Streptococcus phocae sp. nov., a New Species Isolated from Clinical Specimens from Seals

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A new beta-hemolytic streptococcal species, *Streptococcus phocae*, was isolated from organ specimens obtained from seals. This taxon is described on the basis of the results of a study of 22 strains. *S. phocae* was serologically somewhat heterogeneous (group antigen -/F/C). Strains belonging to this species exhibited high levels of DNA-DNA homology to each other, as determined by DNA-DNA hybridization, but low levels of DNA-DNA homology to the type strains of other streptococcal species. A simple scheme for the differentiation of *S. phocae* from other beta-hemolytic streptococci is presented. Strain 8399 H1 (= NCTC 12719) is the type strain of *S. phocae*.

Streptococci have received much attention because of the large number of infections that they cause in humans and animals. These organisms constitute one of the major groups of bacteria that inhabit respiratory systems. Isolation of *Streptococcus* species (alpha-hemolytic, beta-hemolytic, or nonhemolytic) from seals with pneumonia or septicemia has been reported several times. Authors have described isolates that resemble *Streptococcus canis* (2, 3, 21), *Streptococcus zooepidemicus* (2), and beta-hemolytic streptococcal isolates without any further identification (1, 8, 19).

During the summer 1988, there was an epizootic outbreak among harbor seals (*Phoca vitulina*) in northwestern Europe caused by a morbillivirus that is antigenically related to canine distemper virus and is called phocine distemper virus. This disease killed more than 18,000 harbor seals and a small number of grey seals (*Haliochoerus grypus*) (10). In many cases, the final cause of death of seals infected with phocine distemper virus was infection with one or more opportunistic bacterial pathogens. Beta-hemolytic streptococci were isolated from seals from Scotland (16), England, The Netherlands, Germany (10), Denmark (11), Sweden (5), and Norway (13). The most common signs that were observed clinically were dyspnoea, coughing, and nasal discharge. Fifty-four seals were autopsied at the State Veterinary Laboratories of Norway, Central Veterinary Laboratory, in 1988. The most common gross lesions found were pneumonia with areas of consolidation, purulent exudate in the bronchi, interlobular edema, and emphysema (13).

In this paper, we describe the characteristics of streptococcal isolates obtained from dead seals. These strains were classified in the genus *Streptococcus* as a new species, *Streptococcus phocae*. The type strain of this species is strain 8399 H1.

MATERIALS AND METHODS

Samples. Organ samples from 37 seals were examined bacteriologically, and beta-hemolytic streptococci were the bacteria isolated most frequently from lung specimens. Beta-hemolytic streptococci were also isolated from livers, spleens, and kidneys, indicating that the seals had a septicemic condition.

Bacterial strains. A total of 34 streptococcal strains were isolated from seals suffering from pneumonia; 22 of these strains were included in this study. The type strains of various *Streptococcus* species were purchased from the following institutions: American Type Culture Collection (ATCC), Rockville, Md.; Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany; National Collection of Dairy Organisms (NCDO), Reading, United Kingdom; and National Collection of Type Cultures (NCTC), London, United Kingdom. We selected *Streptococcus gordonii* NCTC 3165 and *Streptococcus sanguis* NCTC 7863^T (T = type strains) as representatives of the *S. sanguis* group and *Streptococcus constellatus* ATCC 27823^T, *Streptococcus intermedius* NCTC 27335^T, and *Streptococcus milleri* NCTC 10708^T as representatives of the *S. milleri* group. We also included *Streptococcus equi* subsp. *zooepidemicus* NCTC 4676^T in this study. The type strains used are listed in Table 1.

Media and cultivation. The specimens were cultivated on blood agar plates (Difco Laboratories, Detroit, Mich.) in a normal atmosphere, in an atmosphere containing 10% carbon dioxide, and in an anaerobic atmosphere (85% N₂, 10% H₂, 5% CO₂) for 18 to 24 h at 37°C. The inocula used for biochemical tests were prepared from overnight cultures. All test preparations were incubated at 37°C. Inocula were also incubated at 10 and 45°C to test for growth.

Morphological and colonial characteristics. Colony morphology was observed on blood agar plates. Cell morphology was determined by using Gram-stained smears and electron microscopy. Motility was examined by using phase-contrast microscopy and cultures grown in Todd-Hewitt broth (Difco). The ability to produce spores was investigated by inoculating a culture into Todd-Hewitt broth, heating the preparation at 80°C for 20 min, and then incubating it at 37°C overnight.

Biochemical characteristics. The ability to produce acid from various carbohydrates under aerobic conditions was determined by using the standard broth method, an API 50CH kit, and an API 20 Strep kit (API System, La Balme-les-Grottes, France). The broth used contained 25 g of heart infusion broth (Difco) in 1,000 ml of distilled water, 12 ml of 0.2% bromthymol blue (1 g of bromthymol blue, 25 ml of 0.1 M NaOH, 475 ml of distilled water), and carbohydrate at a final concentration of 0.8%. The API 50CH test and the API 20 Strep test were performed according to the manufacturer's instructions and were read after 4, 24, 48, and 72 h and 7 days. The enzymatic tests and the tests for production of acid from various carbohydrates were read after 4 and 24 h, respectively.

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TABLE 1. RBRs, as determined by DNA-DNA hybridization, between radioactively labelled DNAs from *S. phocae* strains (8190 R2, 8399 H1^T, and 8026 L2) and *S. iniae* ATCC 29128^T and unlabelled DNAs from strains of *S. phocae* and other *Streptococcus* spp.

Strain	RBR (%) with:			
	<i>S. phocae</i> 8190 R2	<i>S. phocae</i> 8399 H1 ^T	<i>S. phocae</i> 8026 L2	<i>S. iniae</i> ATCC 29128 ^T
<i>S. phocae</i> strains				
7908 P2	130	86	89	4
8026 L2	103	54	100	2
8056 P2	83	86	60	2
8059 P2	60	77	76	3
8093	66	50	71	5
8189 R2	46	73	83	1
8190 R2	100	66	64	5
8202 P3	111	49	52	5
8205 H2	42	145	120	2
8252	83	81	122	8
8287	61	70	136	0
8319	103	139	99	6
8399 H1 ^T	70	100	63	2
8408 P2	96	124	103	3
Strains of other <i>Streptococcus</i> spp.				
<i>S. agalactiae</i> NCTC 8181 ^T	0	2	2	0
<i>S. canis</i> DSM 20715 ^T	1	1	2	1
<i>S. constellatus</i> ATCC 27823 ^T	0	0	1	0
<i>S. dysgalactiae</i> NCDO 2023 ^T	3	3	4	4
<i>S. equi</i> NCTC 9682 ^T	3	4	6	3
<i>S. equi</i> subsp. <i>zooepidemicus</i> NCTC 4676 ^T	1	3	3	4
<i>S. gordonii</i> NCTC 3165	1	0	0	0
<i>S. iniae</i> ATCC 29128 ^T	6	3	16	100
<i>S. intermedius</i> NCTC 27335 ^T	1	0	0	0
<i>S. milleri</i> NCTC 10708 ^T	0	0	1	0
<i>S. mutans</i> NCTC 10449 ^T	1	0	1	1
<i>S. pneumoniae</i> NCTC 7465 ^T	1	0	1	0
<i>S. porcinus</i> NCTC 10999 ^T	1	1	1	2
<i>S. salivarius</i> ATCC 13419 ^T	1	0	0	0
<i>S. sanguis</i> NCTC 7863 ^T	0	0	0	0
<i>S. suis</i> NCTC 10235 ^T	0	1	3	4

Hydrolysis of arginine was determined in arginine dihydrolase medium (3 g of yeast extract [Difco], 1 g of glucose, 5 g of arginine, 0.02 g of bromocresol purple, and enough distilled water to bring the volume up to 1,000 ml) and by using the test in the API 20 Strep kit. Hydrolysis of esculin was determined both by the test in the API 20 Strep kit and by inoculating esculin medium (100 ml of heart infusion broth, 0.55 g of esculin) and adding 1 drop of FeCl₃ after incubation for 24 h. Hydrolysis of hippurate was determined both by the test in the API 20 Strep kit and by inoculating hippurate medium (5 g of meat extract [Difco], 1 g of yeast extract, 10 g of peptone, 5 g of NaCl, 2 g of Na₂HPO₄, enough distilled water to bring the volume up to 1,000 ml, 1% sodium hippurate) and adding 50% H₂SO₄ at a 1:1 ratio after incubation for 4 to 5 days. Starch hydrolysis was evaluated by inoculating Mueller-Hinton agar (Difco) and flooding the plates with iodine after 24 h. Acetoin production was determined both by the test in the API 20 Strep kit and in Voges-Proskauer medium (10 g of tryptone, 3 g of Lab-Lemco [Oxoid, Ltd., Basingstoke, England], 1 g of yeast extract, 20 g of glucose, 1,000 ml of distilled water) by adding 4 drops of alpha-naphthol (alcoholic) and 2 drops of 40% KOH. Production of alkaline phosphatase was determined both by the test in the API 20 Strep kit and in phosphate broth

(100 ml of heart infusion broth, 1 ampulla of phenolphthalein phosphate [Oxoid]) containing 1 drop of 1 N NaOH, which was added after incubation for 24 h. Production of β -glucuronidase and production of pyrrolidonylarylamidase were determined by the API 20 Strep tests. Catalase activity was determined by placing a small amount of a plate culture into 1 drop of 3% H₂O₂. Hyaluronidase activity was determined on blood agar that was inoculated with a *Pasteurella multocida* type A strain and with a streak of a test strain and then incubated for 24 h. The capsular hyaluronic acid of type A strains was depolymerized by hyaluronidase (manifested as a reduction in the size of the *Pasteurella* colonies in the region adjacent to the streptococcal streak) (6). The optochin susceptibility test was performed by placing an optochin tablet (Neo-Sensitabs; Rosco, Copenhagen, Denmark) on a densely inoculated blood agar plate. The sizes of inhibition zones were determined after 24 h of aerobic incubation; inhibition zones more than 12 mm in diameter indicated that a strain was susceptible to optochin. Bacitracin resistance was determined by flooding a Todd-Hewitt plate with a bacterial suspension, removing the superfluous liquid, and drying the plate at 37°C for 30 min before a bacitracin tablet (0.04 IU; Neo-Sensitabs) was added. Inhibition zones were then measured after aerobic incubation for 24 h; inhibition zones more than 15 mm in diameter indicated that a strain was susceptible to bacitracin. Salt tolerance was determined by the presence or absence of growth in inoculated Todd-Hewitt broth with and without 6.5% NaCl after 24 h of incubation. Growth in bile was detected in inoculated Todd-Hewitt broth supplemented with 40% bile after 24 h of incubation.

Serological characteristics. All strains were examined serologically with a Streptex kit (Wellcome Diagnostics, Dartford, England), by the standard method described by Lancefield (14), and by the double diffusion test. The composition of the diffusion gel used was as follows: 1 g of Bacto Agar (Difco), 25 ml of phosphate buffer (pH 6.5), and 70 ml of distilled water. The gel with its walls filled by serum and the test strains was incubated at 4°C for 24 h before precipitation lines were read. For the alternative examination Lancefield grouping sera A through V (Wellcome) were used.

DNA-DNA hybridization. DNAs from 14 of the 22 strains included in this study were subjected to a hybridization analysis. DNA-DNA hybridization was performed essentially as described previously (22), except that DNA was extracted by the method of Coykendall (7). The concentration of DNA was adjusted to 200 μ g/ml.

(i) **Dot blot filters.** Eight parallel dots of each single-stranded genomic DNA and a Tris-EDTA buffer control were blotted onto nitrocellulose filters by using the Hybri-Dot System (Bethesda Research Laboratories, Gaithersburg, Md.) and the method of Katafos et al. (12). The filters were stored dry.

(ii) **Labelling of probe DNA.** Mechanically fragmented genomic DNA probes were labelled with [³²P]dCTP to a specific activity of 1×10^8 cpm/ μ g of DNA by using a random priming labelling kit (Amersham International plc, Buckinghamshire, England) according to the manufacturer's recommendations.

(iii) **Hybridization.** Hybridization was performed at 65°C in a plastic bag for up to 16 h. The sodium salt concentration of the prehybridization and hybridization fluids was 0.1 M. Hybridization washes were performed at 65°C at a sodium salt concentration of 0.1 M. The results of the dot blot hybridization experiments were obtained by overnight autoradiography (Hyperfilm MP; Amersham) and scintillation counting (Packard Instrument Co., Inc., Meriden, Conn.) of standard cut

pieces of the nitrocellulose filter, which were cut with a vacuum cutting device which we designed.

(iv) **Quantification of the dot blot hybridization reaction mixtures.** We determined the mean counts per minute for eight parallel reaction mixtures minus the control counts per minute for salmon sperm DNA for each of the strains. The mean value obtained for the autologous strain by definition represented a DNA homology ratio or a relative binding ratio (RBR) of 100%. The mean RBR for each strain was calculated by dividing the mean counts per minute for the strain by the mean counts per minute for the autologous reaction and multiplying the result by 100.

G+C-ratio. The guanine-plus-cytosine (G+C) content of strain 8399 H1^T DNA was determined by the method of Marmur and Doty (15).

RESULTS AND DISCUSSION

Physiological and biochemical characteristics. The 22 strains which we studied were all facultatively anaerobic, catalase-negative, nonmotile, gram-positive cocci that formed chains when they were grown in liquid media. The biochemical characteristics of these 22 strains are shown in Table 2. Biochemically, the strains appeared to be very homogeneous; 16 of them reacted identically in all of the biochemical tests employed. None of the strains produced acetoin. They all produced acid from D-fructose, maltose, D-mannose, N-acetylglucosamine, and ribose, but failed to produce acid from inulin, lactose, mannitol, D-raffinose, salicin, sorbitol, and trehalose. Four strains also produced acid from amidon and glycogen; one of these strains hydrolyzed starch. Two additional strains produced acid from glycerol, lactose, saccharose, melezitose, and D-turanose; one of these two strains also produced acid from galactose. One strain produced pyrrolidonylarylamidase. All of the strains exhibited inhibition zones of ≥ 30 mm with bacitracin tablets. None of the strains grew in 6.5% NaCl broth or in the presence of 40% bile. None of the strains grew after incubation at 10 and 45°C.

Serological characteristics. Five strains precipitated in the presence of antiserum C, and the rest of the strains did not react with any of the Lancefield test sera. Antigens of the 13 strains coagglutinated with antiserum F in the Streptex system. The strains which reacted with antiserum C in the Lancefield system reacted with antiserum C in the Streptex system as well. Four of the strains did not react with any group antiserum.

DNA-DNA hybridization. Table 1 shows the mean RBRs for DNAs from seal streptococci and reference strains of previously described streptococcal species that were hybridized with total genomic DNAs from streptococci isolated from seals (8190 R2, 8399 H1^T, 8026 L2) and *Streptococcus iniae* ATCC 29128^T used as probes. The total genomic DNAs from streptococcal strains 8190 R2, 8399 H1^T, and 8026 L2 exhibited mean RBRs with DNAs from other seal strains ranging from 42 to 130%, from 49 to 145%, and from 52 to 136%, respectively (Table 1). The strains were also compared with type and reference strains of other *Streptococcus* species.

G+C-ratio. The G+C content of the DNA of strain 8399 H1^T was 38.6 mol%.

Biochemically, the strains isolated from seals were very homogeneous. The phenotypic characteristics of these organisms were typical of the genus *Streptococcus*. The results of our biochemical examinations indicated that the 22 strains which we studied were different from other beta-hemolytic streptococci (Table 3).

Serologically, the strains seemed to be somewhat heterogeneous. The procedures used to extract group antigens were

TABLE 2. Biochemical characteristics of 22 *S. phocae* strains and type strain 8399 H1

Characteristic	No. of strains positive ^a	Reaction of <i>S. phocae</i> 8399 H1 ^T
Acid produced from:		
Amidon	4	—
D-Fructose	22	+
Inulin	0	—
Galactose	1	—
Glycerol	2	—
Glycogen	4	—
Lactose	0	—
Maltose	22	+
Mannitol	0	—
D-Mannose	22	+
Melezitose	2	—
N-Acetylglucosamine	22	+
D-Raffinose	0	—
Ribose	22	+
Saccharose	2	—
Salicin	0	—
Sorbitol	0	—
Trehalose	0	—
D-Turanose	2	—
Hydrolysis of:		
Arginine	0	—
Esculin	0	—
Hippurate	0	—
Starch	1	—
Voges-Proskauer reaction	0	—
Production of:		
Alkaline phosphatase	21	+
β -Glucuronidase	0	—
Catalase	0	—
Hyaluronidase	0	—
Pyrrolidonylarylamidase	1	—
Beta-hemolysis	22	+
Susceptibility to optochin	0	—
Susceptibility to bacitracin	22	+
Salt tolerance	0	—
Growth in the presence of 40% bile	0	—
Growth at 10°C	0	—
Growth at 45°C	0	—

^a A total of 22 strains were tested.

different for the Streptex system and the Lancefield method. An enzyme extraction method was used in the Streptex system, while acid extraction was used in the agglutination technique. In addition, the sera used in the two methods were somewhat different. Lancefield antisera A through V were crude rabbit sera (14), while in the Streptex system polystyrene latex particles were coated with purified rabbit antibodies. This might explain the lack of uniformity in the serological results.

Genetically, the highest level of heterologous affinity was the RBR of 16% observed with DNA from *S. iniae*, the species the most frequently isolated from dolphins (17). The strains isolated from seals were clearly distinguished from all other type strains compared (RBRs, 6% or less). Despite the variations in the RBRs of the streptococcal strains isolated from seals, which could have been due to variations in DNA quality and the very stringent conditions used (9, 18), the DNAs of these strains were distinctly separated from the DNAs of all previously described streptococcal species investigated.

The phenotypic and genotypic homogeneity of the strains isolated from seals and the differences between these strains and other species in the genus *Streptococcus* provided substan-

TABLE 3. Biochemical and serological characteristics that differentiate *S. phocae* sp. nov. from other beta-hemolytic *Streptococcus* species

Characteristic	<i>S. phocae</i>	<i>S. iniae</i>	<i>S. canis</i>	<i>S. equi</i>	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>S. porcinus</i>	<i>S. milleri</i> group
Acid produced from:								
Inulin	— ^a	—	—	—	—	—	—	
Lactose	—	+	+	—	+	d	d	
Mannitol	—	+	—	—	—	—	+	
D-Raffinose	—	—	—	—	—	—	—	
Ribose	+	+	+	—	—	+	+	
Salicin	—	+	NT	+	+	d	+	
Sorbitol	—	—	—	—	—	—	+	+
Trehalose	—	+	+	—	+	+	+	+
Hydrolysis of:								
Arginine	—	NT	+	+	+	+	+	+
Esculin	—	+	—	d	d	—	+	+
Hippurate	—	—	—	—	—	+	—	—
Voges-Proskauer reaction	—	NT	—	—	—	+	+	+
Production of:								
Alkaline phosphatase	+	NT	+	+	+	+	+	
β-Glucuronidase	—	NT	+	+	d	d	+	
Pyrrolidonylarylamidase	—	NT	—	—	+	—	—	
Beta-hemolysis	+	+	+	+	+	+	+	+ ^b
Susceptibility to optochin	+	—	—	—	—	—	—	—
Susceptibility to bacitracin (0.04 IU)	+	—	—	—	+	—	—	—
Lancefield group	—/F/C	—	G	C	A	B	E,P,U,V	—/F/A/C/G

^a —, 90% or more of the strains are negative; d, 11 to 89% of the strains are positive; +, 90% or more of the strains are positive; NT, not tested.

^b A total of 5% of the strains are alpha-hemolytic or nonhemolytic.

tial evidence that the *Streptococcus* strains isolated from seals belong to a species which has not been described previously. The name which we propose for this species is *Streptococcus phocae*.

Description of *Streptococcus phocae* sp. nov. *Streptococcus phocae* (pho'cae. L. gen. n. *phocae*, of a seal). The description of *S. phocae* below is based on the characteristics of 22 strains. The cells are gram-positive cocci that are 1 µm in diameter and occur singly, in pairs, or in chains. Nonmotile. Non-spore forming. Colonies on blood agar are circular, entire, and slightly convex with smooth and glistening surfaces. Unpigmented. The colonies range from pinpoint up to 0.8 mm in diameter after aerobic incubation at 37°C for 24 h. Facultatively anaerobic. Produces β-hemolysin. Biochemical characteristics are shown in Table 1. All strains produce acid from D-fructose, D-glucose, D-mannose, maltose, N-acetylglucosamine, and ribose. Some strains produce acid from D-turanose, glycerol, lactose, melezitose, and saccharose. No acids are produced from 5-ketogluconate, arabinose, β-gentobiose, inositol, inulin, L-fucose, mannitol, sorbitol, trehalose, or xylose within 7 days.

Description of the type strain. Type strain 8399 H1 was isolated from the liver of a seal. Its characteristics are identical to those described above and shown in Table 2.

Characteristics which differentiate *S. phocae* from other related streptococcal species. Selected biochemical characteristics that are useful for identification of *S. phocae* and separation of this taxon from other beta-hemolytic streptococcal species are shown in Table 3 (17). *S. phocae* is easily differentiated from all other beta-hemolytic streptococci except *Streptococcus equi* subsp. *equi* by its failure to produce acid from trehalose. It is distinguished biochemically from *S. equi* subsp. *equi* by the production of acid from ribose and by its inability to produce acid from salicin and to hydrolyze arginine. *Streptococcus pyogenes* exhibits inhibition zones of >15 mm with bacitracin tablets (0.04 IU), while most beta-hemolytic streptococci belonging to other groups exhibit smaller inhibition zones (20). The streptococcal strains isolated from seals are all susceptible to bacitracin; in this study, however, other

biochemical characteristics (Table 3) clearly separated these strains from *S. pyogenes*. So far, strains belonging to the *S. milleri* group are the only streptococcal species known to exhibit the F antigen. Biochemically and on the basis of DNA similarity levels, strains belonging to the *S. milleri* group clearly differ from the strains isolated from seals by production of arginine dihydrolase and acetoin (Tables 1 and 3). *S. iniae*, the most closely related species genetically, is easily differentiated from *S. phocae* by biochemical characteristics (Table 3).

Baker et al. described six different *Streptococcus* types isolated from different organs of grey seal pups (4). Types 1, 2, and 5 produced β-hemolysin. It is possible that *Streptococcus* type 1 is genetically related to *S. phocae*.

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REFERENCES

- Andersson, S. S., J. R. Baker, J. H. Prime, and A. Baird. 1979. Mortality in grey seal pups: incidence and causes. *J. Zool.* **189**: 407–417.
- Baker, J. R. 1980. The pathology of the grey seal (*Halichoerus grypus*). II. Juveniles and adults. *Br. Vet. J.* **136**:443–447.
- Baker, J. R. 1989. Natural causes of death in non-suckling grey seals (*Halichoerus grypus*). *Vet. Rec.* **125**:500–503.
- Baker, J. R., S. S. Anderson, J. H. Prime, and A. Baird. 1980. The pathology of the grey seal (*Halichoerus grypus*). I. Pups. *Br. Vet. J.* **136**:401–412.
- Bergman, A., B. Järplid, and B. M. Svensson. 1990. Pathological findings indicative of distemper in European seals. *Vet. Microbiol.* **23**:331–341.
- Carter, G. R., and S. V. Rundell. 1975. Identification of type A strains of *P. multocida* using staphylococcal hyaluronidase. *Vet. Rec.* **96**:343.

7. Coykendall, A. L. 1970. Base composition of deoxyribonucleic acid isolated from cariogenic streptococci. *Arch. Oral Biol.* **15**:365–368.
8. Geraci, J. R., D. J. St. Aubin, I. K. Barker, R. G. Webster, W. S. Hinshaw, W. J. Bean, H. L. Ruhnke, J. H. Prescott, G. Early, A. S. Baker, S. Madoff, and R. T. Schooley. 1982. Mass mortality of harbour seals: pneumonia associated with influenza A virus. *Science* **215**:1129–1131.
9. Gilmour, M. N., T. S. Whitam, M. Kilian, and R. K. Selander. 1987. Genetic relationships among the oral streptococci. *J. Bacteriol.* **169**:5247–5257.
10. Heide-Jørgensen, M.-P., T. Härkönen, R. Dietz, and P. M. Thompson. 1992. Retrospective of the 1988 European seal epizootic. *Dis. Aquat. Org.* **13**:37–62.
11. Heje, N. L., P. Henriksen, and B. Aalbæk. 1991. The seal death in Danish waters 1988. I. Pathological and bacteriological studies. *Acta Vet. Scand.* **32**:205–210.
12. Katafos, F. C., C. W. Jones, and A. Efstradiadis. 1979. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. *Nucleic Acids Res.* **7**:1541–1552.
13. Krogsrud, J., Ø. Evensen, G. Holt, S. Høie, and N. H. Markussen. 1990. Seal distemper in Norway in 1988 and 1989. *Vet. Rec.* **126**:460–461.
14. Lancefield, R. C. 1933. A serological differentiation of human and other groups of hemolytic streptococci. *J. Exp. Med.* **57**:571–595.
15. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109–118.
16. Munro, R., H. Ross, C. Cornwell, and J. Gilmour. 1992. Disease conditions affecting common seals (*Phoca vitulina*) around the Scottish mainland, September–November 1988. *Sci. Total Environ.* **115**:67–82.
17. Sneath, P. H. A., N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.). 1986. *Bergey's manual of systematic bacteriology*, vol. 2, p. 1043–1071. The Williams & Wilkins Co., Baltimore.
18. Southern, E. M. 1975. Detection of the specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
19. Steiger, G. H., J. Calambokidis, J. C. Cabbage, D. E. Skilling, A. W. Smith, and D. H. Gribble. 1989. Mortality of harbour seal pups at different sites in the inland waters of Washington. *J. Wild. Dis.* **25**:319–328.
20. Stoner, R. A. 1978. Bacitracin and coagglutination for grouping of β -hemolytic streptococci. *J. Clin. Microbiol.* **7**:463–466.
21. Stroud, R. K. 1979. Causes of death in marine mammals stranded along the Oregon coast. *J. Wild. Dis.* **15**:91–97.
22. Tønjum, T., G. Bukholm, and K. Bøvre. 1990. Identification of *Haemophilus aphrophilus* and *Actinobacillus actinomycetemcomitans* by DNA-DNA hybridization and genetic transformation. *J. Clin. Microbiol.* **28**:1994–1998.