Chapter 1: Bacterial Life in Sea Ice

1.1 Introduction

Approximately 10% of the ocean surface is covered by sea ice (Weeks & Ackley, 1982), which until the late 80s was considered to be a kind of microbial desert. Recent investigations, however, demonstrated a vast variety of cold-adapted microorganisms, which actively colonise these extreme environments.

Arctic and Antarctic sea ice provide habitats for a vast diversity of diatoms, bacteria, flagellates and protozoa which reside within the internal brine pocket and channel systems. Among prokaryotes, members of three major phylogenetic groups: the *Proteobacteria*, *Cytophaga-Flavibacterium-Bacterioides* (CFB) group, and the high and low mol percent Gram-positive bacteria, are known to inhabit sea ice environments (Bowman et al., 1997a; Brown & Bowman, 2001; Staley & Gosink, 1999). Detection of ammonia-oxidizing, purple sulfur, and iron-transforming bacteria has also been reported (Priscu et al., 1990; Petri & Imhoff, 2001). To date Archaea associated with sea ice have not been found.

Despite the multitude of environmental stress factors such as low temperature and high salinities, studies during the last decade have established that algae and bacterial assemblies contribute significantly to the productivity of the polar seas, (Lizotte, 2001; Rivkin et al., 1989; Helmke & Wheeland, 1995). Close algae-bacteria coupling was demonstrated in a number of cultivation-based and molecular-biological surveys indicating a substantial role of the heterotrophic bacterial component within the microbial sea ice communities (Helmke & Weyland, 1995; Rivkin et. al., 1989; Brown & Bowman, 2001; Kottmeier & Sullivan, 1990; Bowman et al., 1997a).

1.2 Sea ice formation and structure: habitats for microorganisms

Since most studies on the bacterial component of sea ice have taken place in th Southern Ocean, the main part of our knowledge on diversity, taxonomy and physiology of sea ice bacteria is based on the results obtained in Antarctica. Arctic and Antarctic sea ice, however, provide similar but not identical habitats (Horner et al., 1988).

The Arctic Ocean is comprised of $4.1x10^6$ km^2 of shallow shelf seas that are seasonally ice-covered and $8.2x10^6$ km^2 of central deep basins that are usually covered by 3-5 m thick ice throughout the year (Wheeler et al., 1997). Comparative larger fluctuations of sea ice covered areas are known to be characteristic for the Southern Ocean, ranging from 4 $x10^6$ to $20x10^6$ km^2 (Zwally et al., 1983).

The ice-covered regions can be divided independently on the age of ice into ‘fast ice’ which forms and remains attached along the coast, and ‘pack ice’ which includes any area of sea ice, other than ‘fast ice’ (Garrison et al., 1991; World Meteorological Organisation, 1970). The relative composition and structure of ice vary throughout the ice-covered regions and is determined primarily by physical processes. Development of sea ice includes several transition stages such as ‘frazil ice’ and ‘pancake ice’ (for review, Weeks & Ackley, 1982). The basic structural types of sea ice include ‘platelet ice’, ‘congelation ice’, and ‘snow ice’ (Fig. 1). A close correlation between bacterial colonization of sea ice and type of ice has been revealed (Helmke & Weyland, 1995).

‘Frazil ice’ represents the first stage of sea ice growth in open water where dynamic, turbulent mixing is sufficient to produce supercooling in near surface waters. Planktonic microorganisms accumulate within the newly forming ice at concentrations often exceeding that of the underlying water column (Garrison, 1991; Palmisano & Garrison, 1993; Grossmann, 1994). However, physical enrichment mechanisms during sea ice formation were found to be effective only for microalgae but not for bacteria, most likely due to brine drainage (Weissenberger &Grossmann, 1998).

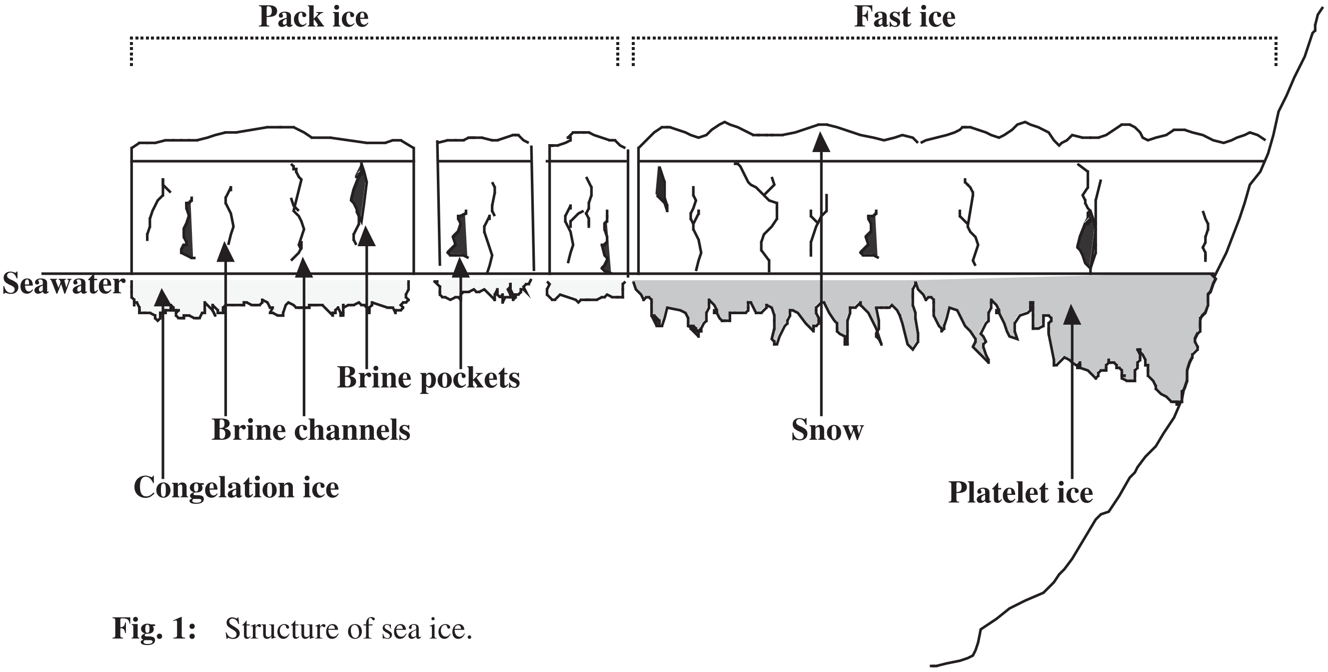

Fig. 1: Structure of sea ice.

With advancing age of the ice, an increase in selective bacterial growth and the number of psychrophilic bacteria were observed (Helmke & Weyland, 1995). Enrichment and sustainment of bacterial biomass within newly formed ice was proposed to depend on their attachment to cells or aggregates of algae and consequently upon the concentration of microoralgae and detritus present in the water column during freezing (Weissenberg & Grossmann, 1998). These results could explain the relatively high percentage of particle-related bacteria in Antarctic and Arctic sea ice reported by several authors (Bowman et al., 1997a; Delille et al., 1996; Sullivan & Palmisano., 1984; Smith et al., 1989) compared to those reported in temperate coastal areas (Unanue et al., 1992).

In coastal regions, near ice shelves, ‘platelet ice’ can form at considerable depth in the water column as water flowing from under ice shelves rises and undergoes supercooling (Dieckmann et. al., 1989). In contrast to the formation of ‘frazil ice’, ‘congelation ice’ forms under an established ice cover when freezing takes place at the ice-water interface. ‘Congelation ice’ is composed of columnar crystals, with long axes oriented vertically, with brine inclusions between adjacent crystals. As ‘congelation ice’ is formed, particulate material is rejected from the ice matrix, so young ‘congelation ice’ has the lowest biomass and displays very low heterotrophic activities which are comparable to water columns (Clarke & Ackley, 1984).

While the initial stock of organisms in sea ice depends on the abundance and composition of the microbial assemblages and particular organic material (POM) within the water column at the time of ice formation, the development of sea ice biota is strongly controlled by the ice-floe formation. A number of dynamic processes, including ice rafting, divergence, pressure-ridge and the break-up of ice floes by waves and sea swell, contribute to the structural heterogeneity of sea ice. Often smaller floes fuse during freezing events, so ice floes may be composites of ice with different histories and structural composition (Lange et al., 1989). Older sea ice belonging to the mainly ‘frazil’ and ‘mixed ice’ has maximal numbers of bacteria (Helmke & Weyland, 1995). They include high proportion of culturable cells and dividing cells as well as large bacteria. Bacteria of these ice types are usually active and significantly contribute to productivity (Helmke & Weyland, 1995).

1.3 Bacterial production in sea ice

A significant contribution of sea-ice microbial communities (SIMCO) to heterotrophic processes in sea ice was demonstrated by several investigations. Bacterial production ranging from 0.01 to 24.4 mg C m^{-3} d^{-1} in different ice cores has been shown to comprise up to 15% of primary production (Helmke & Weyland, 1995; Kottmeier & Sullivan, 1987). In some cases, the bacterial production was reported to exceed algal production (Kottmeier & Sullivan, 1990). Helmke & Weyland (1995) demonstrated that bacteria contribute up to 50% in total turnover of monomeric substrates in sea ice. Furthermore, the high proportion of bacterial ATP to total ATP (on average 34%) also indicates a substantial role for the heterotrophic bacterial component within the microbial sea ice communities (Helmke & Weyland, 1995; Rivkin et. al., 1989).

Dissolved organic material (DOM) and particulate organic matter (POM) provided by sea ice algae assemblages or incorporated from seawater during ice formation present energy sources supporting enrichment of bacterial populations in sea ice. Close algae-bacteria coupling was demonstrated in a number of cultivation and molecular-biological surveys and support the importance of diatoms in providing organic substrates for heterotrophic bacterial metabolism (Brown & Bowman, 2001; Grossi et al., 1984; Kottmeier et al., 1990; Bowman et al., 1997a; Priscu et al., 1990). The estimates of the fraction of primary production released by ice algae as DOM range from 4 to 32% (McConville & Wetherbee, 1983; Kottmeier & Sullivan, 1987; Palmisano & Sullivan, 1985). Most of the organic nitrogen in ice-algae assemblages, where diatoms are known to be dominating (Palmisano et al., 1993), is primarily produced in the form of high molecular weight organic materials, which can not be directly assimilated by bacteria. Hydrolysis of these macromolecules through the action of extracellular enzymes is a first and often limiting step of organic nitrogen utilisation by microorganisms (Hoppe, 1991).

The investigations on the decomposition of particulate organic matter (POM) in sea ice by means of fluorogenic model substrates have been reported. Leu-MCA hydrolysis times were comparable with those for eutropic water samples of a temperate region (Hoppe et al., 1988, Helmke & Weyland, 1995). Compared to the water column beneath sea ice, higher activities of extracellular protease, phosphatase and α- and β-glucosidases were demonstrated in diverse sea-ice samples (Helmke & Weyland, 1995). In addition, production of a vast variety of cold-active biopolymer degrading enzymes, which can contribute significantly to the hydrolysis of the major organic constituents (α- and β-linked polysaccharides, proteins, esters) by Arctic sea ice isolates, was reported (Groudieva et. al., unpublished).

Temperature optima for reported enzymes vary from 30°C to 60°C (Helmke & Weyland, 1995). Degradation of particular matter is controlled less by specific temperature adaptation of the extracellular enzymes than by the formation of enzymes which are governed by the adaptation of the producers (Helmke & Weyland, 1986; Helmke & Weyland, 1995).

Activity of ammonia-oxidizing bacteria (Priscu et al., 1990) and presence of purple sulfur bacteria (Petri & Imhoff, 2001) in sea ice have also been reported suggesting that chemoautotrophs may play an important role in primary production in sea ice. Capacity for both respiratory metabolism and photoheterotrophic growth enables bacteria to take advantage of minor quantities of oxygen in the dark, to reduce the oxygen tension further due to their respiratory activities and to use DOM as phototrophic electron donors and carbon sources (Petri & Imhoff, 2001).

Since the importance of bacterial heterotrophy and respiration in sea ice was demonstrated, sea ice was considered as one of the potential sources of organic material in the Arctic Ocean (Wheeler et al., 1997). The mean concentration of dissolved organic matter (DOC) in sea ice from the central Arctic is 316 ± 99µM (Melnikov, 1997). If the lower 10 cm melts annually in the central basin, this would contribute about 32 µM DOC to the upper m^3 of the surface water. This is about 30% of the ambient surface water DOC but only 3% of DOC integrated over the upper 100 m. Hence, the average contribution of ice formation and ice