American-Eurasian J. Agric. & Environ. Sci., 8 (3): 253-256, 2010
ISSN 1818-6769

Role of Sponge Associated Actinomycetes in the Marine Phosphorous Biogeochemical Cycles

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Abstract: Phosphorous that sustains marine life become inaccessible as most of them become insoluble due to the formation of complexes with cations and thus the availability of soluble phosphorous decreases to a greater extent. There are reports envisaging marine microbes to play a crucial role in solubilizing these phosphates by the excretion of organic acids, phosphatase and phytase enzymes. In spite of the action of these microbial products, an enormous quantity of insoluble phosphate precipitates to the ocean sediments thus posing a hindrance to the phosphorous biogeochemical cycle. It has been previously evidenced that local ecosystem e.g. coral reefs accomplish a faster cycling of insoluble phosphates. Thus the results of the present study put forth a possible hypothesis that sponge associated actinomycetes and on the whole microbial community of the coral reefs can play a potential role in solubilizing the phosphates and thus increasing the efficiency of phosphorous cycle. This present work opens a new door step for the *in vivo* study that can be focused on further possible mechanism of phosphate accumulation and solubilization by bacterial communities associated with benthic invertebrates.

Key words: Phosphate solubilizers · Actinomycetes · Marine phosphorous cycle · Inorganic insoluble phosphorous

INTRODUCTION

Phosphorous is an essential nutrient sustaining marine primary and secondary productivity. It is required by all organisms for biological synthesis and energy transfer processes [1-3]. Phosphorous being a major component of seawater is a limiting nutrient as inorganic phosphates in association with metal cations become insoluble and therefore inaccessible to organisms [4]. In aquatic environment the total phosphorous (TP) occurs in three forms: Dissolved Inorganic Phosphate (DIP), Dissolved Organic Phosphorous (DOP) or Particulate Phosphorous (PP) [5] with DOP as the predominant form [6]. The phosphorous in the marine ecosystem passes through different stages of biogenic cycle beginning with their migration to final drainage basins and ending with fixation in bottom sediments and diagenesis. Thus various aspects of phosphorous geochemistry in natural environments are being actively discussed in scientific literature. This work was an attempt to place a new claim where sponge associated actinomycetes can be actively involved in the solubilization of inaccessible phosphorous and thus making it available both for its host sponge and for the biogenic cycle for sustainability of human life.

MATERIALS AND METHODS

Bacterial Strains and Media: The 11 actinobacterial cultures that have been isolated from sponge *Dendrilla nigra* and maintained with the series name Marine Actinobacterial Depository (MAD01-MAD11) at the Marine Bioprospecting Laboratory, Bharathidasan University, Trichy, India [7] was used in the present study. The actinobacterial cultures were maintained on actinomycetes isolation agar at 4°C. The cultures were characterized by 16S rRNA sequencing and deposited in Genbank with accession numbers GQ246762 - GQ246755, EU939309 and EU828548.

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Screening for Phosphate Solubilizers: Pinpoint inoculation of the actinomycetes cultures were made on PVK medium with tricalcium phosphate as the insoluble inorganic phosphorous source. The inoculated plates were incubated at 27°C for 7 days. The cultures capable of forming clear zone around the colonies were scored as phosphate solubilizers.

Phosphate Solubilizing Efficiency: The actinobacterial cells were inoculated in a 250ml Erlenmeyer flask containing 100ml of PVK and NBRIP broth and incubated at 27°C for 7 days at 250 rpm. Uninoculated broth served as a control. After incubation, the cell free supernatant was obtained by centrifugation at 8000xg for 15min at 4°C. The resulting supernatant was filtered through a 0.22µm filter and the clear filtrate obtained was subjected for analytical technique in order to study the phosphate solubilizing efficiency of the isolates.

Release of Soluble Phosphorous: The amount of soluble phosphorous liberated was determined in the culture filtrate using molybdate blue method [8]. The intensity of the blue color developed was measured spectrometrically at 730nm. The amount of soluble phosphate was expressed as µg/ml. Change in initial pH of the culture broth was also recorded with a pH meter at the end of all the experiments.

RESULTS AND DISCUSSION

The 11 actinobacterial cultures used in the present study for screening the phosphate solubilizing ability were classified as *Streptomyces* sp., *Nocardiopsis* sp., *Acinetobacter* sp. and unidentified bacterial (yet to be characterized) species. All the strains grew well on actinomycetes isolation agar plates supplemented with 2% NaCl and antibiotics (cycloheximide and nalidixic acid). The bacterial isolates MAD04 (*Nocardiopsis* sp.), MAD06 (*Streptomyces* sp.), MAD09 (*Nocardiopsis* sp.) and MAD11 (*Streptomyces* sp.) produced halo on PVK medium. But all other isolates did not produce any visible halos on agar plates but had the ability to solubilize tricalcium phosphate on PVK and NBRIP broth (Table 1). All the isolates showed potent phosphate solubilizing ability on the broth medium. The efficiency of the phosphate solubilizers was assessed and observed that MAD06 (*Streptomyces* sp.) showed maximum phosphate solubilizing activity with the liberation of 367.32 µg/ml soluble phosphorous and reduction of initial pH from 7.0 to 4.0.

In order to describe the role of sponge associated actinobacterial strains on biochemical phosphorous cycle, certain considerations regarding the marine phosphorous cycle are taken into account. It has been studied that as the concentration of carbon-di-oxide in the atmosphere increases it results in chemical weathering and intensifies dissolved phosphorous supply from continents to the ocean [9]. Thus contributing to the total amount of phosphorous dissolved in oceanic water to approximately 100 Gt. A major share of dissolved mineral phosphorous occurs in oceanic water as HPO^{4-} (87%) and PO_4^{3-} (12%) ions which form monocharge complexes with calcium and magnesium [10]. Such complexes become insoluble and this accumulates in sediments becoming unavailable for living forms. On contrary to this the dissolved phosphorous near sea mouths immediately involves in the biogenic cycle. Thus it has been believed that phosphorous of deep waters are less intensely involved in the general biogeochemical recycling. It has been estimated that the complete mixing of oceanic water

Table 1: Characterization of the actinobacterial isolates by 16SrRNA sequencing and the phosphate solubilizing activity of the isolates given by the reduction in the pH and by the release of soluble phosphorous

	Results of BLAST search			Reduction in initial pH (7.0)		
Strain	Identity	General classification	The most similar species	PVK medium	NBRIP	Release of soluble phosphorous (µg/ml)
MAD01	98%	Actinobacteria	*Streptomyces* sp. LD48	5.5	5	BD
MAD02	YTBC	YTBC	YTBC	5	5	BD
MAD03	97%	Ã- proteobacteria	*Acinetobacter lwoffi* ATCC 17925	5	5	BD
MAD04	98%	Actinobacteria	*Nocardiopsis dassonvillei DSM 40465*	6	6	342.41
MAD05	99%	Actinobacteria	*Streptomyces gougerotii* DSM403247	4	4	327.12
MAD06	99%	Actinobacteria	*Streptomyces rutgersensis* DSM40077 T	6	5	367.23
MAD07	97%	Actinobacteria	*Streptomyces clavuligerus* MTCC 7037	6	5	160.14
MAD08	96%	Actinobacteria	*Nocardiopsis* sp. DSM44659	5	6	261.73
MAD09	98%	Actinobacteria	*Nocardiopsis listeri* DSM40297	6	5	323.94
MAD10	YTBC	YTBC	YTBC	4	3	206.47
MAD11	99%	Actinobacteria	*Streptomyces rochei*	5.5	5	310.86

BD- Below detection

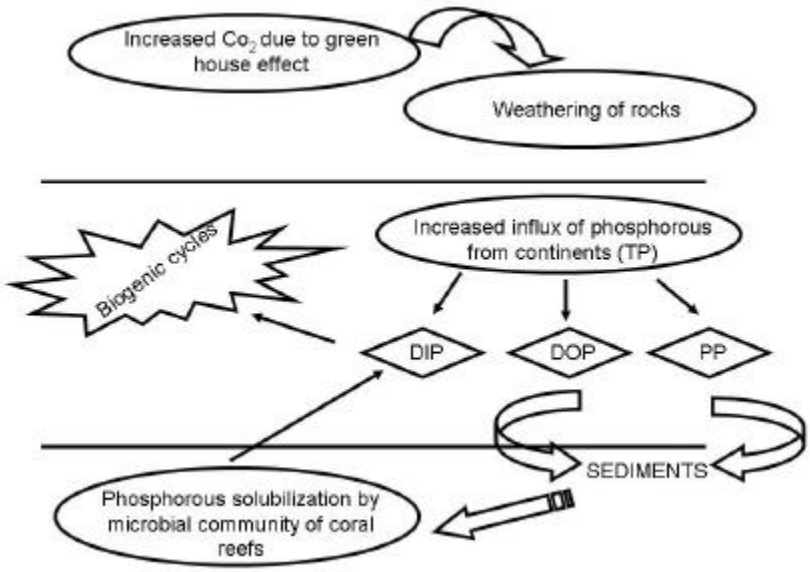

Fig. 1: Proposed possible role of sponge associated actinomycetes in the marine phosphorous biogeochemical cycle

takes approximately 1000 yrs. In a previous study of Sorokin, [11] it has been envisaged that the biogeochemical phosphorous cycle may be accomplished much faster within a day or several hours in local ecosystem eg: coral reefs.

As sponges are filter feeders they invariably filter a large volume of seawater and accumulate the insoluble phosphates. The largest amounts of microorganism with in the mesohyl of many demosponges [12]. Solubilizes the phosphates and thus make it available both for the host and accomplishes a faster phosphorous cycle thus envisaging a possible involvement of the sponge associated microbes (Figure 1). It has long been known that populations of marine heterotrophic bacteria are capable of mineralizing Dissolved Organic Phosphorous (DOP) compounds as sources of phosphorous in the processes of enzymatic dephosphorylation [13, 2].

A voluminous research has been carried out with marine heterotrophic bacteria that are capable of hydrolyzing DNA [14, 15, 16] phytin [14,17-21] and glycerophosphate [22, 23]. Thus the present study carried out is novel in its own way as sponge associated actinomycetes have been exploited for the solubilization of inorganic phosphorous. To the best of our knowledge this is the first report drawing an ecological background with respect to phosphorous biogeochemical cycle for the presence of sponge associated actinomycetes. Further studies needed to be carried out in order to study the *in vivo* accumulation of inorganic phosphorous by the marine sponges and the solubilizing ability of the sponge associated bacterial community.

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