UTILIZATION OF VANADIUM BOUNTIFUL BIOFOULING TUNICATE FOR THE ENRICHMENT OF AZOTOBACTER SP., - AN ANALYSIS

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ABSTRACT: Tunicates / Ascidians belong to the Class Ascidiacea of the sub-phylum Urochordata are dominant members in macrobiofouling benthic community in the marine ecosystem and are placed in between invertebrates and vertebrates. These amazing sedentary creatures have the ability to accumulate more vanadium content in its body than its environment due to the presence of specialized vanadocytes in their blood. This vanadium is essential for ascidians as chemical weapon to lead a successful life in the pressurized environment. Vanadium is also an essential nutrient for some microorganisms for the functioning of nitrogenase enzymes. Hence the present study is aimed to analyse the vanadium content in selected tunicates and its effect on the growth of Azotobacter sp., in *in-vitro* condition. In the present study, variation in accumulation of vanadium was recorded among the members of the Class Ascidiacea. The highest accumulation was recorded in *Phallusia nigra*.

Azotobacter sp., was isolated using Ashby's medium and was identified by performing the biochemical tests such as Gram staining, Catalase test, Oxidase test, Citrate Utilization test, Indole test, Methyl red test, Voges Proskauer test and Triple Sugar Iron test. The growth of azotobacter species was gradually increased with increasing the concentration of dry weight of the selected tunicate and the Optical Density was measured. Besides, the pH and Indole acetic acid production was recorded in different groups, such as Group 1(Azotobacter) Group II (Azotobacter with Standard) and Group III (Azotobacter enriched with tunicates). The members of the Class Ascidiacea can be used as non-conventional bioresource from the marine environment for the enrichment source of non-symbiotic bacteria, Azotobacter sp.,

Key words: Ascidians, Enrichment source, Azotobacter, Biofertilizer

1.INTRODUCTION

Ascidians / tunicates (Subphylum Urochordata) are the largest and most diverse group in the marine ecosystem contributes a part in the food chain, provide food and space for the survival of other organisms and serve as an indicator to assess the quality of water by accumulating the unusual range of metals than its environment. These amazing creatures can be used as an excellent biomarker as they lead sessile mode of life in its habitat throughout their life following a brief planktonic larval stage[14,19] **.** Besides, primitive mode of feeding habits(filter, ciliary and mucous), lack of excretory system and the presence of specialized vanadocytes in the blood are ensuing bioaccumulation in its body tissue. These specialized cells have the ability to accumulate an unusual range of vanadium from the environment[20,3] that provides antifouling defences to the ascidians [13]

There is a fact that each and every species needs a specific type of nutrient/ mineral to regulate the metabolism of an organism. As per the available literature, vanadium is an essential element for some organisms including bacteria, fungi and animals .Robson *et al.*,(1986) have noted the naturally occurring vanadium containing enzymes, haloperoxidases and bromoperoxidases in algae and iodoperoxidase in lichens and nitrogenase in A*zotobacter*. Bishop and Premkumar (1992) have reported that the enzyme nitrogenase needs specific cofactor, iron –vanadium(Fe/V) in its active sites. Vanadium- irons have many structural roles reflected by its structural and electronic analogy to phosphorus**.** Rubio and Ludden (2005) and Newton (2005) have found that Azotobacter and several photosynthetic nitrogen fixers carry additional forms of nitrogenase whose cofactor contains vanadium (V-nitrogenase) or only iron(Fe-nitrogenase). Hence the present study is aimed to analyse the presence of vanadium content in the body tissues of selected ascidians and its effect on the growth of azotobacter in *in-vitro* condition

II. MATERIALS AND METHODS

The commonly available tunicates were collected, from the Thoothukudi coast and identified using the taxonomical keys of **[6]** . The collected samples were dried, powdered and stored in sterilized containers for the estimation of metals. The body tissue (1 g) of each species was subjected to acid digestion in a crucible using a mixture of 3ml of the solution containing 1 part sulphuric acid and 3 part of 60% perchloric acid. The digested sample was then evaporated to near dryness and the residue was dissolved in 20ml of 2N hydrochloric acid. This solution was subjected to centrifugation, filtered and analyzed by Atomic Absorption Spectrophotometer (Model AAS: 6300) at a specific wavelength for vanadium content in the sample. The value is expressed as µg/g in dry weight of the body tissue.

From various sites of the field, 10g of the rhizospheric soil was collected and Azotobacter sp. was isolated using the specific medium, Ashby's medium by Pour plate culture method. This species was confirmed by performing the biochemical tests such as the Gram Staining test. Catalase Test, Oxidase Test, Citrate Utilization Test, Indole Test, Methyl Red Test, Voges Proskauer Test, Triple Sugar Iron Agar Test, and Urease Production Test.

Different groups, Group I(Azotobacter as control), Group II (azotobacter sp. with vanadium pentoxide) and Group III(Azotobacter with *P.nigra)* of culture media were prepared. The growth of Azotobacter species in these culture media was analyzed by measuring the Optical density, pH. production of Indole Acetic Acid(IAA)using Spectrophotometer pH meter and Salkowisky reagent.To find the optimization of experimental animal, different concentration(10mg,20mg,30mg,40mg,50mg,60mg,70mg 80mg,90mg and 100mg) of fine powder of *P.nigra* was added in the Azotobacter enriched culture medium, subsequently the Optical Density and pH was measured.

III. RESULTS AND DISCUSSION

The results on the accumulation of Vanadium in the body tissue of selected ascidians showed that the maximum amount 40.46/ µg/g and 26.66 µg/g was recorded in *P.nigra* and *P. arabica* respectively belonging to the family Ascididae(Figure 1). The present finding is the supportive evidence with the result of Tamilselvi *et al.*, (2010) and Abdul Jaffar Ali (2014). As per the available literature, vanadium is an essential element for ascidians and its function as anti-predatory in nature. Ruppert(2004) reported that the blood of phlebobranchian contain specialized vanadocytes and associated vanabin, the protein in the blood plasma may be the reason for accumulating more vanadium ion from the seawater. Later these vanadocytes are deposited just under the outer surface of the tunic where they function as protection to avoid predation.

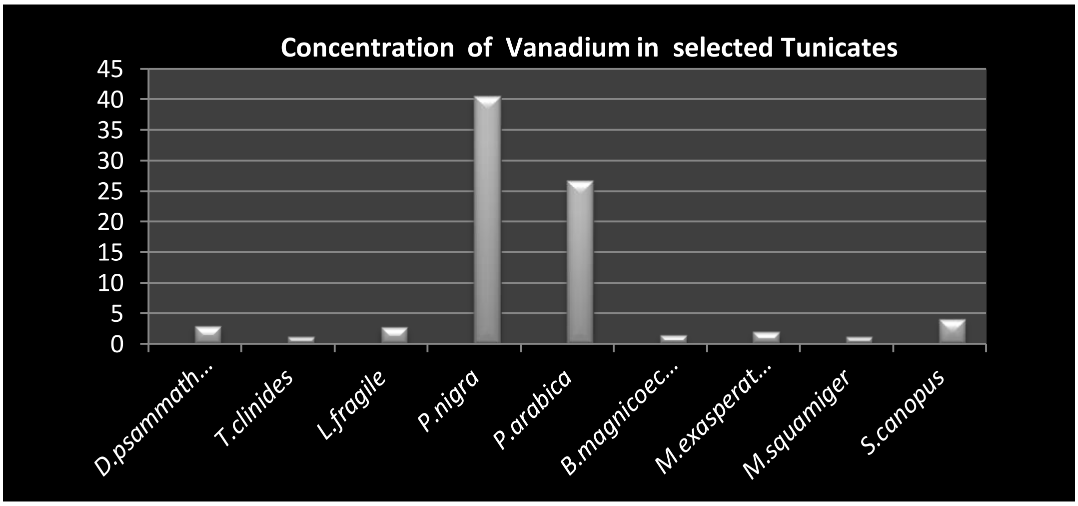

Figure .1The amount of vanadium in selected tunicates. Values are expressed as(µg/g)

S.no	Name of the test	Inference	Result
1.	Gram Staining	Pink colour	-
2	Catalase	Appearence of effervescence bubbles	+
3	Oxidase	Developent dof eep blue colour	+
4	Citrate Utilization	Developent of deep blue	+
5	Indole	Light cherry colour	+
6	Voges Proskauer	Developent of pink colour	+
7	Triple Sugar Iron Agar	Developent of black colour	+
8	Methyl red	Developent of pink colour	+

Table 1:Biochemical test for Azotobacter sp.,

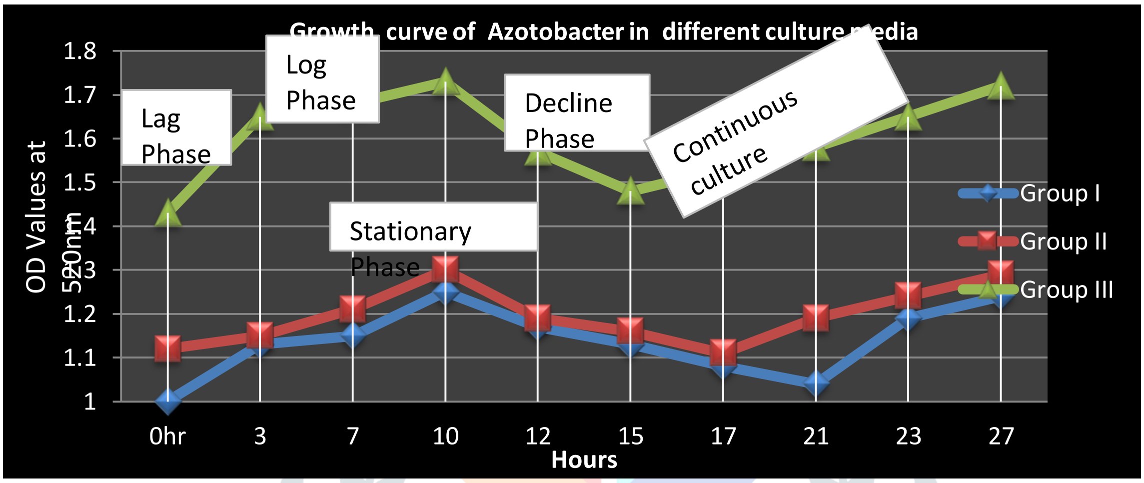

Figure.2 Growth curve for Group I,Group II and Group III inoculated with Azotobacter sp.,

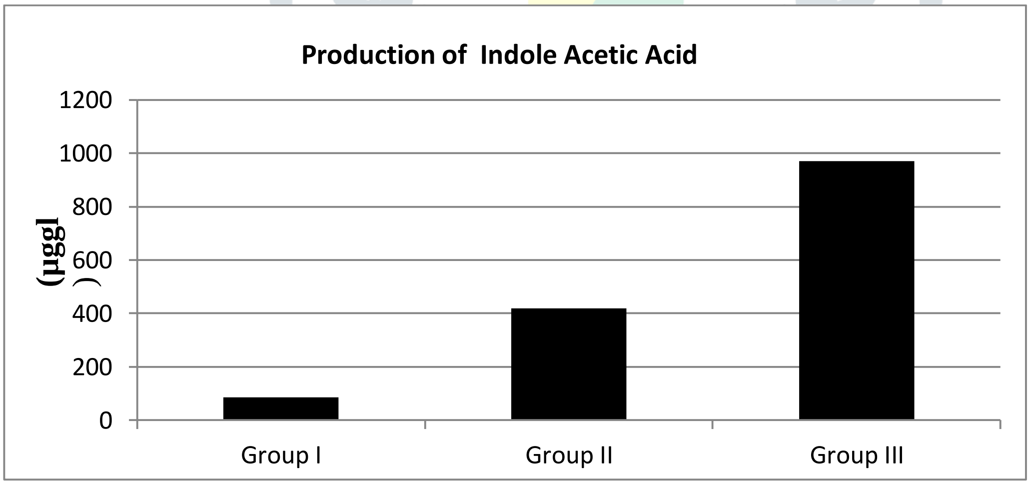

Figure.3 Production of Indole acetic acid in different culture media inoculated with Azotobacter sp.,

The biochemical test for Azotobacter sp. was given in table 1. Azotobacter is a gram-negative, rod-shaped, aerobic and heterotrophic bacteria found in soil, water and sediments [18] and has the ability to fix atmospheric nitrogen non-symbiotically. Free nitrogen-fixing bacteria of the genus Azotobacter sp., [5]and reported that all the species have the capacity to produce oxidase and catalase for the protection of nitrogenase. The same result was also obtained for the tests catalase and oxidase.

A typical growth curve for Group I, Group II, and Group III revealed that well-marked phases could be seen including lag, log, stationary and decline. The growth of Azotobacter sp. is plotted against the time factor is represented in Figure 2..The observed maximum OD value for Group III, Group II and Group I was 1.73, 1.3 and 1.25, respectively at 10^{th} hours of incubation period. Notable to see that, its maximum growth peak could be seen in Group III which indicates the availability of more nutrients that ensuring the growth of Azotobacter sp. in the culture medium. After a while, the OD value was gradually decreased until the culture received with fresh culture as supplementation The time duration for providing continuous culture was vary for Group I, Group II and Group III. The pH is gradually increased with increasing the incubation time. Generally, the range of pH value was in between 7.62 and 8.29.

The present findings revealed that Azatobacter enriched with *P.nigra* showed the maximum optical density and this value was gradually increased with increasing the concentration of *P.nigra*. Vanadium is not only an essential element for some other organisms, but also required to some bacteria such as *Azobacter* sp., for nitrogen-fixation Besides, that variation in the OD value was also noted between Group I, Group II and Group III. Bishop and Premakumar (1992) have documented that the enzyme nitrogenase needs specific cofactor in its active sites and are, iron – molybdenum protein (Fe-Mo), iron (Fe) and iron- vanadium (Fe/V). The present investigation pointed out that the need of vanadium bountiful source for the enrichment of azotobacter sp. This fact can be well illustrated with the growth curve that obtained from vanadium sources(Group II and Group III) inoculated with the Azotobacter sp., Even though all the groups (Group I, Group II and Group III) showed a well-marked growth curve consisting of lag, log, stationary and decline phase. a remarkable variation was noted at each phase and particularly in group III A steep line of lag phase and log phase in Group III signified the enriched nutritional medium that might have facilitated the rapid growth of the Azotobacter sp., in the medium. It could be compared with the profuse amount of vanadium in *P.nigra* that might have enhanced the metabolism via nitrogenase system found in Azotobacter sp. The steady and slower rate of lag and log phase in Group I indicates the less availability of nutrients in the medium showed less lag phase. In this experimental work, the continuous culture was also made by providing additional respective broths to Group III, Group II and Group I at 15 hours, 17hrs and 21st hours respectively.

Many investigators have experimentally proved that sveral species of Azotobacter produce IAA and other phytohormones such as gibberellins and cytokines in vitro condition[8].T he result of the present experimental work is also a supportive evidence for the production of IAA by the Azotobacter species(Figure 3). The maximum (970 µg/l)production was recorded in Group III followed by Group II(420 µg/1) and Group I(85.7 µg/l) . The enriched broth with vanadium bountiful *P.nigra* might have induced the azotobacter species to synthezixe more IAA.

IV. CONCLUSION

To conclude, Azotobacter sp. plays a major role in increasing the production of yield in agriculture as it synthesizes growth promoting substances such as Indole acetic acid, gibberellin, and other substances. In the present work, high production of Indole acetic acid was obtained using vanadium bountiful, biofouling tunicate *P.nigra.* A mass production of indole acetic acid can be possible by utilizing vanadium-rich tunicate. This kind of utilization not only solve the socio-economic problems of the country but also replace the existing problems left by the chemical fertilizers. Besides, new vistas will be opened to prepare the liquid biofertilizer using marine biofouling organisms to make the environment a green.

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