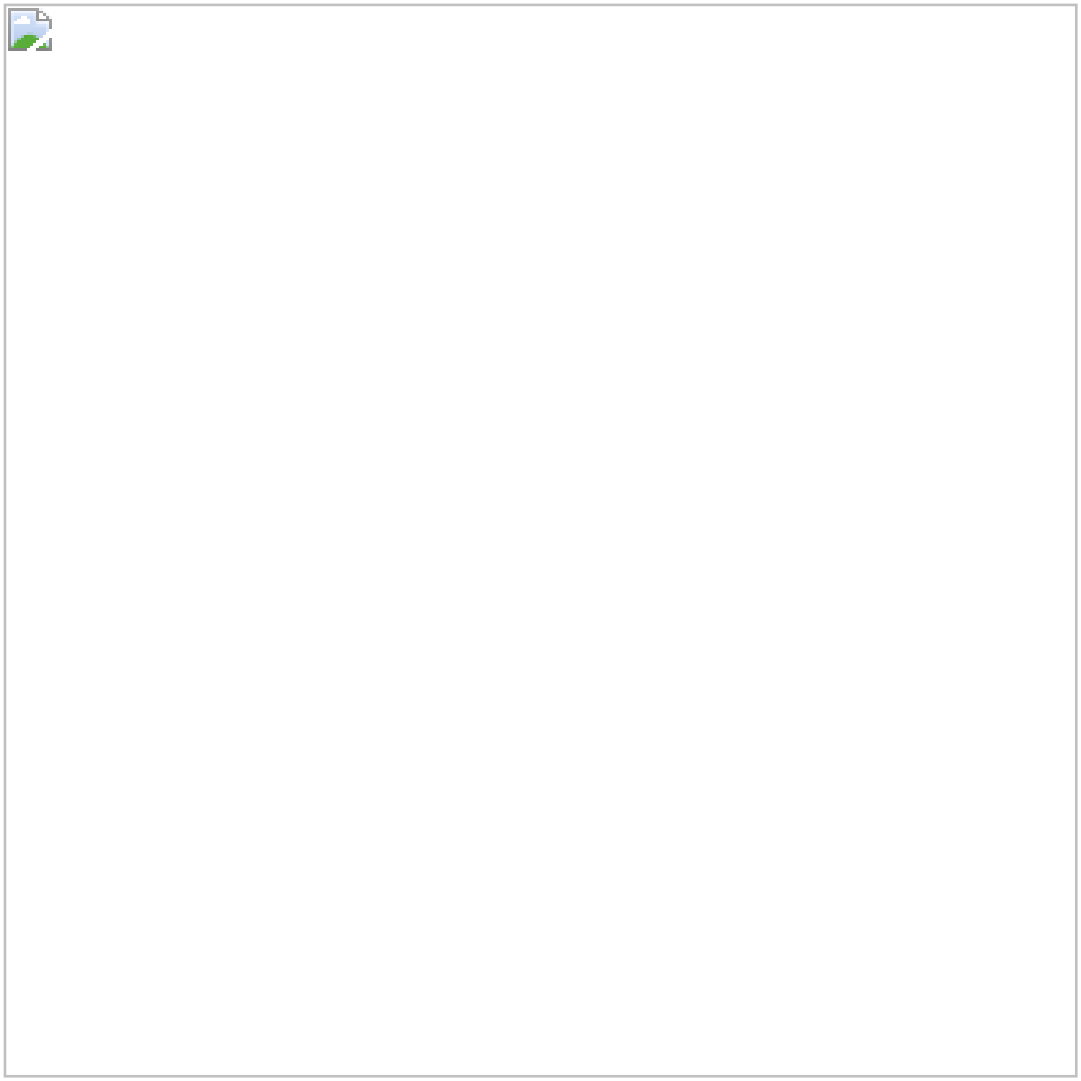

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MICROFLORA ASSOCIATED WITH THE SKIN OF THE BOWHEAD WHALE (*BALAENA MYSTICETUS*)

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ABSTRACT: A study of the microbiological flora isolated from cultures of normal and lesional skin tissue samples collected from 19 bowhead whales (*Balaena mysticetus*) over a 4 yr period is presented. These cultures were obtained from 30 tissue samples (17 normal, 13 lesion) and 248 swab samples (157 normal, 91 lesion). Seven hundred-thirty bacterial and yeast isolations were made (285 normal, 445 lesion). Distribution revealed that 56% of the gram positive bacterial isolates, 75% of the gram negative bacterial isolates and 64% of the yeast isolates recovered were associated with lesional skin. It was found that 80% of one group of *Corynebacterium* sp. isolates, 90% of the *Acinetobacter* sp. isolates and 94% of the *Moraxella* sp. isolates were associated with lesional skin. Although the primary yeasts recovered were *Candida* spp., they were found on both normal and lesional skin. Enzymatic assays of isolates from normal and lesional skin demonstrated production of enzymes capable of causing necrosis. The majority of the microorganisms recovered were facultative anaerobes and many of them could be considered potential pathogens of mammalian hosts.

Key words: *Balaena mysticetus*, bowhead whale, bacterial flora, skin microflora, survey, skin lesion microflora.

INTRODUCTION

The bowhead whale (Balaena mysticetus) is a large (up to approximately 20 m in length) baleen whale that had been widely distributed in the northern circumpolar seas. It was subjected to intense commercial hunting until about 1915 by which time and the population had been greatly reduced. Of the remaining animals, those that utilize the Bering, Chukchi and Beaufort Seas (the Western Arctic stock) are the most numerous (Braham, 1984). The population has been protected from commercial harvest since formation of the International Whaling Commission (IWC) in 1947; however, a Native harvest has been allowed (Tillman, 1980).

The western Arctic stock now numbers an estimated 7,800 animals and is subjected to a small subsistence harvest by Alaskan Eskimo with the harvest under regulatory control by the IWC, the U.S. National Oceanic and Atmospheric Administration and the Alaska Eskimo Whaling Commission (International Whaling Commission, 1988). This stock of bowhead whales also is subject to increasing industrial activity (related to oil and gas exploration and development) throughout much of its range, particularly in the Beaufort Sea. To increase an available data base needed for proper management, many studies have been conducted or are underway presently. These include a major effort conducted in cooperation with the subsistence hunters regarding the censusing of spring migrating whales at Point Barrow, Alaska (Clark and Ellison, 1988; Zeh et al., 1988; George et al., 1988) and regarding basic morphological studies useful in helping predict likely impacts should the animal encounter an oil spill (Haldiman et al., 1985; Henry et al., 1983; Tarpley et al., 1987).

Concerns regarding potential impacts from contact with spilled oil have focused primarily upon impacts to the skin in view of the finding that bowhead whales examined during butchering have dozens to hundreds of roughened areas (1 to 4 cm diameter) on their skin surface (Albert, 1981; Haldiman et al., 1985). These areas of roughened skin surface are speculated to be likely sites for adherence of spilled oil (Albert, 1981). A preliminary study uti-

lizing formalin fixed skin samples in the laboratory showed that oil would adhere to these roughened areas of the epidermis (Haldiman et al., 1981). This, along with the finding of large numbers of microorganisms in the depths of these areas of roughened epidermis, lead to added concern that contact with spilled oil could result in adherence of oil at these sites, causing further damage to the epidermis and allowing the resident microorganisms access to the blood. Thus, an effort was made to identify the bacterial and mycotic flora associated with both normal skin and roughened skin of the bowhead whale; the results are reported herein.

MATERIALS AND METHODS

During the years 1983 to 1986, a 19 subsistence harvested bowhead whales taken near Barrow, Alaska were examined for skin microflora. Both time and swabs of lesions were collected. Two hundred seventy-eight samples were obtained; 30 skin samples and 248 swabs. Skin samples (full thickness epidermis, approximately 2 × 2 cm) were collected from 30 sites on five whales. Of the 30 skin samples, 17 (57%) were from normal skin and 13 (43%) were from lesional skin. Two hundred forty-eight skin surface swabs were taken from different sites on 19 whales. Of the 248 swabs, 157 (63%) were from normal skin and 91 (37%) were from lesional skin.

All samples were collected, frozen and shipped frozen from North Slope Borough (Barrow, Alaska 99723, USA) to the College of Veterinary Medicine, University of Georgia (Athens, Georgia 30602, USA). Upon arrival, samples were transferred to a −70 C freezer and held until cultured.

Tissue samples were thawed at room temperature (25 C), then swabbed using a sterile swab. The swab was suspended in 1 ml of 3% NaCl, vortexed and immediately cultured on both bacterial and mycotic media. After the first year of the study, all specimens were collected using swabs. Swab samples were collected on site using Culturettes® (Marion Laboratories, Kansas City, Missouri 64114, USA). These swabs were thawed as above, transferred to a tube, combined with 1 ml of 3% NaCl, vortexed and immediately cultured as above.

Specimens were cultured on a battery of both selective and nonselective media. These media included Bismuth glucose glycine yeast extract agar (yeast selective), Sabouraud-dextrose agar (mycotic-nonselective), thiosulfate citrate bile salt sucrose agar (vibrio-selective), MacConkey agar (gram negative-enteric bacteria-selective), 5% Casman bovine blood agar (general bacteria-nonselective), trypticase yeast extract agar with 3% NaCl (general marine bacteria-nonselective), Cytophaga agar with 0.85% NaCl and Cytophaga agar with 3% NaCl (cytophaga-selective). All media were obtained in dehydrated form from BBL Division of Becton Dickinson and Co. (Cockeysville, Maryland 21030, USA) and prepared as directed, except for Cytophaga agar which was made as described by Reichenbach and Dworkin (1981).

The plates were inoculated with 1 drop (approximately 0.05 ml) of the prepared saline suspension and then streaked for isolation. Replicate plates were inoculated for the varying incubation temperatures used. Replicate plates were incubated at 37 C for 48 hr, 37 C anaerobic for 72 hr, 30 C for 72 hr, 20 C for 144 hr, 20 C anaerobic for 92 hr and 5 C for 216 hr. Each bacterial type noted on each plate was initially transferred to trypticase soy agar with appropriate salinity and maintained at both its original incubation condition and at 30 C until it was determined that the isolate would grow at 30 C. Isolates were then broadly grouped according to gram stain and cellular morphology. Following grouping each morphologic type was speciated using specific schema and reference sources (Edwards and Ewing, 1972; Gunn et al., 1982; Kriss, 1963; Koneman et al., 1983; Lodder and Rij, 1967; Odds, 1979; Pickett and Pederson, 1970; Farmer et al., 1985; Kreig and Holt, 1984; Sneath et al., 1986; Lennette et al., 1985).

Following identification, isolates from both lesion and normal skin samples were examined for their ability to produce hemolysin, gelatinase, elastase, chondroitinase or to hydrolyze Tween-80 (lipase). These studies were completed at 30 C, 20 C and 5 C to determine whether temperature affected enzyme production. Procedures utilized have been published previously (Hsu et al., 1981; 1983).

Comparisons of differences between more than two percentages were done with a chi-square:one-way analysis of variance (Zar, 1984). If this statistic was significant, the percentages were transformed into arcsines and each arcsine was compared to every other arcsine using Tukey's test. Comparisons of differences between two percentages were done with a Z statistic for binomial parameters (Zar, 1984). The association of dicotomous variables was done using a phi correlation (Mattson, 1981). The correlations were incorporated into a matrix.

RESULTS

Two hundred seventy-eight samples (skin samples and swabs) were examined.

TABLE 1. Gram positive bacteria found in lesional and normal skin samples from bowhead whales (*Balaena mysticetus*).

Group	Skin isolates		
	Lesional	Normal	% isolated from lesions[c]
Lesional *Bacillus* spp.[a]			
B. brevis	11	4	
B. cereus var. *mycoides*	10	5	
B. cereus	28	21	
Total	49	30	62.0[d]
Non-lesional *Bacillus* sp.[b]			
B. subtilis	12	18	
B. megaterium	34	37	
B. pumilis	9	12	
Total	55	67	45.1[d]
Lesional *Corynebacterium* spp.			
Corynebacterium spp. group B-1	6	1	
Corynebacterium spp. group B-3	13	4	
Corynebacterium spp. group F-1	6	2	
C. equi	10	3	
C. pseudotuberculosis	6	0	
Corynebacterium spp. group F	4	1	
Total	45	11	80[e]
Non-lesional *Corynebacterium* spp.			
Corynebacterium spp. group A_1–A_2	1	2	
Corynebacterium spp. group A_4	1	1	
Corynebacterium spp. group D_2	3	2	
Corynebacterium spp. group J–K	0	1	
C. minutissium	3	2	
C. striatum	3	2	
C. mycoides	2	2	
Corynebacterium spp. group A_5	12	9	
Corynebacterium spp. group ANF-3	9	9	
C. aquaticum	4	4	
Total	38	34	53[d]
Staphylococcus spp.			
S. cohnii	3	0	
S. epidermidis	6	6	
S. warneri	7	4	
S. hominis	6	5	
S. capiti	2	1	
S. haemolyticus	2	5	
S. sapophyticus	5	4	
S. hyicus	1	0	
Total	32	25	60[d]
Miscellaneous species			
Rhodococcus spp.	5	4	
Micrococcus spp.	3	6	
Total	8	10	44[d]

[a] Species in which >50% of isolates come from lesional skin.
[b] Species in which ≤50% of isolates come from normal skin.
[c] Percentage data transformed to arcsines and compared using Tukey's test at a 5% probability level.
[d] Percentages with same superscript are not statistically different.
[e] Percentages with different superscript are statistically different from all others.

TABLE 2. Yeasts found in the lesional and normal skin samples from the bowhead whale (*Balaena mysticetus*).

Group	Skin isolates		
	Lesional	Normal	% isolated from lesions
Lesional *Candida* spp.[a]			
C. famata	11	8	
C. krusei	19	8	
C. parapsilosis	7	3	
C. rugosa	10	5	
C. viswanathii	3	0	
C. lipolytica	8	7	
Total	58	31	65[c]
Non-lesional *Candida* spp.			
C. humicola	2	2	
C. intermedia	1	1	
C. pseudotuberculosis	1	1	
C. stellatoides	4	2	
C. utilis	5	3	
C. guillermondii	2	0	
Total	15	9	63[c]
Lesional *Cryptococcus* spp.			
C. gastricus	11	11	
C. luteolus	5	2	
C. albidus	10	8	
Total	26	21	54[c]
Non-lesional *Cryptococcus* spp.			
C. laurentii	3	4	
C. neoformans	2	0	
C. terreus	0	1	
C. uniguttulatus	3	1	
Total	8	6	57[c]
Rhodotorula spp.			
R. glutinis	8	3	
R. rubra	6	0	
R. minula	4	0	
Total	18	3	86[c]
Miscellaneous spp.			
Saccharomyces cerevisiae	7	5	
Torulopsis glabrata	3	3	
Torulopsis inconspicua	1	2	
Torulopsis candida	2	0	
Torulopsis pullulans	1	0	
Geotrichum candidum	2	1	
Trichosporon beigelli	2	2	
Hansenula anomola	5	1	
Total	23	14	63[c]

[a] Species in which >50% of isolates come from lesional skin.
[b] Species in which ≤50% of isolates come from normal skin.
[c] Percentages with same superscript not significantly different.

Seven hundred thirty isolations were made representing 22 genera and 80 species and/or groups of bacteria and yeast. Of the 730 isolations 61% (445) were from lesional skin and 39% (285) were from normal skin. The 730 isolates were further examined and classified as gram positive bacteria, gram negative bacteria or yeast. Two hundred twenty-nine of 406 (56%) of the gram positive bacterial isolates, 68 of 91 (75%) of the gram negative bacterial isolates and 148 of 233 (64%) of the yeast isolates were from lesional skin. A comparison of these groups of microorganisms shows that they are statistically different from each other ($P < 0.005$).

A list of the gram positive bacteria isolated is presented in Table 1. Many of the microorganisms were more frequently isolated from lesions than from normal skin. In Tables 1 and 2 the microorganisms within certain genera were divided into those where >50% of the isolates were from lesional skin and those where ≤50% of the isolates were from lesional skin. For all groupings the percentage of total isolates which were from lesions is noted. A chi square one-way analysis of variance of the percentages for all groupings showed that the group of *Corynebacterium* spp. where >50% of the isolates were from lesions is significantly different ($F = 4.38$, df = 5/400, $P < 0.001$) from the flora of normal skin. In this group 80% of the isolates were from lesions.

Data listing the association of yeasts with bowhead whale skin are presented in Table 2. No significant differences were noted among these genera with regard to their occurrence on lesion versus normal skin ($F = 1.33$, df = 5/227, $P > 0.05$). This suggests that yeast are probably ubiquitous members of the microflora present on bowhead whale skin.

A grouping of gram negative bacteria isolated from lesional and normal bowhead whale skin is presented in Table 3. Chi square one-way analysis of the variance of the percentages indicates a significant difference ($F = 3.04$, df = 3/87, P

TABLE 3. Gram negative bacteria isolated from lesional and normal skin samples from the bowhead whale (*Balaena mysticitus*).

Group	Skin isolates		
	Lesional	Normal	% isolated from lesions[a]
Pseudomonas spp.			
P. cepacia	1	0	
P. diminuta	3	5	
P. picketii	1	1	
P. vesicularis	3	1	
P. maltophilia	2	1	
P. stutzeri	7	2	
Total	17	10	63[b]
Moraxella spp.			
M. phenylpyruvica	8	1	
Moraxella spp.	3	0	
M. osloensis	4	0	
Total	15	1	94[c]
Acinetobacter			
A. calcoaceticus variety *lwoffi*	14	1	
A. calcoaceticus variety *antratum*	4	1	
Total	18	2	90[c]
Miscellaneous spp.			
Flavobacterium multivorum	5	9	
Flavobacterium meningosepticum	1	0	
Alcaligenes faecalis	2	0	
Pasteurella haemolytica	1	0	
Bordetella bronchisepticum	0	1	
Providencia stuarti	1	0	
Escherichia coli	4	0	
Total	14	10	64[b]

[a] Percentage data was transformed to arcsines and compared using a Tukey's test at a 5% probability.
[b] Percentages with the same superscript are not statistically different.
[c] Percentages with different superscript are statistically different from all others.

< 0.05) in the flow of normal versus lesional skin. The percentages of isolates of *Acinetobacter* sp. (90%) and *Moraxella* sp. (94%) found associated with lesions, while not significantly different from each other, were both significantly ($P < 0.05$) larger than the percentage of any other group of isolates of gram negative bacteria.

The microorganisms associated with the skin were divided into two major groups, those recovered both from normal and lesional skin and those recovered only from lesional skin. These microorganisms were evaluated at 30C for their ability to produce hemolysin, elastase, gelatinase, lipase and chondroitinase. The results of these studies are tabulated in Tables 4 and 5. "Z" tests of proportions were used to analyze the patterns of enzymatic activity (Table 6).

When examining microorganisms found on both lesional and normal skin, it was noted that the percentage that had the enzymes necessary for hemolytic activity of blood cells (38%) was significantly larger in those found in the lesions ($Z = 2.41$, $P = 0.015$) than the same composition of organisms found on normal skin (28%). Similarly, of the organisms which produced gelatinase 70% of the organisms associated with lesions were gelatinase positive compared to 59% of the same group of organisms on normal skin ($Z = 2.61$, $P = 0.008$). When examining microorganisms found on both lesional and normal skin, the prevalence of those producing both lipase and elastase and associated with lesional skin was not different from that of those same microorganisms when associated with normal skin. Among the microorganisms found on both lesional and normal skin there was no correlation between the production of elastase and lipase; however, there was a correlation between the production of elastase and the production of both hemolysin and gelatinase (Table 6). Chondroitinase was not produced by any of the isolates.

DISCUSSION

The dominant species of bacteria found on the lesional and normal skin of the bowhead whale show similarities to the environmental findings reported by Atlas (1982). Similar data, although directed at limited examination of the respiratory system of the bowhead whale, included some organisms noted in the current investigation (Johnston and Shum, 1981). The wide variation of organisms recovered from

TABLE 4. Results of enzymatic studies of microorganisms isolated from both normal and lesional skin of the bowhead whale.

Organism	Skin isolates		Enzymes			
	Lesional	Normal	Hemolysin	Gelatinase	Elastase	Lipase
Candida krusei	19	8	–[a]	Nd[b]	Nd	–
Candida parapsilosis	7	3	–	–	–	–
Candida rugosa	10	5	–	–	–	+
Candida humicola	2	2	–	+[c]	–	–
Candida intermedia	1	1	–	+	–	+
Candida lipolytica	8	7	+	+	–	+
Candida stellatoidea	4	2	–	+	–	–
Candida utilis	5	3	–	–	–	+
Cryptococcus gastricus	11	11	+	+	+	+
Cryptococcus luteolus	5	2	–	–	+	+
Cryptococcus uniguttulatus	3	1	–	–	–	–
Hansenula anomala	5	1	–	+	–	–
Cryptococcus albidus	10	8	–	–	–	–
Cryptococcus laurentii	3	4	+	+	+	–
Rhodotorula glutinis	8	3	–	–	–	–
Trichosporum beigelii	2	2	–	–	–	–
Pseudomonas diminuta	3	5	+	+	–	–
Pseudomonas vesicularis	3	1	–	–	–	+
Pseudomonas maltophilia	2	1	–	+	–	+
Pseudomonas stutzeri	7	2	+	–	–	+
Moraxella phenylpyruvica	8	1	–	–	–	+
Acinetobacter calcoaceticus variety *lwoffi*	14	1	–	–	–	+
Acinetobacter calcoaceticus variety *antratum*	4	1	–	–	–	+
Flavobacterium multivorum	5	10	–	+	+	–
Bacillus brevis	11	4	–	–	–	–
Bacillus pumilus	9	12	+	+	+	+
Bacillus cereus variety *myoides*	10	5	–	+	–	+
Bacillus subtilis	12	18	–	+	–	–
Bacillus megaterium	34	37	+	+	–	+
Bacillus cereus	28	21	–	+	–	+
Corynebacterium spp. group B-1	6	1	–	+	–	–
Corynebacterium spp. group B-3	13	4	+	+	+	+
Corynebacterium spp. group F-1	6	2	–	–	–	+
Corynebacterium equi	10	3	–	+	–	+
Corynebacterium spp. group E	4	1	–	–	–	–
Corynebacterium spp. group A_1–A_2	1	2	–	+	–	–
Corynebacterium spp. group A_1	1	1	+	+	–	–
Corynebacterium spp. group B_2	3	2	–	+	–	+
Corynebacterium minutissium	3	2	–	+	–	–
Corynebacterium striatum	3	2	–	+	–	–
Corynebacterium mycetoides	2	2	+	–	+	–
Corynebacterium spp. group A_3	12	9	–	+	–	+
Corynebacterium spp. group ANF-3	9	9	+	+	+	+
Corynebacterium aquaticum	4	4	+	+	+	+
Staphylococcus epidermidis	6	6	–	–	–	+
Staphylococcus warneri	7	4	–	–	–	–
Staphylococcus hominis	6	5	–	+	–	–
Staphylococcus capitis	2	1	–	–	–	–
Staphylococcus haemolyticus	2	5	–	+	–	–
Staphylococcus saphrophyticus	5	4	–	–	–	–
Rhodococcus spp.	5	4	–	+	–	–
Micrococcus spp.	3	6	–	–	–	–

[a] Negative.
[b] Not done.
[c] Positive.

TABLE 5. Results of enzymatic studies of microorganisms isolated from only lesional skin of the bowhead whale.

Organism	Skin isolates		Enzymes			
	Lesional	Normal	Hemolysin	Gelatinase	Elastase	Lipase
Candida guilliermondii	2	0	−[a]	−	−	−
Candida viswanathii	3	0	−	+	−	−
Cryptococcus neoformans	2	0	+[b]	+	+	−
Rhodotorula rubra	6	0	−	−	−	+
Rhodotorula minuta	4	0	−	−	−	−
Torulupsis candida	2	0	−	−	−	−
Torulupsis pullulans	1	0	−	−	−	−
Moraxella spp.	3	0	−	−	−	−
Moraxella osleonsis	4	0	−	−	−	−
Escherichia coli	4	0	−	−	−	−
Staphylococcus cohnii	3	0	−	−	−	−
Staphylococcus hyicus	1	0	+	−	−	−
Flavobacterium meningosepticum	1	0	+	+	−	−
Corynebacterium pseudotuberculosis	6	−	−	−	−	−

[a] Negative.
[b] Positive.

these skin samples was of interest but not unexpected. The fact that from 50% to 75% of the major groups of microorganisms came from lesional skin tends to indicate that these sites serve as a source of nutrients for these organisms. It was noted also that 80% of one group of *Corynebacterium* spp. isolates in the study were associated with lesional skin and were significantly different from other groups of gram positive organisms which were isolated.

The genera of gram positive bacteria associated with the bowhead whale skin included six *Bacillus* spp., 16 species or defined groups of the genus *Corynebacterium* and eight *Staphylococcus* spp..

Species of the genera *Candida*, *Cryptococcus* and *Rhodotorula* were the dominant yeasts isolated from bowhead whale skin. Other genera were found in smaller numbers (Table 2). There was no statistical difference noted in the frequency of isolation of yeast from lesional or normal skin. Members of this group of organisms and particularly of the genus *Candida*, are known to be efficient degraders of hydrocarbons (Atlas, 1982).

The gram negative bacterial flora from bowhead whale skin represented species from the genera *Pseudomonas* (6), *Moraxella* (3), *Acinetobacter* (2), *Flavobacterium* (2) and *Alcaligenes*, *Pasteurella*, *Providencia* and *Escherichia* (1 each). The genera *Acinetobacter* and *Moraxella*, when recovered, were found associated with lesional skin 90% and 94% of the time, respectively. *Pseudomonas* spp. and *Flavobacterium* spp. were common isolates from arctic waters (Atlas, 1982) and were of questionable significance on lesional skin.

The lesional skin which we compared to normal skin in this study consisted of examples of the six types reported by Haldiman et al. (1981). Currently these investigators have refined their observations to include some indications of ecdysis-like processes around some lesions and suggest that this "molting" may be a part of a healing process (Haldiman et al., 1987). They further suggest that the occurrence of physiologic ecdysis could predispose the skin to development of lesions. Some of the microbial isolates were found only on lesional skin (Table 5), suggesting a close association between the organism and the lesion. Other microbial isolates (Table 4) occurred both on lesional and normal skin suggesting a potential role as an opportunistic pathogen.

When microbial isolates were evaluated for the presence of several enzymes (he-

TABLE 6. Intercorrelational matrix of gelatinase, elastase, lipase and hemolysin reactions of organisms isolated from bowhead whale skin.

Enzyme	Gelatinase	Elastase	Lipase	Hemolysin
Gelatinase	—	0.27[a]	0.12[b]	0.34[a]
Elastase		—	0.19[b]	0.58[a]
Lipase			—	0.17[b]
Hemolysin				—

[a] Significant positive correlation ($P < 0.05$) indicating the two variables vary in concert.

[b] Non-significant correlation ($P < 0.05$) indicating that the two variables vary independently.

molysins, gelatinase, lipase, elastase and chondroitinase) often associated with pathogenic organisms, it was found that isolates from lesions produced hemolysins (38%) and gelatinase (70%) more frequently than organisms associated with normal skin. There also was a further correlation of these two enzymes with elastase suggesting that the lesions were active sites of necrosis. The presence, as a result of bacterial growth, of one or all of these three enzymes in a lesion could produce skin necrosis. Elastase action would destroy tissue organization while gelatinase and hemolysins degrade cell proteins which results both in necrosis and would provide a source of nutrients for further bacterial growth and multiplication. The enzymatic studies suggest that the organisms associated with lesions on the skin of bowhead whales are capable of causing necrosis of epidermal tissue. All genera of organisms isolated from bowhead whale skin contain one or more species which have been shown to produce disease in other warm blooded hosts. This suggests that, if given the opportunity, most of the isolates from this study could cause disease in an appropriate host.

The ultimate validation of these results would be to reproduce skin lesions on bowhead whales in a controlled exposure study attempting to prove Koch's postulates. This is neither practical nor feasible. In view of the presence of the numerous roughened areas of epidermis on a whale, and in view of the finding of several species/types of microorganisms present in these lesions that are potential pathogens, it is important that such findings be considered when assessing the potential risk to the bowhead whale from contact with environmental contaminants such as spilled oil.

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