Vol. 16: 199-206, 1993 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published September 9

Occurrence and stability of plasmids in *Aeromonas salmonicida* ss *salmonicida* isolated from salmonids with furunculosis

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ABSTRACT: The fish pathogen *Aeromonas salmonicida* ss *salmonicida* was first recorded in Norwegian farmed salmonids in 1985. Plasmid profiling was investigated as a possible method for use in epizootiological work. A total of 383 different isolates was investigated over a period of 6 yr. Plasmid profiling of the isolates revealed 1 to 4 large plasmid bands varying in size from 52 to 105 MDa. Repeated plasmid screening of isolates (even a single isolate) grown under varying conditions and DNA-DNA hybridization, suggested that only one large plasmid was present but that it could occur in up to 4 configurations. An alternative possibility was that there were 4 large plasmids, each sharing common sequences, but that the individual plasmids did not always replicate to the stage where visible bands were produced. Different growth media or length of incubation of culture had no apparent effect on the occurrence of the different large plasmid bands. The DNA of the large plasmid bands was susceptible to mechanical stress and UV-light exposure. A group of 4 small plasmids of 3.4, 3.5, 3.6 and 4.2 MDa characterized most of the isolates whereas only 7.3 % of the isolates contained plasmids of intermediate size (6.5 to 40 MDa). Overall, the plasmid content of *A. salmonicida* ss *salmonicida* seemed to be very constant worldwide, and thus plasmids in this organism would generally be of little epizootiological value.

INTRODUCTION

Plasmids often represent the most variable part of the genome in a bacterial cell (Elmerich 1990). This variation in the plasmid profile has been used to subgroup isolates in epidemiological studies of outbreaks of salmonellosis among both humans and animals (Holmberg et al. 1984, Olsvik et al. 1985, Sørum et al. 1990). In bacterial fish pathogens, plasmid variation also occurs within species and subspecies. For example, the bacterium causing cold-water vibriosis, *Vibrio salmonicida*, contains from one to several different plasmids. However, the variation noted in plasmid profiles among isolates was limited, with a pair of profiles predominating (Sørum et al. 1988). Identical plasmid profiles were found in isolates of *V. salmonicida* from all areas in the eastern and western Atlantic Ocean from which the bacterium has been isolated (Sørum et al. 1993). On the other hand, isolates of atypical *Aeromonas salmonicida*, an organism that can cause disease in farmed fish (Austin & Austin 1987) show considerable plasmid variation. Plasmid profiling among isolates of atypical *A. salmonicida* may be useful for dividing the isolates into subgroups (Bast et al. 1988, Belland & Trust 1989, Sørum unpubl. results)

The primary aim of this inyestigation was to evalute the use of plasmid profiling in epizootiological work in outbreaks of furunculosis in salmonids caused by *Aeromonas salmonicida* ss *salmonicida*. The investigation was started in 1985 when furunculosis was thought to have been introduced into Norwegian salmonid farming with the import of infected smolts from Scotland. The initial results showed an apparent variation in plasmid profiles of the *A. salmonicida* ss *salmonicida* isolates. The variation was, however, found to be inconsistent, even within isolates, and thus plasmids proved, at the time, to be ineffective as an epizootiological tool. Other workers have shown that the plasmid content of *A. salmonicida* ss *salmonicida* is homogeneous (Bast et al 1988, Belland & Trust 1989).

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In particular, the small plasmids have been considered to be characteristic for *A. salmonicida* ss *salmonicida*. However, little attention has been paid to the larger plasmids of this bacterium. Also, the number of strains of *A. salmonicida* ss *salmonicida* investigated in earlier studies has been small (Toranzo et al. 1983, Hackett et al. 1984, Bast et al. 1988, Belland & Trust 1989).

In addition to the primary goal of evaluating the epizootiological use of plasmid profiling in a large collection of clinical isolates of *Aeromonas salmonicida* ss *salmonicida*, a second aim of this study was therefore to analyze the stability of the large plasmids of the bacterium and to study the factors influencing this stability.

MATERIALS AND METHODS

Bacterial strains. A total of 383 isolates of *Aeromonas salmonicida* ss *salmonicida* was included in this study. The Norwegian isolates were obtained from the kidney or gross lesions of farmed Atlantic salmon *Salmo salar* or rainbow trout *Oncorhynchus mykiss* suffering from furunculosis. Isolates from other countries and a reference isolate are listed in Table 1.

Table 1 *Aeromonas salmonicida* ss *salmonicida* isolates (46) obtained from countries other than Norway

Isolate	Country	Year of isolation
ATCC 14174		
2827/85, B2402/85	Sweden	1985
2827/85, B2403/85	Sweden	1985
30/87	Sweden	1987
78/87	Sweden	1987
417/87	Sweden	1987
428/87	Sweden	1987
524/87	Sweden	1987
2755/85 Rindsholm	Denmark	1985
2755/85 Veldal	Denmark	1985
33DK	Denmark	1990
34DK	Denmark	1990
35DK	Denmark	1990
37DK	Denmark	1990
38DK	Denmark	1990
39DK	Denmark	1990
40DK	Denmark	1990
41DK	Denmark	1990
42DK	Denmark	1990
43DK	Denmark	1990
260/86	Finland	1986
261/86	Finland	1986
265/86	Finland	1986
266/86	Finland	1986
275/86	Finland	1986
1244/86	Finland	1986
1302/86	Finland	1986
1262	Finland	1986
1153	Finland	1986
4948/86	Finland	1986
250/87	Scotland	1987
85/09/2078	Scotland	1985
3010/85, B83016L	Scotland	1983
3010/85, B83016	Scotland	1983
3010/85, B85093	Scotland	1985
3010/85, B850100	Scotland	1985
3010/85, B85112	Scotland	1985
Ft X	Scotland	
2893/85	Switzerland	1985
425/86 Fi 20/86	Switzerland	1986
2754/85, 72/78	France	1985
2754/85, 88/68	France	1985
3001/85, 41/84 R	France	1984
2834/85, A140 85	Ireland	1985
2834/85, A141 85	Ireland	1985
123/86	USA	1986

The Norwegian isolates were originally grown on 5 % bovine blood agar incubated aerobically at 15 °C for 2 d. Subsequently, the isolates were grown on Luria agar (LA), in Luria broth (LB), or in Brain Heart Infusion Broth (Difco Laboratories, Detroit, MI, USA) (BHIB) at 15 or 22 °C. The isolates were stored on LA slants at 4 °C and at -70 °C in LB supplemented with 10 % glycerol. When using stored isolates, 5 % bovine blood agar was the medium used for regrowth. LB was the routine medium used for culture of all isolates for plasmid isolation.

Plasmid isolation. Plasmids were isolated using 2 different small-scale procedures. A modified method of Kado & Liu (1981) was employed for all isolates one or more times. Modifications involved the incubation conditions with the lysis solution, and the phenol/chloroform extraction step. The time of incubation with the lysis solution was varied from a few minutes to more than 1 h. The temperature for incubation with the lysis solution varied from 55 to 65 °C. In the phenol extraction step, both acid and buffered phenol at pH 7.0 were used, and the phenol contact time was varied. Plasmid isolation was performed for the first time as close to the primary isolation as possible. The mini-Birnboim procedure (Birnboim & Doly 1979) was also used in plasmid isolation from selected strains in addition to the modified method of Kado & Liu (1981). A large-scale SDS-method (Maniatis et al. 1982) was used to isolate plasmid DNA from strain 2921/89. The plasmid DNA was run in a cesium chloride/ethidium bromide (CsCl/EtBr) gradient to purify it.

Plasmid electrophoresis. The plasmids from all isolates were separated by electrophoresis in 1 % agarose gel in a vertical gel apparatus. The buffer used was Tris-borate-EDTA (TBE) (0.089 M boric acid, 0.0025 M EDTA, 0.089 M Tris, pH 8.0). Electrophoresis was carried out at 120 V for 3.5 h. Alternatively, for better separation of the small plasmids, 0.8 % agarose gel in a horizontal gel apparatus was used for a selected group of isolates. In this case, electrophoresis was carried out at 50 V for 8 h using Tris-acetate buffer (0.005 M NaAc,

0.001 M EDTA, 0.04 M Tris, pH 7.8). Gels were stained with ethidium bromide (EtBr) and photographed under UV light. The sizes of the plasmids were estimated by including *Escherichia coli* reference plasmids of known size in the gels. The plasmids Sa (23 MDa), RP4 (34 MDa), R1 (62 MDa), a strain containing pDK9 (140 MDa) and a plasmid of 105 MDa, and *E. coli* V517 with plasmids of 1.4, 1.8, 2.0, 2.6, 3.4, 3.7, 4.8 and 35.8 MDa (Macrina et al. 1978) were used as standards.

Two-dimensional electrophoresis, as described by Hintermann et al. (1981), was used to distinguish between covalently closed circular plasmid DNA and relaxed, open, circular plasmid DNA. After separating a plasmid preparation in a horizontal 0.8 % agarose gel in Tris-acetate buffer, the gel was stained with EtBr as described, and photographed. The gel was then exposed to UV light from a hand lamp (UV-50 series, UVP International, Inc., San Gabriel, CA, USA) emitting short wave (254 nm) radiation, at a distance of 15 to 30 cm for 5 min. The gel was then turned 90°, and subjected to electrophoresis for another 8 h at 50 V before being restained and photographed under UV light.

Variation in growth conditions and treatment of the isolated plasmid DNA. Efforts were made to determine whether plasmid profiles varied as a result of different growth or lysis conditions of the bacteria, or after exposure of the plasmid DNA to UV illumination or mechanical stress.

To test the effect of growth conditions, 3 strains of *Aeromonas salmonicida* ss *salmonicida* were grown aerobically in 3 different broth media for 48 h at 15 °C before harvesting the cells. The media were BHIB (Difco), LB, and seawater yeast peptone (SWYP) broth. SWYP broth was prepared with artificial seawater as described by Hendrie et al. (1970). In addition, 2 strains (one from the spleen; the other from a muscle lesion of the same fish), which had shown varying plasmid profiles on repeated plasmid isolations, were grown aerobically on LB at 15 °C, and harvested at different points in the growth curve (i.e. after 20, 22.5, 43.5, 47 and 67 h).

To investigate combined effects on the plasmid profiles of the lysis step and the other steps of the plasmid DNA isolation procedures, cells were harvested from 2 colonies on an agar plate and lysed directly in the wells of an agarose gel. The colony material was mixed with 15 µl of a lysozyme solution [lysozyme, 7500 U ml^{-1}; RNase I, 0.3 U ml^{-1}; 0.05 % bromphenol blue in TBE buffer; 20 % Ficoll Type 400 (Pharmacia, Uppsala, Sweden)] which had been previously placed in the empty agarose well. The suspension was incubated at room temperature for 5 min before a 30 µl layer of 0.2 % SDS in TBE buffer and 10 % Ficoll Type 400 was carefully placed on top of the bacterial suspension. After a gentle mixing in the well, a 100 µl volume of a mixture of 0.2 % SDS and 5 % Ficoll Type 400 was added as an overlay without disturbing the contents of the well. The wells were sealed with hot agarose, and the gels run in TBE buffer.

To test whether mechanical stress during isolation procedures produced conformational changes in the plasmid DNA, resulting in more than one band from the same plasmid, plasmid DNA was flushed through plastic pipettes and syringe needles (25G × $^{5}/_{8}$) a varying number of times before the samples were electrophoresed as described.

To investigate the effect of UV illumination on plasmid DNA, plasmid DNA samples were spotted in droplets on a plastic sheet and illuminated with UV light of 254 nm from a hand-lamp (UV-50 series, UVP International, Inc., San Gabriel, CA, USA) for 7 min at a distance of 10 cm. The samples were then electrophoresed as described.

DNA-DNA hybridization. Gels with separated plasmid DNA were dried in a gel drier (Bio Rad, Model 443, Slab Dryer, Bio Rad Laboratories, Richmond, CA, USA) and stored at room temperature. Probe DNA was prepared from plasmid DNA of *Aeromonas salmonicida* ss *salmonicida* isolate 2921/89 purified in a CsCl/EtBr gradient, and separated in low melting point agarose (Sea Plaque GTG agarose, FMC BioProducts Rockland, ME, USA) (LMA) on a horizontal gel apparatus. This strain had only one large plasmid band of 52 MDa which was excised from the LMA gel. The plasmid DNA was labelled with ^{32}P directly in the LMA using an oligolabelling kit (Bethesda Research Laboratories, Inc, Gaithersburg, MD, USA). Hybridization tests were performed at 42 °C overnight without any prehybridization. The gels were washed in 5 × SSC, 0.1 % SDS at 65 °C. They were then wrapped in thin plastic, and X-ray films (Kodak XR, Rochester, NY, USA) were exposed to them for 2 d.

RESULTS

All the isolates in the collection had one or more large plasmid bands sized 52, 72, 85 or 105 MDa, some isolates yielding all 4 of the large plasmid bands (Fig. 1). This variation in the large plasmid band profiles occurred even in isolates from the same fish farm. Even isolates obtained from the kidney and muscle lesions of the same diseased fish showed variation in the number and size of their large plasmid bands. During one disease outbreak, isolates of *Aeromonas salmonicida* ss *salmonicida* were isolated from the kidney, the spleen, and from muscle lesions in 6 fish with clinical signs of furunculosis. On original testing, only isolates from 4 fish gave identical plasmid profiles. On

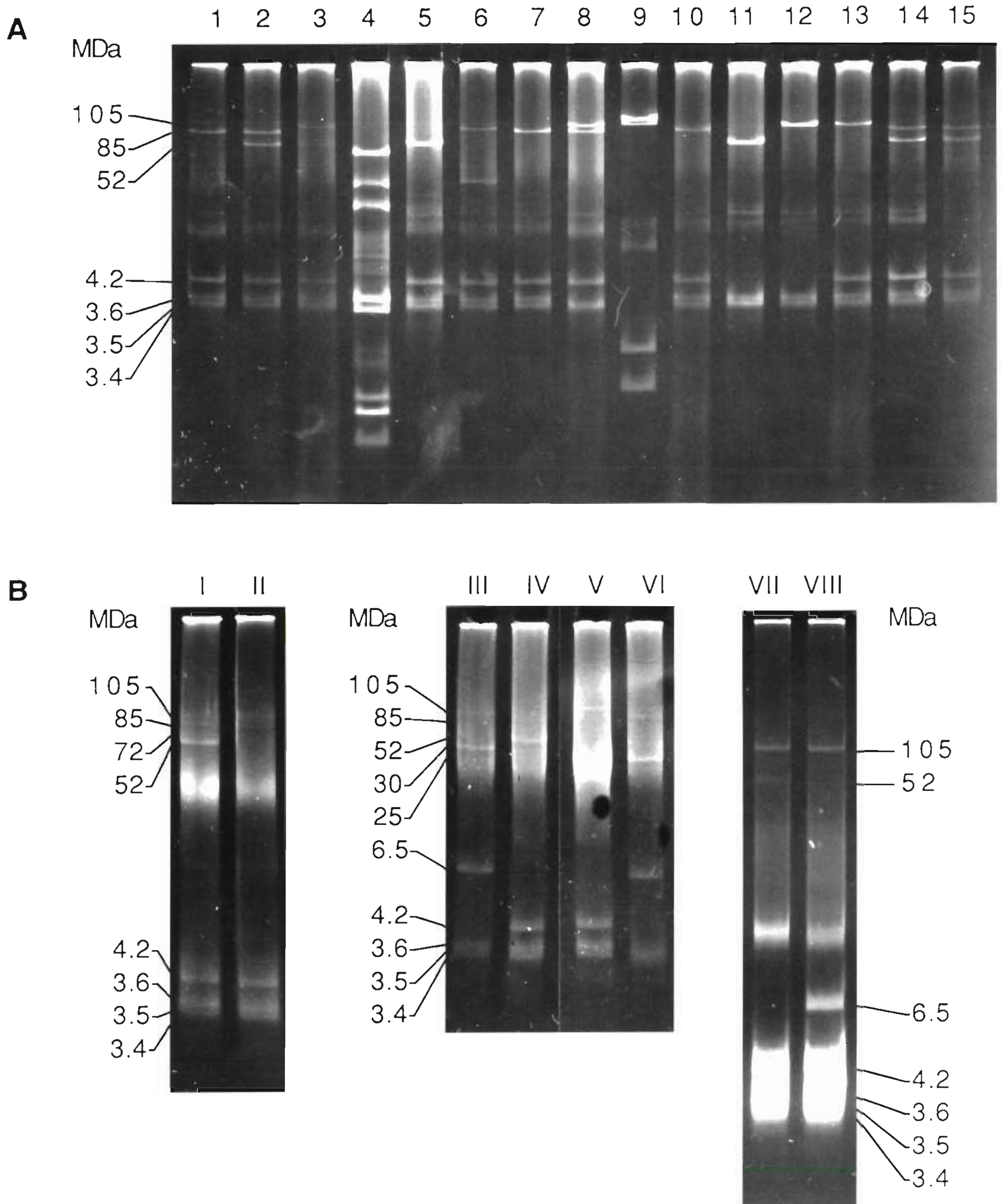

Fig. 1. Plasmid profiles of *Aeromonas salmonicida* ss *salmonicida* isolates after plasmid isolation by the Kado & Liu (1981) method. Plasmid sizes are given in megadaltons (MDa). (A) Lanes 1 to 3, 5 to 8, and 10 to 15 contain regular plasmid profiles of *A. salmonicida* ss *salmonicida*. Lane 4 contains standard plasmids from *Escherichia coli* V517 with sizes of 35.8, 4.8, 3.7, 3.4, 2.6, 2.0, 1.8 and 1.4 MDa. Lane 9 contains standard plasmids of 140 (pDK9) and 105 MDa. The isolates in lanes 11 and 12 both lack the 4.2 MDa plasmid. The isolate in lane 8 lacks the 3.6 MDa plasmid. The 3.4 and 3.5 MDa plasmids comigrate in this gel. Weak bands of the open circle plasmids of the small plasmids of 3.4 to 4.2 MDa can be seen between the large and small plasmids. (B) Lane I contains all 4 large plasmid bands seen in *A. salmonicida* ss *salmonicida* in one profile. Lanes III and VI contain intermediate sized plasmids (these are R-plasmids; data not shown) of 30 and 25 MDa, respectively, in addition to the regular plasmids of *A. salmonicida* ss *salmonicida*. The profiles in lanes III and VI contain a 6.5 MDa plasmid instead of the 4.2 MDa plasmid commonly seen. Lane VIII contain a 6.5 MDa plasmid in addition to the regular plasmids seen in *A. salmonicida* ss *salmonicida*. Lanes II, IV, V and VII contain ordinary profiles of *A. salmonicida* ss *salmonicida*

Table 2. Large plasmid profiles of isolates from kidney (k), spleen (s), and muscle lesions (m) of salmonids from a single furunculosis outbreak at one farm. The plasmid isolation procedure for all the isolations reported in this table was the modified method of Kado & Liu (1981)

Fish no.	Plasmid profile (MDa)	
	First isolation	Second isolation[a]
1 k	–[b]	85
s	85, 52	105
m	105	52
2 k	105	105, 85, 52
s	105	105, 85, 52
m	105	105, 85
3 k	105	105, 85, 52
s	105	85, 52
m	105	85
4 k	105	85, 52
s	105	–[c]
m	105	–[c]
5 k	105	85, 52
s	105	105, 85, 52
m	105	105
6 k	105	105, 85, 52
s	105	52
m	105, 52	52

[a] The second plasmid profile test was performed more than 10 mo after the first one
[b] Plasmid profiling was not performed
[c] The isolate was lost during storage

Table 3. Occurrence and variation in profiles of large plasmids from isolates of *Aeromonas salmonicida* ss *salmonicida*. The plasmid isolation procedure used for all of the isolates in this table was the modified method of Kado & Liu (1981)

Plasmid profile (MDa)	Occurrence of profiles[a]	Isolates with var. profile[b]
105	228 (38.1)	26/52
85	110 (18.4)	32/45
52	65 (10.9)	10/16
105, 52	62 (10.4)	6/9
85, 52	53 (8.8)	12/18
105, 85, 52	28 (6.3)	7/7
105, 85	24 (4.0)	2/3
72	6 (1.0)	5/5
72, 52	6 (1.0)	1/2
105, 85, 72, 52	4 (0.7)	1/1
85, 72	2 (0.3)	–[c]
85, 72, 52	1 (0.2)	1/1
Total	599[d]	103/159

[a] Number of times a particular plasmid profile was observed in all testings and (in parentheses), the percentage of times that a particular plasmid profile was observed relative to the total number of times profiles were prepared
[b] Number of isolates that yielded a plasmid profile on retesting that differed from that on the initial testing relative to the number of isolates that were retested. Note: retesting was done from 1 to 8 times, depending on the isolate
[c] None of isolates with this plasmid profile was retested
[d] Total number of plasmid profiles observed as a result of the original and repeat testings

a second testing more than 10 mo later, all but one of the isolates yielded plasmid profiles that differed from the ones that were originally present (Table 2).

When plasmid profiles for a given isolate were repeatedly examined over time using the same plasmid isolation procedure (modified method of Kado & Liu 1981), it was found that the number of large plasmid bands in a gel varied. For example, while strain 3736/89 had only one large plasmid band of 105 MDa when first investigated, it showed 3 bands of 52, 85 and 105 MDa the second time plasmid isolation was performed. After the third plasmid isolation, only 2 bands of 52 and 105 MDa were visible in the gel.

The different plasmid profiles and their relative frequency of occurrence are presented in Table 3. Among the plasmid profiles made from the entire collection of isolates, one profile with a lone 105 MDa plasmid band (in addition to the small plasmids) was the most frequently seen (38.1 %) (Table 3).

Of the 383 isolates, 159 were plasmid-profiled more than once. Of these, 116 isolates were plasmid-profiled twice with 80 of them (69 %) yielding plasmid profiles that differed between the 2 testings. After several plasmid isolations, the plasmid profiles with the 105 MDa band were the most stable, with plasmid variation occurring in only half of the isolates showing this profile (Table 3).

The Kado & Liu (1981) method of plasmid isolation gave the best results with regard to the large plasmids. Use of a freshly prepared lysis solution with pH adjusted to 12.55 and incubation for lysis at 65 °C for close to 1 h produced the clearest plasmid profiles. Extraction with acid phenol using the shortest possible contact time gave clear and reproducible results. Sometimes 2 successive extractions were necessary. The mini-Birnboim method gave a high yield of the small plasmids, but a low yield of DNA from the large ones. In addition, the mini-Birnboim method gave 1 extra band from each of the 4 small plasmids. These bands migrated more slowly than the supercoiled plasmid DNA, and probably represented a relaxed form of the small plasmids. In conclusion the modified method of Kado & Liu (1981) gave the clearest and most easily read plasmid profiles of the 2 methods employed.

The amount of DNA in each of the large plasmid bands in a given isolate, visualized by the intensity of

each band, often varied. When the large plasmid occurred as a single band, the band intensity was strongest.

A group of 4 small plasmids of 3.4, 3.5, 3.6 and 4.2 MDa was seen in the majority of the 383 *Aeromonas salmonicida* ss *salmonicida* isolates investigated. The 2 smallest plasmids of 3.4 and 3.5 MDa were seen in every isolate, while the 3.6 MDa plasmid was lacking in 4 isolates (1 %), and the 4.2 MDa plasmid in 39 isolates (10.2 %). Nine of the 46 isolates (20 %) from countries other than Norway lacked the 4.2 MDa plasmid. One of the isolates (Ft 337) lost the 4.2 MDa plasmid during the study. One isolate lacked both the 4.2 and 3.6 MDa plasmids.

Plasmids intermediate in size between the large and small plasmids were seen in 28 (7.3 %) of the strains investigated. These plasmids varied in size from 6.5 through 10, 19, 23, 25, 30, 35, 38 and 40 MDa. No variation of the bands of plasmids of intermediate size were seen on repeated plasmid isolations. Sixteen isolates, all of them from Norway, had a 6.5 MDa plasmid instead of the 4.2 MDa plasmid. One isolate had both the 4.2 MDa plasmid and the 6.5 MDa plasmid (Fig. 1). Of the isolates with a 6.5 MDa plasmid, 7 from the same local area in Norway had a 25 MDa R-plasmid (Fig. 1) with 3 antibiotic resistance determinants. Another 5 of the strains with a 6.5 MDa plasmid had a 30 MDa R-plasmid (Fig. 1) with a tetracycline resistance determinant, and were isolated from another local area on the western coast of Norway (data not shown). Of the 46 strains originating from outside. Norway included in this study, 9 harboured intermediate-sized plasmids.

The 2-dimensional electrophoresis method involving UV radiation of the gel after regular electrophoresis showed that the smaller plasmids migrated as expected in the second phase of the electrophoresis. However, the larger plasmid bands of 50 MDa and more, did not migrate during the second phase of electrophoresis (data not shown).

Three different isolates that had all shown a 105 MDa plasmid band in the first plasmid preparation were grown in 3 different broth media (BHIB, LB, and SWYP) before plasmid isolation. One isolate produced 2 large plasmid bands of 105 and 52 MDa in SWYP, while only the 105 MDa band was produced in the 2 other media. Only the 105 MDa band was produced from the other 2 isolates.

Two strains (4190/88 3B and 3M) from the spleen and a muscle lesion, respectively, of the same fish which had shown varying plasmid profiles on repeated plasmid isolations were harvested at 20, 22.5, 43.5, 47 and 67 h. The length of incubation time had no effect on plasmid profile (data not shown). The storage of purified plasmid DNA at 4 °C for periods of up to several weeks before agarose gel electrophoresis did not seem to affect the appearance of plasmid bands in agarose gel.

Electrophoresis of plasmids from bacteria lysed in the wells of the agarose gel did not give any large plasmid bands (data not shown).

No large plasmid bands could be detected after UV illumination, or following mechanical stress of isolated plasmid DNA using the aforementioned syringe and needle procedure (data not shown).

Large plasmid bands of all observed sizes, hybridized with the plasmid of 52 MDa from strain 2921/89 when the latter was used as probe. This strain had shown only this particular 52 MDa plasmid band after several isolations. None of the control plasmids from *E. coli* hybridized with the probe (Table 4).

DISCUSSION

The plasmid content of the large number of *Aeromonas salmonicida* ss *salmonicida* isolates examined in this study was very homogeneous when all of the plasmids present in the organism were considered. Most of the variation that occurred was that involving the profiles yielded by the large plasmid(s) (i.e. those ranging in size from 52 to 105 MDa). Unlike the single dominant plasmid profiles seen in *Salmonella* isolates during outbreaks of salmonellosis in man and animals (Holmberg et al. 1984, Olsvik et al. 1985, Sørum et al.

Table 4. Homology between the DNA of the 52 MDa plasmid band of *Aeromonas salmonicida* ss *salmonicida* isolate 2921/89, used as radioactive labelled probe, and the large plasmid bands of other *A. salmonicida* ss *salmonicida* isolates and plasmid DNA of 2 *Escherichia coli* strains

Isolate	Origin[a]	Homology[b]	No homology[b]
2921/89	N	52	
3736/89	N	85	
2442/89	N	52 85	
2685/89	N	52 85	
250/86 Fi	N	85	
2893/85	CH	85 105	
2755/85 V	DK	52 85	
30/87	S	85 105	
34 DK	DK	52	
37 DK	DK	52 85	
42 DK	DK	105	
E. coli K 12			pDK9[c]
E. coli V 517			1.4, 1.8, 2.0, 2.6, 3.4, 3.7, 4.8, 35.8

[a] N: Norway; CH: Switzerland; DK: Denmark; S: Sweden
[b] Sizes of plasmids in MDa
[c] pDK9 has a size of 140 MDa

1990), the plasmid profiles in cases of furunculosis varied to a considerable degree within outbreaks, among isolates from a single diseased fish, and even within a given isolate. Our studies indicated that the large plasmids were susceptible to strees (both physical and mechanical), as was the large (170 MDa) plasmid of another fish pathogen, *Vibrio salmonicida*, investigated earlier by Sørum et al. (1992).

Susceptibility to physical and mechanical stress may explain why large plasmid profiles obtained using the gentle plasmid extraction technique of the Kado & Liu (1981) procedure were superior to those that resulted when the harsher mini-Birnboim method was used. Other factors tested, including the effect of the growth medium, the effect of age of the culture, and the effect of length of storage of the isolated plasmid at 4 °C appeared not to account for the large plasmid variability.

Large plasmids have also been described from *Aeromonas salmonicida* ss *salmonicida* isolates by other workers. For example, Bast et al. (1988), in a study of 35 isolates, reported that 1 or more large plasmids (50 to 90 MDa) were always present, with some of the isolates possessing plasmids varying in size from 63 to 77 or from 77 to 83 MDa. Likewise, a large plasmid (70 to 145 kb, equal to approximately 47 to 97 MDa) was also reported in each of 14 isolates studied by Belland & Trust (1989). Further, based on restriction enzyme analysis, there was considerable relatedness between these large plasmids.

In the present study, the hybridization tests indicated that all of the large plasmids shared common sequences, thus supporting the possibility that the different large plasmid bands may actually have represented different configurations of the same plasmid. Another possibility is that there were in fact up to 4 different large plasmids, each of them with common sequences, but that the individual plasmids did not always replicate to the stage where visible bands were produced. If the latter is true, this would constitute a hitherto unknown method of plasmid expression. Although unequivocal explanations for the large plasmid variability observed in this study are still not available, it is clear that the large plasmids would be useless as epizootiological markers in view of their 'within-isolate' variability.

When the intermediate and small-size plasmids carried by *Aeromonas salmonicida* ss *salmonicida* are considered, they appear to provide only limited potential for use as epizootiological markers. As already mentioned, the plasmids of intermediate size (6.5 to 40 MDa) appeared to be stable on repeated testing but only 28 (7.3 %) of the isolates, 9 of them from outside of Norway, carried these plasmids. The scope for unique plasmid profiles with respect to intermediate-size plasmids was thus rather limited. The same conclusion also appears warranted with respect to epizootiological usefulness of the small plasmids. It also has already been indicated that most of the isolates carried 4 small (3.4 to 4.2 MDa in size) plasmids, only 1 % (4) of the isolates lacking the 3.6 MDA plasmid, 10 % (39) of the isolates lacking the 4.2 MDa plasmid, and 0.003 % (1) of the isolates lacking both of these plasmids. These data indicate that the isolates were very homogeneous with respect to the small plasmids. Further, some of this variability may have developed after collection and holding of the isolates. (One isolate, for example, lost its 4.2 MDa plasmid during the study and a number of the other isolates that lacked this plasmid had been held in culture collections for some time.) Because of the homogeneity in the small plasmid profiles, the value of the small plasmids as epizootiological markers would be limited.

In summary, as a result of our 6 yr of study with 383 isolates of *Aeromonas salmonicida* ss *salmonicida* we conclude that the plasmid content of this organism is remarkably uniform and that, except in the limited number of cases where plasmid additions or deletions occur, their usefulness for epizootiological purposes is limited. The high degree of variability observed with the large plasmids was of no value for epizootiological work because the variability occurred within isolates.

Acknowledgements. We gratefully thank the following for donating isolates of *Aeromonas salmonicida* for the study: Dr A. McKay, Marine Laboratory, Aberdeen, Scotland; Dr M. Alexander, Institute of Aquaculture, University of Stirling, Stirling, Scotland; Dr P. Vestergård, Statens Veterinære Serumlaboratorium, Århus, Denmark; Dr Inger Dalsgaard, Danish Institute for Fisheries and Marine Research, Royal Veterinary and Agricultural University, Copenhagen, Denmark; Dr C. Michel, Institut National de la Recherche Agronomique, Jouy-en-Josas, France; Dr J. McArdle, Fisheries Research Centre, Dublin, Ireland; Dr O. Ljungberg, Statens Veterinærmedicinska Anstalt, Uppsala, Sweden; and Dr T B. Jøssund, Oppland, Norway. We acknowledge the technical assistance of Anne Berit Hvaal and Kari Dommarsnes. This study was supported by a grant from the Norwegian Agricultural Sciences Foundation to H.S.

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Responsible Subject Editor: T. Evelyn, Nanaimo, B.C., Canada

Manuscript first received: October 7, 1992
Revised version accepted: May 7, 1993