25% magnesium trisilicate were used to enumerate SSB. The plates were incubated at room temperature (30 ± 2°C) for 3 days and the colonies were counted. The colonies exhibiting clear solubilisation zones in medium containing magnesium trisilicate were silicate solubilising ones. The ability of two elite isolates (Isolate 1 from soil and an isolate 2 from talc) of silicate solubilising *Bacillus* sp to produce clear zone in these agar media supplemented with magnesium trisilicate was tested. A loopful of the SSB was streaked on the agar media and the plates were incubated at room temperature (30 ± 2°C) for 4 days. The width of bacterial growth was measured at upper, middle and lower portions and the mean was arrived. The extent of clearing zone was measured by scale on one side of the bacterial growth. Since acidolysis is involved in the dissolution process, the acid producing ability of these cultures was tested in the liquid media. The media containing magnesium trisilicate were prepared and dispensed separately in 100 ml quantities in 250 ml Erlenmeyer flasks. The flasks were sterilised at 121°C for 15 min, cooled, inoculated with a loopful of the cultures and were incubated at room temperature (32 ± 2°C). After one week the cultures were centrifuged to remove the cells and debris and the pH of the clear supernatants was determined in a digital pH meter (Everflow scientific instrument). An aliquot of 100ml of the culture filtrate was titrated against 0.05 N NaOH to determine the titrable acidity.

RESULTS AND DISCUSSION

Soil and talc, although harboured millions of bacteria, exhibited only a few thousands silicate solubilising bacteria.. After 3 days of incubation a larger number of total bacteria with fast growth was observed in nutrient agar but the colonies with solubilisation zone were lesser. In Bunt and Rovira medium larger colonies appeared but

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Table 1. Enumeration of Silicate Solubilising Bacteria (SSB) in field soil in different medium containing magnesium trisilicate*

Samples	Total bacteria (10^6)	Silicate solubilising bacteria (10^4)	% SSB
Soil			
Nutrient agar	65	40	0.61
Bunt and Rovira medium	67	90	1.34
Glucose medium	83	20	0.24
Soil extract medium	54	290	5.37
Talc			
Nutrient agar	392	8	0.02
Bunt and Rovira medium	576	6	0.01
Glucose medium	25	2	0.08
Soil extract medium	202	3	0.01

*mean of two samples; population mean of three replicate plates

Table 2. Growth clearing zone and titrable acidity produced in different media by two different silicate solubilising *Bacillus* sp

Medium	*Bacillus* sp isolate 1				*Bacillus* sp isolate 2			
	Growth (mm)	Clearing zone (mm)	pH	Titrable acidity*	Growth (mm)	clearing zone (mm)	pH	Titrable acidity*
Bunt and Rovira medium	18	1	7.69	2.5	7	22	3.82	52.0
Glucose agar	5	25	7.50	0.5	6	2	7.50	0.5
Soil Extract Agar	13	3	7.30	0.7	3	2	7.30	0.5

*ml of 0.05 N consumed

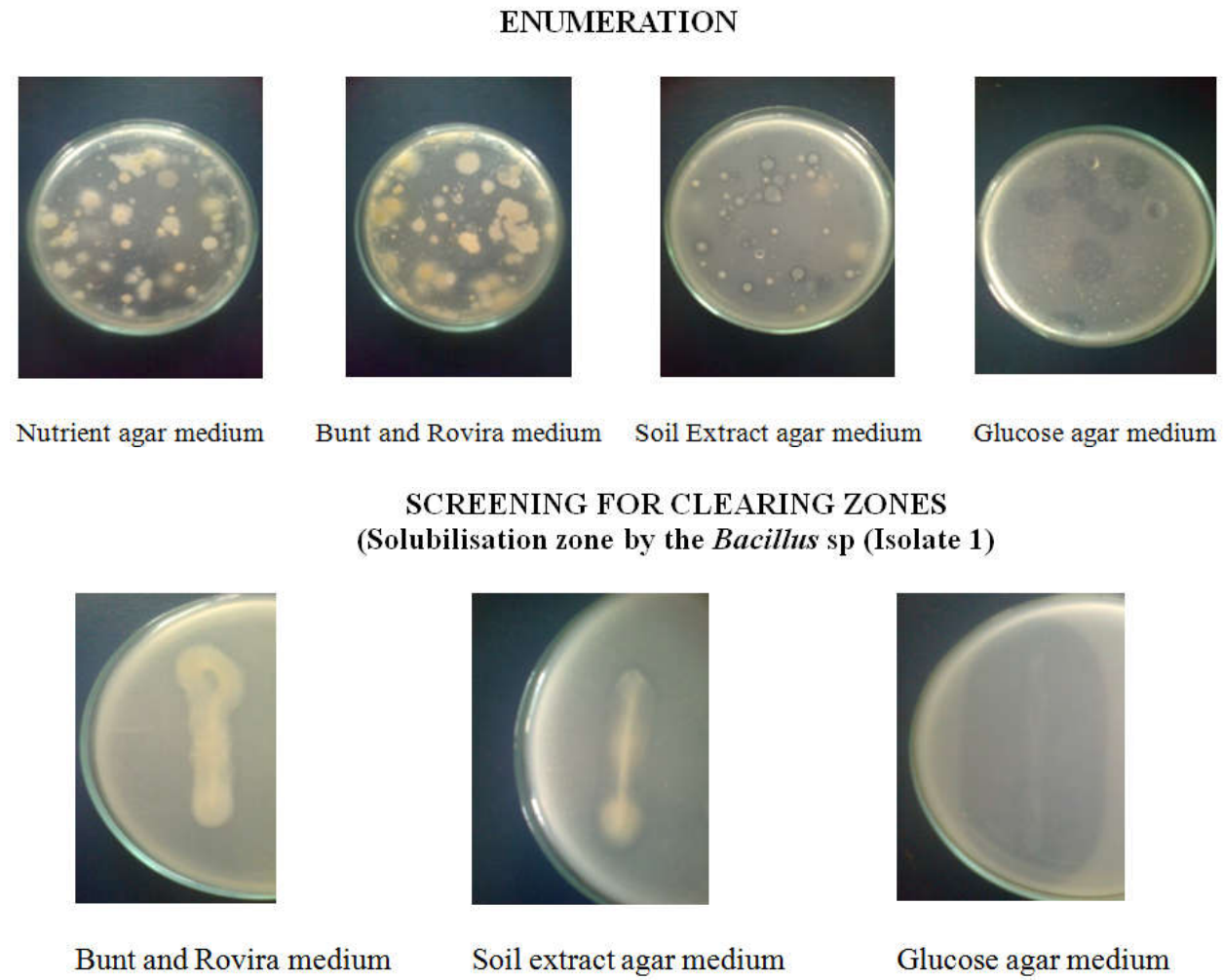

Figure 1: Enumeration of SSB and screening for solubilisation potential

the solubilisation zone was small and restricted. However the colonies in plain glucose agar was tiny and small but the solubilisation zones were comparatively larger and clear (Figure 1, Table 1). Over the same period of incubation, colonies in soil extract agar exhibited good growth with clear and larger solubilisation zones facilitating easy enumeration. Therefore soil extract agar supplemented with magnesium trisilicate appears to be more ideal for enumeration of SSB compared to the other media. Testing of two different isolates of silicate solubilising *Bacillus* sp in Bunt and Rovira, Soil extract agar and plain glucose agar with 0.25% magnesium trisilicate revealed a faster growth in the former two media but a very restricted small solubilisation zones. In the glucose medium despite a slow growth the solubilisation zone appeared earlier and was clear and larger than the other two media indicating the suitability of this medium for rapid screening of the isolates. The culture filtrate of the isolate 1 exhibited a slight increase in pH from the initial 7.0. The titrable acidity was also only slightly higher in Bunt and Rovira medium than the other two media (Table 2).

However the culture filtrate from the isolate 2 showed a sharp decline in pH to 3.82 in Bunt and Rovira medium indicating a higher acidity produced which was also evident from the titrable acidity as 100 ml of the culture filtrate consumed 52.0 ml of 0.05 N NaOH . This isolate did not produce the same level of acidity in the other two media. Several mechanisms of dissolution of silicates by bacteria have been suggested. Acidolysis, alkaline hydrolysis, ligand degradation, enzymolysis, capsule adsorption, extracellular polysaccharides and redox have been shown to play a role in microbial dissolution of silicates. But acidolysis is the main and largely accepted mechanism of weathering silicate minerals (Jongmans *et al*., 1977). Although all types of organic acids have been shown to be involved in dissolution of silicate, gluconic acid was identified as the most effective agent (Sheng *et al*., 2008). It is likely the plain glucose medium with silicate might have favoured the production of more gluconic acid even though total acidity was lower. The acid produced might have also diffused rapidly in this medium as it contains only glucose and no other salts. In the other media the salts present might have interfered with the production of acid and or its diffusion.

The results revealed the difference among the *Bacillus* sp in their ability to produce acid. Further the Bacillus isolate 1 that produced a relatively less acidity exhibited a larger zone in glucose medium. Therefore it is likely that acidolysis which is considered as important in silicate solubilisation may operate in one bacteria but other mechanisms may play a role in other organisms. It is concluded that for isolation and enumeration of silicate solubilising bacteria the soil extract agar supplemented with silicate appears to be more ideal. Based on the earliness of appearance and the larger width of the zone plain glucose agar medium is more ideal for rapid screening for efficiency despite a slow growth of the bacteria in this medium. However it is always better to test the isolates in more than one media for assessing the growth and solubilisation so as to avoid discarding the likely best isolates as the solubilisation potential differed with media.

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