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Production of Certain Hydrolytic Enzymes by Psychrophilic Bacteria from the Antarctic Krill, Zooplankton and Seawater

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Abstract

Psychrophilic bacteria isolated from krill, zooplankton and water samples collected in the Indian Ocean Sector of Southern Ocean during the Ninth Indian Expedition (1989-1990) were enumerated and several strains subjected to various biochemical tests. Presence of different enzymes was examined from about 500 of these strains. Bacterial numbers were the highest in the krill gut samples; moderate on zooplankton surfaces and low in water and the ice samples. *Pseudomonas, Vibrio. Chromobacterium, Aeromonas, Acinetobacter* and *Moraxella* were the genera among the Gram-negative organisms. Nearly 8% of the isolates were Gram-positive and over 15% were unidentifiable. Notably, the bacterial strains collected from zooplankton were found to possess large number of hydrolytic enzymes compared to those strains collected either from water or krill samples. Based on these results, the functional role of bacterial enzymes in relation to trophodynamics of euphausiids and their role in the post-harvest technology of krill is discussed.

Introduction

Our understanding of bacterial processes in the Southern Ocean has radically changed over the last few decades. The contentions of earlier investigators were that bacteria would strongly be inhibited by low temperatures and that oxidation of dissolved organic matter would be extremely low. However, recent application of new enumeration and assay techniques in the Southern Ocean ecological studies has highlighted the presence of various, highly active and abundant bacterial communities in all the available ecological niches (Hanson *et al.,* 1983) and microbial heterotrophy is now recognised as an important pathway of. Carbon and energy transfer (Hempel, 1985: Holm-Hansen, 1985 and Tanner, 1985). Although there can be no doubt that krill pervades the foodweb of the maritime Antarctic ecosystem, the trophic structure of marine communities in this system appears to be more complex (Hopkins, 1985 and Vincent, 1988) than earlier views would suggest. Oxidation and biotransformation of organic debris derived from the krill moults and fecal pellets by the bacterial communities is an important aspect in the trophodynamics of the Southern Ocean. Tanoue and Hara (1986) proposed two new pathways of organic material one of which is a new foodchain including nonliving particulate + dissolved organics + bacteria - choanoflagellate - krill - vertebrate. Much of the current interest with the krill, *Euphausia superba* is for its exploitable resource potential. Hence, microbiological studies, in this regard, attain greater importance primarily in the understanding of nutrient regenera-

tion within the Antarctic marine systems and also in the post-harvest handling of krill as the bacterial enzymatic activities can be detrimental to the quality of processed products.

Very few microbiological studies are available from the Indian Ocean sector of the Southern Ocean. Since the knowledge of distribution of bacteria in different niches is necessary to gain valuable insights on their ecological role, the present study was undertaken i) to examine the quantitative distribution pattern of bacteria in the water column, sea-ice, krill and zooplankton; ii) to characterise biochemically some of the strains isolated from these samples; and iii) to record the pattern of extracellular enzyme production in these strains with a view to assess their role in nutrient regeneration as well as in the spoilage of krill.

Materials and Methods

Water, krill and zooplankton samples were collected from five different sampling stations located between 65°S and 45°S in the Indian Ocean sector during the return voyage of the Ninth Indian Scientific Expedition (1989- 90). Water samples were collected from 6 different depths using modified JZ-bacteriological samplers. Zooplankton and krill were collected by hauling the Heron-Tranter net both horizontally and vertically upto a depth of 200 m.

Viable populations of bacteria from several water, ice, zooplankton and krill samples were enumerated on the seawater nutrient agar or Zobell marine agar. Standard microbiological methods such as a) membrane filtration technique for counting bacterial cells in seawater and ice samples and b) serial dilution and spread plate techniques for enumerating bacteria from zooplankton, body surfaces of krill, whole krill homogenates as well as bacteria in the alimentary tracts of krill were followed. All plates were incubated at refrigerated temperature (5°C) for 10 days, checked periodically for growth and viable counts recorded. Total direct counts of bacteria from the water samples were determined by epifluorescence microscopic method by using acridine orange as the fluorescent dye (Persons et al/., 1984).

Strains of bacteria from all these sources were selected randomly, purified and studied for their taxonomical and enzyme properties. Analytical Profile Index 20e strips (API, France) were used for identification of these strains. Criteria recommended by the API Company were followed for their characterisation. Production of a few hydrolytic enzymes by these psychrophyllic bacteria was studied following procedures recommended by Cowan and Steel (1974), McFaddin (1980), Baumann and Baumann (1981) and Espeche *et al.,* (1987). All these tests were carried out by incubating the cultures at 18°C. Results were recorded after 10 days of incubation.

Results

Vertical distribution pattern of bacterial populations at five stations within the Antarctic Convergence is represented in Fig.l. Surface waters had uniform bacterial counts (41.25 to 59.20 x 10^2 ml^{-1}). But higher counts were observed at 100m (47 to 76.50 X 10^2 ml^{-1}) than at any other depths and their numbers were generally low at 200m. Total counts as enumerated

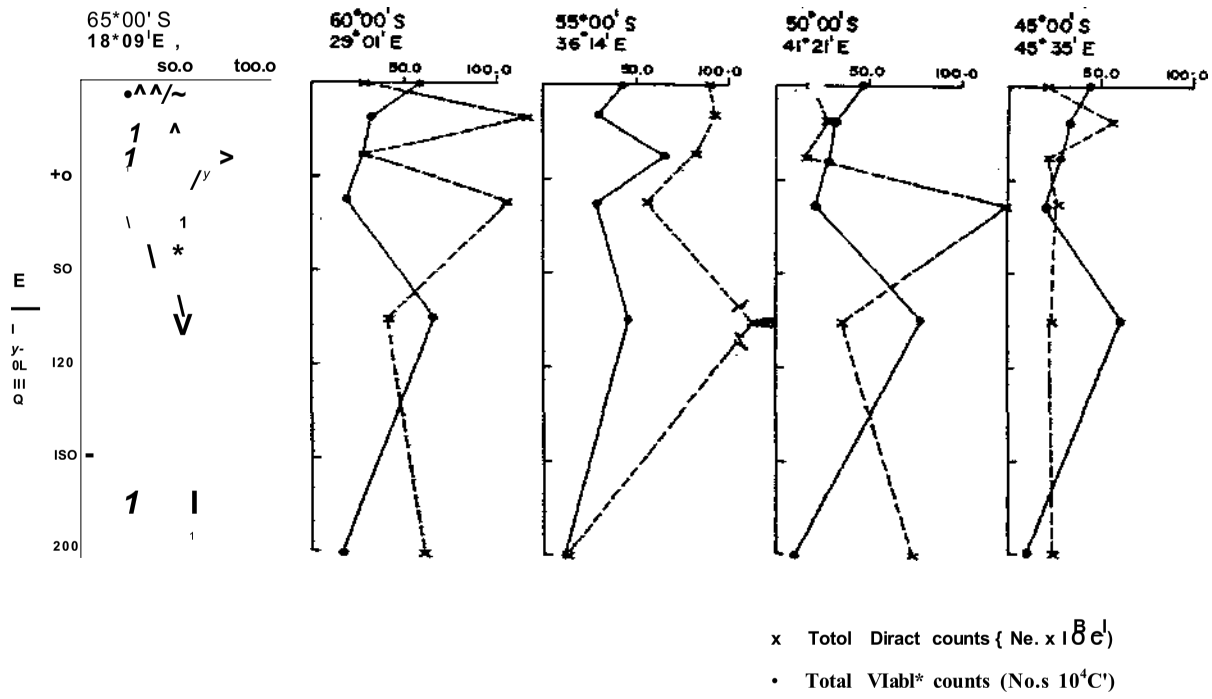

Fig. I. Vertical distribution of bacteria in Antarctic waters.

Table 1: Viable Bacterial Numbers in Different Samples and their Relative Abundance Compared with Water

Sample type	Minimum	Maximum	Mean	Relative abundance
Water	412.50	592.00	468.20	1.00
Flake ice	300.00	1200.00	700.00	1.40
Soft ice	350.00	1020.00	750.00	1.60
Cone ice	400.00	600.00	500.00	1.06
Bard ice	43.00	60.00	46.50	0.09
Zooplankton	70.00	150.00	100,00	213.58
(Krill a)gut	249.50	1055.00	621.00	1326.42
b)homogenate	305.00	755.00	452.00	965.40
c)body surface	0.50	16.00	2.00	4.27

Bacterial population numbers are expressed as no. ml^{-1} for water and ice; no. x 10^3 g^{-1} wet wt. of zooplankters, gut contents and homogenates of krill and no. cm^{-2} of external surface of the krill. Mean viable numbers are the averages of atleast 10 samples.

by the epifluorescence microscopic method were about 3 - 4 orders of magnitude higher than the viable counts enumerated by the plating method. Mean population numbers, their ranges and relative abundance in various types of samples are furnished in Table 1. Compared to the bacterial counts from surface waters, krill guts harboured over 1300 times more bacterial populations followed by the krill homogenates and zooplankton. It was seen that the freshly formed flake and soft ice sampled at the first station had similar bacterial counts and hard ice appeared to be the least preferred habitat.

Over 500 strains of bacteria were isolated from all these samples and they were later screened for different extracellular enzymes. Of these, results from 438 isolates were ultimately available for interpretation. Table 2 gives the percentage of isolates from various sources producing different enzymes. Proteolytic activity is pronounced in most of these psychrophiles. Majority of the strains isolated from zooplankton showed multienzyme activities. Whereas, distribution of this activity was moderate in the strains from krill and water. In Table 3, the types of bacteria identified from different samples are furnished. Strains belonging to *Pseudomonas, Aeromonas, Chromobacterium, Vibrio, Moraxella* and *Acinetobacter* dominated the generic composition. Many of the gram-negative strains tested by API method were unidentifiable. Four types of Gram-positive bacteria contributing nearly 8% of the total, were recorded. Genera wise distribution of enzyme production is represented in Table 4. Strains of *Pseudomonas, Aeromonas* and *Vibrio* were versatile in their enzyme production.

Discussion

Bacterial heterotrophy, once considered negligible in Antarctic waters, is now emerging as an important pathway of secondary production. Detrital material, particularly derived

Table 2: Distribution Pattern of Extracellular Enzymes among the Psychrophylic Bacteria from Different Sources
[Results expressed as percentage of strains positive for the following enzymes]

Enzyme	Source		
	Krill	Zooplankton	Water
No. of strains	182	119	137
Gelatinase	90.65	84.03	62.04
Protease	86.81	82.35	71.53
Lipase	30.76	61.35	31.39
Amylase	18.13	61.35	47.45
Cellulase	34.06	69.75	47.45
Cellobiase	50.54	80.67	65.69
Alginate layse	19.78	69.75	25.55
Chitinase	19.23	61.35	36.49
B - galactosidase	25.27	61.35	36.49
Deoxyribose nuclease	28.02	69.75	25.55
Urease	10.44	0	0

Table 3: Major Taxonomic Groups of Bacteria from Different Sources
[Figures in parentheses are the percentages]

Bacterial taxon	Sample source		
	Krill	Zooplankton	Water
Pseudomonas	39(21.42)	30 (25.21)	31 (22.63)
Vibrio	28(15.38)	29 (24.36)	24(17.52)
Aeromonas	20(10.99)	18(15.12)	21(15.33)
Acinetobacter	17 (9.30)	0	15(10.95)
Chromobacterium	12(6.50)	9 (7.56)	8(5.85)
Moraxella	9 (4.95)	0	11 (8.03)
Gram positive	13(7.14)	9 (7.56)	8(5.83)
Unidentifiable	44(24.17)	24(20.17)	19(13.87)
Total number of strains	182	119	137

from krill, appears to enter the foodweb at many points. Quantitative distribution and importance of microbial communities is still to be understood realistically (Clarke, 1985). From the ecological view point, presence of large population of bacteria in the alimentary canals of krill, as observed in the present study, can be due to very favourable conditions existing in the guts for the bacteria and that these bacteria, in turn, may be aiding in the digestion of food particles in the krill guts. Rakusa and Zdanoski (1989), who observed a

Table 4: Generawise Pattern of Enzyme Production from Different Groups of Bacteria Isolated from Krill, Zooplankton and Water Samples
[Results expressed as percentage of strains positive for these enzymes. Figures in parentheses are the number of strains examined]

Enzymes	P (100)	V (81)	A (59)	Ac (32)	Ch (29)	M (20)	Un (87)	Gp (30)
Gelatinase	71.0	100	84.8	65.6	100	100	54.0	83.3
Protease	78.0	75.3	84.8	65.6	100	100	85.0	80.0
Lipase	71.0	45.6	67.8	65.6	0	80	78.2	60.0
Amylase	43.0	45.6	47.5	18.8	82.8	0	48.3	60.0
Cellulase	29.0	22.2	0	18.8	82.8	60	48.3	33.3
Cellobiase	36.0	45.6	0	37.5	82.8	80	41.4	83.3
Alginate lyase	14.0	22.2	25.4	65.6	82.8	20	33.3	66.7
Chitinase	21.0	53.0	25.4	0	0	0	33.3	26,6
B-galactosidase	22.0	74.0	84.8	0	0	0	65.5	53.3
DNAse	64.0	0	0	81.1	0	0	20.7	43.3
Urease	0	0	13.6	40.6	0	0	6.9	0

P-Pseudomonas; V-*Vibrio; A-Aeromonas;* Ac- *Acinitobacter, Ch-Chromobacterium;* M- *Moraxella;* Un- unidentifiable and Gp-gram positive

staggering 260,000 times higher bacterial counts in the krill digestive tracts than in the surrounding water, are also of similar opinion. The ecological role of bacteria associated with net-zooplankton needs to be elucidated. The fact that bacteria abound in such high numbers and most of them appear to possess multienzyme systems, may be suggestive of these bacteria performing an important function in the assimilation of zooplankton exudates. Understanding the role of free-living bacteria is of great relevance in the Antarctic ecosystem as these communities act as reservoir of cells involved in the dynamic exchange between the exposed (zooplankton surfaces, particles etc.) and protected (guts of fish, krill, birds, etc.) habitats (Karl *et a.,* 1991). Another interesting aspect that needs to be studied is the taxonomy of the Antarctic psychrophiles. Basically, studies on microbial community structure and their enzyme production capabilities are of fundamental nature and significant in highlighting their ecological functions, In this context, a better understanding of the natural microflora from where the krill is harvested becomes a prerequisite for dealing with microbiological problems in krill processing technology (Turckiewicz *et a.,* 1982). Bacterial degradative activities such as proteolysis, lipolysis, production of amylases, ureases, etc., can, at times, determine the success of krill processing. One can envisage microbiological activities to be economically adverse as they can cause deleterious effects to the shelf life of raw and frozen krill, quality of processed krill protein concentrates, peeling wastes and certain holding equipments. Presence of active proteolytic and lipolytic enzymes especially of psychrophyllic pseudomonads is thought to be the primary cause for the onset of spoilage of krill stored atO to 2°C (Kelley *et al,* 1980 and Espeche *et al*., 1987). Bacteria associated with the krill spoilage are reported to withstand the frozen storage at -30°C for

more than 2.5 months and can cause spoilage even at such low temperatures (Gounot, 1976). Rapid deterioration of krill after its harvest has been attributed more to autoproteolysis than to bacterial activity (Fevolden and Eidsa, 1981). However, the spoilage can be more harmful due to bacterial activity.

From the results of the present study, it can be suggested on the one hand that these psychrophilic bacteria are fully capable of breaking down organic material produced in the Antarctic ecosystems. On the other, their degradative capabilities need to be taken note of in the post harvest technology of the krill not only from the point of spoilage aspects but also from the point of employing their enzymes in the efficient utilisation of wastes arising from krill processing. This inference is made since many of the examined enzymes were found to be present in most of the psychrophil'es, irrespective of their source of origin.

Enormous peel wastes arising from the krill processing can be used for the production of chitin and its derivatives. Bailey and Wynn-Williams (1982) noticed some of the Antarctic bacteria to grow and multiply rapidly on the krill moults. Psychrophilic chitinoclastic bacteria can be employed to process these exoskeletal wastes. During this investigation, over 34% of the total isolates were found to possess the ability to hydrolyse chitin and utilise it as the sole source of carbon and energy for their growth. This information is important from the view point of employing psychrophilic bacteria for the processing of chitin and its derivatives.

Microbiology and microbial processes in the Southern Ocean started receiving attention very lately. Application of new enumeration and assay methods has revealed that Antarctic ecosystems harbour a wide range of bacteria; their abundance comparable with temperate latitudes as well as these microorganisms perform several useful ecological functions (Vincent, 1988). Studies related to the association of bacteria with euphausids have also been initiated only lately. Importance of such studies lies in understanding the role these microbes play especially in the breakdown of food particles as well as in the recycling of moult and fecal material originating from the krill, *Euphausia superba* . Microorganisms intimately associated with krill, whose basic function is biotransformation of organic matter, can be problematic in the quality of krill catch and its products. Present study, therefore is of relevance in the handling and post harvest technology, besides highlighting the importance of bacteria in the biological processes of the Southern Ocean foodweb.

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