

STUDY OF THE ROLE OF MICROBES AS SOURCE AND SINK OF
DIMETHYL SULPHIDE IN DONA PAULA BAY

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By
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CERTIFICATE

This is to certify that the thesis entitled, "**STUDY OF THE ROLE OF MICROBES AS SOURCE AND SINK OF DIMETHYL SULPHIDE IN DONA PAULA BAY**", submitted by Ms. SREE S. KUMAR for the award of the degree of Doctor of Philosophy in Marine Science is based on her original studies carried out by her under my supervision for the partial fulfillment of the award of the Doctor of Philosophy Department of Marine Science during the academic session 2008 - 2009.

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DECLARATION

As required under the university ordinance 0.19.8 (iv), I state that the present thesis entitled **“STUDY OF THE ROLE OF MICROBES AS SOURCE AND SINK OF DIMETHYL SULPHIDE IN DONA PAULA BAY”** is an original research work and carried out by me at National Institute of Oceanography, Dona Paula, Goa and that no part thereof has been published or submitted in part or in full, for any other degree or diploma in any university or institute. To the best of my knowledge the present study is the first comprehensive work of its kind from Dona Paula Bay. The literature related to the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

SREE S. KUMAR

Dedicated to my

'AMMA'

“ DMS (P) research reminds one of expanding circular ripples on the surface of a pond after having thrown a stone into the water: one will hardly understand the behaviour of the outer rings unless one turns the sight onto the core rings and links their formation with the external forcing, the thrown stone ”.

Simo, 2004

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Chapter 1

INTRODUCTION

Introduction

Among the three major habitats of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest inhabitable space for living organisms, particularly microbes. Due to its large volume and vast area, influence of the oceans on climate is profound. Microbes account for more than 90% of ocean biomass and constitute a hidden majority of life that flourishes in the sea. There are more than a billion micro-organisms living in each litre of seawater, and it is now known that microbes dominate the abundance, diversity and metabolic activity of the ocean. Marine microbes shape the chemical composition of the Earth's oceans and atmosphere. They influence our climate, are the major primary producers in the ocean provide us with a source of medicines and natural products and dictate much of the flow of marine energy and nutrients. Marine microorganisms have an extensive role in the mineral cycling.

Sulfur appears in nature in multitude of compounds either as inorganic or organic forms. The inorganic form occurs abundantly throughout the earth's crust. The main source of "S" is from igneous pyrite (FeS_2) in magma. The crust formed from the magma mixes with atmospheric O_2 and in due course weathers away, forming sulphate, which is carried to the ocean. The organic forms are the constituents of many proteins, vitamins and hormones. These sulfur compounds undergo frequent metabolic transformations, both chemically and biologically. Most micro-

organisms can reduce sulfate to sulfide, an assimilatory process, to produce -SH groups used in certain organic molecules. The reduction of sulphate to a more reduced sulphur species is a necessary prerequisite for the formation of volatile sulphur compounds and their emission to the atmosphere. Thus, biogeochemical processes can be considered as the driving force of the atmospheric sulphur cycle (Fig.1).

Understanding the biogeochemical sulphur cycle in the marine environment has drawn considerable interest among microbial ecologists. Interest in bacteria mediating the bio-geochemical cycle of dimethyl sulphide and dimethyl sulfoniopropionate [both together termed as DMS (P)] has grown as it has been shown that oceanic emission of these compounds can impact global temperature. Substantial attention has been focused in recent years on the biogenic production of the organic sulphur molecule dimethyl sulphide (DMS) and its flux to the atmosphere from marine systems. DMS represents 95% of the natural marine flux of sulfur gases to the atmosphere, and it is estimated that the flux of marine DMS supplies about 50% of the global biogenic source of sulfur to the atmosphere. Photochemical reactions and ultraviolet radiation can oxidize DMS. This interest has been generated by the speculation that in the remote marine atmosphere, oxidation products of DMS dominate aerosol fractions and these may play a significant role in climate regulation, by altering cloud optical properties (Andreae et al. 1983; Bates et al. 1987; Charlson et al. 1987). In addition, DMS is reported to be a potent

chemoattractant for some crustaceans (copepods) and birds (shearwaters and petrels) as a marker for their potential food supplies (Nevitt et al. 1995; Steinke et al. 2006). The DMS concentration results from the combined effects of dimethylsulphoniopropionate (DMSP) production and removal processes (Cantoni and Anderson, 1956; Challenger and Simpson, 1948).

DMSP, a tertiary sulphonium compound is found in many groups of algae including the chlorophytes, rhodophytes, prymnesiophytes, dinophytes, diatoms, chrysophytes, and prasinophytes (Malin and Kirst, 1997). Various functions have been attributed for DMSP, primarily as an osmolyte (Vairavamurthy et al. 1985), anti-predation (Wolfe et al. 1997), anti-bacterial activity (Sieburth, 1960), and as a methyl donor in the synthesis of nitrogen based metabolites (Chillemi, et al., 1990). DMSP is released by damaged phytoplankton cells due to physical stress (e.g. turbulence, zooplankton grazing or viral lysis) and subsequently gets transformed by phytoplankton and bacterial enzymes to DMS. Enzymatic cleavage by DMSP lyase (dimethylpropiothetin dethiomethylase; enzyme classification [EC] number 4.4.1.3.) is thought to be the major process for DMS production in marine environment. DMSP lyase isozymes have been found in various marine organisms including bacteria (de Souza and Yoch, 1995; van der Maarel et al., 1996), fungus (Bacic and Yoch, 1998), and micro- and macroalgae (Cantoni and Anderson, 1956; Stefels and van Boekel, 1993; Steinke et al. 1998, Niki et al. 2000; Wolfe, 2000). Because

each molecule of DMSP contains five atoms of carbon, DMSP synthesis is also important in the carbon cycle; its production is estimated to account for 3–10% of the global marine primary production of carbon (Kiene et al. 2000), and its degradation supplies about 3–10% of the carbon requirements of heterotrophic bacteria in surface waters (Simó et al. 2002). This nutrient is made available to bacteria over space and time although not all species of bacteria are equally likely to obtain this nutrient (Kiene et al., 2000; Simo, 2001). Many bacteria have DMSP-lyase and are thought to play a significant part in converting the algal DMSP to DMS, while other types of bacteria consume the DMSP. Therefore, heterotrophic bacteria may play a key role in controlling the fate of DMSP once this compound is released from algal cells. Additionally, the build-up of acrylate within the microzone or mucus layer surrounding a cell (Noordkamp et al. 2000) may result in concentrations harmful to microbial organisms (Slezak et al. 1994). And from bioenergetics point of view, use of relatively more reduced substrates by microorganisms could provide the greatest growth yield and sulphur compounds can serve as electron acceptors for diverse microbial communities.

Most of the ecological studies on DMS (P) production and utilization are from temperate open ocean waters (Malmstorm et al. 2004 and the references therein). The few known studies on estuarine DMSP have also been carried out in temperate waters. It has been inferred from all these studies that the dynamic distribution of these sulfur compounds with

higher DMS (P) concentrations is in the estuarine region (Sciare et al. 2002; Hu et al. 2005) compared to the open ocean. Annual cycle data on DMS (P) concentrations as well as data from coastal areas are limited. Knowledge of DMS (P) distribution and its dependence on environmental factors inclusive of the biological processes are imperative for understanding the ecological impact of DMS (P) on the marine sulfur cycle, especially in the estuarine waters. Once released, DMSP is quickly dispersed and gets diluted by diffusion and turbulence (Alldredge and Cohen, 1987) in the estuary. The factors controlling the production of DMS by marine algae and microbes are still poorly understood, but it is apparent that complex biological, chemical and physical factors regulate the production of DMS from DMSP and also the flux of DMS into the atmosphere (Andreae, 1990; Belviso et al. 1993; Brimblecombe and Shooter, 1986; Dacey and Wakeham, 1986; Kiene, 1990; Kiene and Bates, 1990; Watson et al. 1991; Curran et al. 1998). Results from work over the last decade or so have revealed a fascinating picture of how DMSP and its degradation products are involved in many different levels of ecosystem dynamics. Just one example of these interesting interactions is the recently discovered involvement of DMSP, and its degradation products (eg DMS) in chemosensory and chemotactic behaviour among diverse species from bacteria to birds (Nevitt et al. 1995; Wolfe and Steinke, 1996; Zimmer – Faust et al. 1996; Wolfe et al. 1997).

In Indian waters, most of the studies on DMS (P) have been restricted to its distribution in the Central Indian Ocean, the Arabian Sea, and the Bay of Bengal. The only study from near coastal waters Off Candolim, Goa is that of Shenoy and Kumar (2007); Shenoy and Patil, (2003). No holistic study has been made either from the biological point of view despite of the factor that the whole process of DMSP cycle is biologically mediated or on annual cycle of these sulfur compounds. Bacterioplankton communities in tropical estuarine systems are frequently exposed to varying environmental conditions, particularly during the seasons (Ram et al. 2007). However, no attempt has been made to gauge the bacterial population associated with these sulfur containing labile dissolved organic compounds. The goal of this study was to identify and quantify the bacteria assimilating DMS (P). The present study also attempted to understand the factors contributing towards DMS (P) concentrations in these waters. To accomplish this goal a station was selected in Dona Paula Bay as this pristine bay is biogeochemically very active zone. The study has also made an attempt to quantify the temporal distribution of DMS (P) and to understand the role of microbes in the assimilation and flux of DMS (P).