Studies on the vibro-disease of rainbow trout
(Salmo gairdneri irideus). 1. Therapeutic
effect of nitrofuran derivatives.

by Koichiro Hayashi, Shigeo Kobayashi,
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Studies on the Vibrio-Disease of Rainbow Trout (Salmo gairdneri irideus)
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Studies On The Vibrio-Disease of Rainbow Trout (*Salmo gairdneri irideus*)

I. Therapeutic Effect of the Nitrofuran Derivatives

By Koichiro HAYASHI, Shigeo KOBAYASHI, Tokiro KAMATA, and Hisao OZAKI

The Vibrio-disease in rainbow trout resembles furunculosis in fish. It has often been observed causing serious damage in several areas in rainbow trout farms. We note, for instance, a report concerning the conditions or circumstances of the occurrence of the present disease in "A report On the Survey of Diseases in Rainbow Trout (Niji-Masu Gyo-Byo Chosa Hokoku-Sho)" compiled by Tama Sub-Station of the Tokyo Fisheries Experimental Station (Tokyo-

1) An outline was published at the sixteenth meeting of the Yoson-Bu (Trout Culture Section) held in May, 1963 in the city of Hikone.

2) Shiga-Ken, Samegai Yoson Shiken-Jo (Samegai Trout Culture Experimental Station, Shiga-Ken)
To Suisan Shiken-Jo Tama Bunjo) (1963). The disease is caused by infection from Vibrio bacteria. There are several reports already available on this disease on subjects such as the properties of the pathogene, symptoms of the disease, treatment, or countermeasures (Hoshina '56, '57, '58; Endo '60, '61, '62; Kishi et al '58; Murae et al '59; and Uchida '61). Recently Hoshina ('65) made a detailed report on this disease.

On the other hand nitrofuran derivatives, on account of their superior antibacterial properties, are used widely as food preservatives in accordance with the Food Sanitation Act in bean curds, bean filling, meat products, and especially in marine products. They are also used in the treatment and prevention of diseases in domestic animals such as poultry.

Over a period extending from 1962 to 1963, the authors tested the effects of nitrofuran derivatives against Vibrio-disease which broke out among rainbow trout in Samegai Trout Culture Experimental Station in Shiga-Ken. They were able to confirm their effectiveness in the prevention and treatment of the present disease. This report deals with their work.

The authors wish to express their thanks to the staff members of the above-mentioned station for their assistance and cooperation during the test. They also wish to thank the Ueno Chemicals Ltd. who presented them with nitrofuran derivatives.

I Pathogenic Vibrio Bacteria and Their Pathogenic Properties

In order to test the effects of nitrofuran derivatives, the authors began the experiments first by isolating pathogenic Vibrio and then by examining its various properties.
I. Experimental Method

Isolation of Pathogenic Vibrio

The surface of the diseased section of one-year and two-year rainbow trout (body lengths approximately 15cm and 30 cm, respectively), which had been raised at Samegai Rainbow Trout Culture Experimental Station, and which had been affected by Vibrio disease, was disinfected with alcohol, and washed with sterilized physiological salt solution; then the skin and muscle were separated with a sterilized knife, and the bacteria were fished out with a platinum loop from the muscle which had started to disintegrate and liquefy. The bacteria, then, were smeared over a flat culture ground whose composition is shown in Table 1. They were cultivated at 23°C and pure culture was obtained from the resulting colony. In the case of fish which had been severely affected, the bacteria were similarly isolated from other areas such as blood, kidney, liver, and contents of intestines.

Bacteriological Examination of Isolated Strains

The examination of the various properties of the isolated bacterial strains was carried out in accordance with the methods described in such reference materials as Bergey's Manual of Determinative Bacteriology 7th ed. (‘57), Shaw & Clarke (‘55) or Leifson (‘63).

Pathogenic Properties of Isolated Bacteria

In order to confirm the pathogenic properties of the isolated pure bacteria, specimens were collected twice from the colony with a platinum loop, cultivated at 23°C for 48 hours in a slanted cul-
ture ground made of the materials shown in Table 1, and mixed with 10 ml of sterilized physiological salt solution. 0.1 ml of this bacterial suspension (number of bacteria - approximately $10^8$/ml) was injected in the dorsal muscle or abdominal cavity of 10 rainbow trout in each case - a total of 20 specimens which hatched 9 months before - and the daily number of deaths after the injection was observed. The water temperature during this period was kept at near 8°C. The observation was continued for 4 days. As contrast, specimens, which had been injected similarly with 0.1 ml of sterilized physiological salt solution, were used.

2 Experimental results and Discussion

Isolation of the Pathogenic Vibrio Bacteria

The fish, infected with the Vibrio bacteria, presented symptoms similar to those reported by Hoshina (’56, 65). In serious cases the tissues in the focus liquefied and were filled with blood. Blood spots and discoloration were also observed. In every case a large number of bacteria was present. Although a large number of bacterial strains was isolated from various tissues, the strains shown in Table 2 were selected for our study. As the pathogenic bacteria were being isolated from various tissues of the diseased fish, microscopic specimens were also prepared. An examination of the latter confirmed the presence of an extremely large number of Vibrio bacteria in the blood, muscles, kidney, liver, or contents of the intestines.

Vibrio bacteria, wholly identical in shape to those observed in the direct smear test of various tissues, were obtained in a large number from the plate culture. At the same time a small
number of bacilli, which showed no pathogenic property, was also obtained. It was decided to subject these to bacteriological examinations at the same time and use them in our experiments to compare them with the pathogenic Vibrio. These non-pathogenic bacteria probably invaded the focus as a result of secondary infection. However, infection by pathogenic Vibrio concurrently with that by the aforementioned bacilli may affect the symptoms in fish in some way.

**Pathogenic Properties of Vibrio Bacteria**

The pathogenic characteristics of the isolated bacterial strains are shown in Table 2. An examination of the table shows that only Vibrio bacteria showed pathogenic properties, and that the others did not show any pathogenic properties. The amount of Vibrio bacteria used in the inoculation was approximately $10^7$ for every rainbow trout of 8-9 months. Fish began to die 3 days after the inoculation when the water temperature was near $8^\circ C$, and after two days in near $10^\circ C$ water. Though the difference was slight, in comparison to the case where inoculation with Vibrio bacteria was made in the abdominal cavity, the number of deaths was larger in the case where the inoculation was made into the dorsal muscle.

The point of inoculation with the pathogenic bacteria of the dead fish reddened and was somewhat swollen in every case. Further, reddening was observed in the oral cavity and fins in some cases. It is needless to mention that the original pathogenic Vibrio were again isolated from the dead fish.

In order to determine the minimum lethal dosage of pathoge-
genic Vibrio in rainbow trout, pathogenic Vibrio suspension (M-1 bacterial strain) in sterilized physiological salt solution was prepared and repeatedly diluted ten times. 0.1 ml of each of the diluted solution was inoculated in rainbow trout in a way identical to that described in a previous section. The results are shown in Table 3. An examination of the table appears to suggest that the fatal dose for rainbow trout ranging in weight 100-150g is tens of thousands. It should be noted that since the experiments were carried out at low temperature ranging 6-8°C, the extent of the increase in fish in the number of virulent bacteria which was inoculated into the muscle of rainbow trout or the degree of toxicity produced is unknown. In the light of the fact that the rainbow trout were dead in 2-3 days after the inoculation, even though M-1 strain had multiplied during this period, one can hardly imagine the extent of its increase to be too significant. Thus one may conclude that the observation indicates the strength of the pathogenic property of the M-1 strain. The nature of the toxin, which is produced by the pathogenic Vibrio, has not been established. It may be interesting to conduct a comparative study with endotoxin (regarded as polysaccharide-lipid complex) produced by Vibrio comma (cholera bacteria).

**Bacteriological Characteristics of the Isolated Strains**

The main features of the bacteriological properties of the isolated pure bacterial strains are shown in Table 4.

All the strains mentioned in Table 4 grow well in ordinary agar culture medium. They are aerobic or facultative anaerobic bacteria. They possess polar flagella and move vigorously. They are Gram-negative, produce no spores, and present rod-like or vibrioid appear-
There have already been several reports concerning vibrioid bacteria from several species of fish. Of these the best known are *Vibrio anguillarum* isolated mainly from saltwater fish and *Vibrio piscium* from freshwater fish which was reported by David ('27).

Schäperclaus ('54) reports one more -- *Vibrio aaser* as the pathogenic bacteria of Hechtseuche in Norway. However, the ones which are causing general concern at the present time are the former two.

In view of the fact that the *Vibrio* bacteria under study have been isolated from freshwater fish, one must first compare them with *V. piscium*. They differ a great deal in their reaction to sugar. *V. piscium* does not act on sugar. It is known that *Vibrio* bacteria under study produce acid from sugar. We may regard this phenomenon as indicating a significant difference between the present *Vibrio* bacteria and *V. piscium*.

The *Vibrio* bacteria, which had been isolated from rainbow trout in Japan, have been reported by Hoshina ('56, '57, '58, '65) and other research workers. Hoshina ('57) investigated the *Vibrio* bacteria from rainbow trout, concluded them to be a new mutant from *V. piscium*, and named it *Vibrio piscium var japonicus*. This follows the observation that unlike *V. piscium* it produces acid from sugar.

Thus, we must examine if the *Vibrio* bacteria under study are identical to the *Vibrio* bacteria, which have been isolated by Hoshina from similar rainbow trout of Japan. Since all four bacterial strains (M-1, M-5, L-1, and L-3), which the authors and other workers had isolated in the present study, have shown absolutely identical bacteriological properties, they may tentatively be regarded as belonging to the same species. Further, it was observed
that their properties almost coincided with various properties reported by Hoshina, i.e., we refer to such phenomena as the liquefaction of gelatin, formation of nitrite from nitrate, \( \beta \)-hemolysis of blood, and formation of acid by fermenting sugar of various kinds without producing any gas. Further, they decompose sucrose, mannose, and arabinose together and produce acid. Thus they belong to Type III if one follows the fermentation type defined by Heiberg.

Smith ('61), noting that \textit{V. piscium var japonicus} named by Hoshina is not strong in its action in the formation of indol or \( \text{H}_2\text{S} \), that it produces nitrite from nitrate, and that it produces acid from various kinds of sugar without producing gas, suggests that it may be more appropriate to regard it as a mutant of \textit{V. anguillarum} rather than that of \textit{V. piscium} and to name it as \textit{V. anguillarum type C}. The observation by Smith that the Vibrio bacteria of Hoshina resemble \textit{V. Anguillarum} is natural from the point of view of their biochemical properties. However, \textit{V. anguillarum} is originally of a saltwater variety and is reported in saltwater fish. It develops well when the salt concentration in the culture ground is in the range 0.5-6\%. Schäperclaus reports that it cannot survive when the concentration falls below 0.07\% or rises above 8.5\%. van Duijn ('56) reports that it does not grow at less than 0.25\%. It is reported that the optimal salt concentration is in the range 1.5-3.5\%.

On the other hand, the pathogenic Vibrio bacteria, which have been isolated by the authors and their co-workers, develop well in somewhat lower NaCl concentration in the range 0.1-3.0\%. If one takes into account this observation only, one may say that
they resemble \textit{V. piscium} rather than \textit{V. anguillarum}. Further, they differ from \textit{V. anguillarum} with respect to such phenomena as the negative response to Voges-Proskauer reaction, formation of nitrite, or the fact that they do not congeal milk. If the Vibrio bacteria of Hoshina's and consequently those of the authors' resemble \textit{V. anguillarum} as maintained by Smith, it may be said that they also resemble \textit{V. comma} as shown in Table 5. Thus we are rather inclined to feel that it be regarded as an aquatic type of \textit{V. comma}, and that it may be convenient to give it such a name as \textit{Vibrio ichthyocholerae}. The authors were unable to acquire Vibrio static agent and consequently have not tested its reaction against it. There is room for argument as to which of the two species the present pathogenic Vibrio belong; however, there is no room for argument against the fact that it belongs to the Vibrio family.

Table 5 compares the main characteristics of the Vibrio bacteria mentioned in the preceding sections.

The non-pathogenic bacterial strains isolated, M-2, M-3, M-4, M-6, and L-1 do not form distinct water soluble pigments, and possess polar flagella. Because of their oxidative decomposition action on sugar, they are regarded as belonging to the \textit{Pseudomonas}. The two bacterial strains, L-2 and K-2, possess polar flagella, produce acid from litmus milk, congeal and digest milk slowly, and decompose glucose with the formation of gas. Thus, they are regarded as belonging to the \textit{Aeromonas}. A reference to Bergey's Manual suggests that the bacteria resemble \textit{Aeromonas liquefaciens}.

\textbf{II Effects of Nitrofuran Derivatives on the Pathogenic Vibrio}

In order to establish the extent of the in vitro and in
vivo effects of nitrofuran derivatives on the isolated pathogenic Vibrio the following experiments were carried out.

1. Experimental Method

The Effective Concentration of Nitrofuran Derivatives on Isolated Bacterial Strains

The isolated bacterial strains were cultivated at 23°C for 48 hours in a sloped surface of ordinary agar. A specimen of bacteria was collected by dipping a platinum loop once and added to 10 ml of ordinary bouillon and the test bacterial suspension was prepared. A drop of the suspension was inoculated into the next culture medium to which a nitrofuran derivative had been added. The nitrofuran derivative (Ueno Chemicals) was added in varying concentrations into the bouillon with the following composition.

The Composition of the Ordinary Bouillon

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Bonito extract</td>
<td>2g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2g</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.5g</td>
</tr>
<tr>
<td>Tap Water</td>
<td>1,000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Sterilized for 15 min. at 15 lb.

The cultivation was carried out for 72 hours at 23°C after the inoculation of the test bacteria, and the effective concentration was obtained on the basis of the development of the bacteria.

The Resistivity Against Infection by Inoculation of Pathogenic Vibrio in Fish to Which Furazolidone was Administered

The following experiment was carried out to ascertain if
the rainbow trout, to which one of the nitrofuran derivatives, fura-
zolidone, had been administered, developed resistance against in-
fection by pathogenic Vibrio.

Rainbow trout (11-12 months after hatching) weighing 100-
150g were raised for two weeks with feed to which 0.02% furazolidone
had been added. Materials ordinarily used were used as feed. Five
rainbow trouts, raised in this manner, were assigned to each of the
sections shown in Table 7 to which a fixed quantity of pathogenic
vibrioid suspension with varying degree of concentration was ino-
culated, i.e., the bacteria of M-1 strain were collected from the
sloped surface of the culture medium made of ordinary agar by
dipping the platinum loop twice, and the suspension was prepared
by mixing them with 10 ml of sterilized physiological salt solution.
This was used as the mother liquid and diluted with sterilized physi-
ological salt solution to make the concentration of the resultant
solutions $10^{-1}$, $10^{-2}$, and $10^{-3}$, thereby making a series of inocu-
lant of pathogenic Vibrio 0.1 apart in concentration, and 0.1 ml
of each of the solution was injected into the dorsal muscle of the
rainbow trout. 5 control rainbow trouts, which had been
raised with ordinary feed without furazolidone, were added to each
section, inoculated similarly with the 1/10 series of pathogenic
Vibrio, and the results were compared. After the inoculation, dead
fish were watched for and taken out of the water immediately.

**Concentration of Furazolidone in the Body of the Fish to Which It
was Administered**

In the present experiment the authors are concerned with the
case where 0.02% of furazolidone is added. However, the knowledge
on the concentration, at which the nitrofuran derivative is maintained in fish, when it is administered as an additive to feed, is necessary not only for controlling the development of the pathogenic bacteria effectively but also for the proper administration of the nitrofuran derivative.

Thus the authors extracted serum, muscles, and liver of rainbow trout, which had been continuously fed with furazolidone for two weeks or two months and made an attempt at quantitative determination microbiologically of the amount of furazolidone derivative in them. That is to say: the serum, muscles, and liver were pulverized and diluted 0, 2, 4, and 8 times in bouillon, to each of which an extremely small quantity of bouillon suspension of M-1 bacterial suspension was inoculated, and the mixtures were cultivated at 23°C for 24 hours; the approximate quantities of furazolidone were estimated by observing the conditions of growth of the M-1 bacterial strain. Previously the authors had confirmed the fact that the serum and muscles, to which furazolidone had been added at the rate of 0.25 ppm, inhibited the growth of the M-1 bacterial strain. Thus the judgement was made setting this concentration as a standard. The serum, muscles, and liver of control rainbow trout, not fed with furazolidone, were inoculated with the M-1 bacterial strain, treated in a similar manner.

2. Experimental Results and Discussion

Concentration of Nitrofuran Derivatives required to Inhibit Growth of Isolated Bacterial Strain

The minimal concentration of various nitrofuran derivatives added to bouillon required to inhibit the growth of isolated bac-
terial strains are shown in Table 6.

An examination of the table shows that, of the nitrofuran derivatives tested, there is hardly any difference in effects between "furaskin"*, furazolidone, and z-furan, and that they are very effective against the pathogenic Vibrio, 0.25 ppm being sufficient to inhibit the latter's growth. However, they do not show any antibacterial action against non-pathogenic bacterial strains, M-2, M-3, and M-4. Only exception was K-2 bacterial strain on which they showed considerable inhibitive effects. AF2 was the most effective in controlling the growth of the pathogenic Vibrio - being effective at 0.1 ppm. The above experiments indicate that in a test tube all of the nitrofuran derivatives exert severe inhibitive effects on the growth of pathogenic Vibrio.

Resistance Against Pathogenic Vibrioid Infection in Fish to Which Furazolidone was Administered

Table 7 shows the resistance in rainbow trout which were infected by inoculating them with pathogenic Vibrio suspension of varying concentrations after continuous feeding of furazolidone for two weeks.

An examination of the table shows that, in the case of the specimens to which furazolidon had not been administered, dead fish appeared from the third day after inoculation with Vibrio bacteria and continued to appear to the sixth day, and that the death rate was high in all of the inoculated sections. On the other hand, one notes that the death rates were clearly lower among the fish which had been fed with furazolidone, that the number of deaths decreased with the decrease in the amount of the inoculated bacteria, and that there was a delay in the appearance of dead fish in compa-

*Translator's remark: as transliterated.
rison to the control groups. These observations clearly show that the increase in the resistance was due to the administration of furazolidone.

Further, it is not likely that a violent infection of the kind described above, in which a large dose of pathogenic bacteria is injected directly into the muscles, can occur under natural breeding conditions. In view of the fact that the administration of furazolidone is effective against sudden and massive infections as those described above, it is clear that the administration of furazolidone will be an effective suppressive or preventative means against slow infection by the pathogenic bacteria under natural conditions.

**Furazolidon Concentration in the Body of Fish Administered Furazolidone**

The serum, muscles, and liver collected from rainbow trout, to which furazolidon had been administered over periods of two weeks or two months, in every case inhibited the growth of the M-1 bacterial strain when it had not been diluted by bouillon. However, when they were diluted more than two times, growth in the M-1 bacterial strain was observed. In the light of these observations, the authors inferred that the samples contained approximately 0.25 ppm of furazolidone. The results did not differ between the samples which had been administered furazolidone for two weeks and those administered for two months. It was established that furazolidone was not accumulated in the body, and that furazolidone was retained in the body in a concentration much lower than that which had been anticipated. The relation between the quantity of furazolidone added to the feed, quantity absorbed, or the speed with which it is elimi-
nated is an important matter for research in order to administer furazolidone effectively. However, these questions will be left for future study. Similar observations were made on the various tissues of rainbow trout to which furazolidone had not been administered. The results indicated that in every case they were incapable of inhibiting the growth of the M-1 bacterial strain.

There was no unfavourable effect on the growth or health of fish even though the administration of furazolidone had been continued as long as three months. Vibrio-disease did not break out among the fish to which furazolidone had been administered. Further, in the case of fish in which the disease was progressively worsening, the administration of the present chemicals brought about a shrinkage in the affected part which eventually healed. These observations also suggest the effectiveness of the nitrofuran derivatives against Vibrio-disease.

**Conclusions**

During 1962-1963 the authors isolated from rainbow trout which had been affected by Vibrio-disease, which had broken out in Samegai Trout Culture Experimental Station in Shiga-Ken, pathogenic Vibrio and co-existing non-pathogenic bacteria found in the focus, investigated the effects of nitrofuran derivatives on these bacteria, and observed the resistance of rainbow trout against infection by pathogenic Vibrio bacteria.

1. Certain of the isolated pathogenic Vibrio showed almost identical bacteriological properties as those reported by Hoshina and other investigators. The present Vibrio bacteria were examined in comparison with other Vibrio bacteria which have already been reported.
2. The muscular injection of several thousands of the isolated pathogenic Vibrio brought about deaths at 6-10°C in 2-6 days in rainbow trout weighing 100-150g.

3. The nitrofuran derivatives exert extremely powerful anti-bacterial effects on the pathogenic bacteria. 0.25 ppm of furaskin, furazolin, and $\varepsilon$-furan and 0.1 ppm of AF$_2$ in bouillon inhibited their growth.

4. All of the nitrofuran derivatives showed almost no anti-bacterial effects on the non-pathogenic bacteria tested (Pseudomonas sp. and Aeromonas sp.) with the exception of one bacterial strain.

5. Rainbow trout, which had been raised for 2 weeks with feed to which 0.02% of furazolidone had been added, showed considerable resistance against infection by inoculation of pathogenic Vibrio.

6. Even after administering feed with 0.02% furazolidone additive for three months, no abnormality in health, growth and other factors of rainbow trout was observed.

7. When rainbow trout was raised with the feed with 0.02% furazolidone, the concentration of furazolidone in the body of rainbow trout remained at about 0.25 ppm. This was recognized as the effective concentration in controlling the growth of pathogenic Vibrio.

On the basis of the results described above the authors concluded that the nitrofuran derivatives are effective agents in the prevention and treatment of Vibrio-disease in rainbow trout.
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no Eisei Saikin-Gaku teki Kenkyu. Yonago I-Gaku Zasshi 10 (6),
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To Suisan Shiken-Jo, Okutama Bunjo), 1963: A Report On a Survey
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Kogyo KK. Tokyo).
Table 1

Table 1. Composition of medium used for isolation of pathogenic *Vibrio*.

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<thead>
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<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
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<td>Yeast extract</td>
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<tr>
<td>Bonito extract</td>
<td>1g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1g</td>
</tr>
<tr>
<td>Agar</td>
<td>15g</td>
</tr>
<tr>
<td>Tap water</td>
<td>1,000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Table 2

Table 2. Strains isolated from diseased fish and their pathogenicity.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Isolated strain</th>
<th>Form</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>M-1</td>
<td>curved</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>M-2</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M-3</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M-4</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>M-5</td>
<td>curved</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>M-6</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td>Intestine</td>
<td>I-1</td>
<td>curved</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>I-2</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>L-1</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L-2</td>
<td>rod</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L-3</td>
<td>curved</td>
<td>+</td>
</tr>
<tr>
<td>Kidney</td>
<td>K-1</td>
<td>curved</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>K-2</td>
<td>rod</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3

Table 3. Bacterial numbers which are fatal to rainbow trout. Virulent strain (M-1) was inoculated into the mid-portion of the dorsal muscle.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Amount of injection ml</th>
<th>Bacterial number</th>
<th>Mortality %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>$5 \times 10^5$</td>
<td>5/5 100</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>0.1</td>
<td>$5 \times 10^4$</td>
<td>5/5 100</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>0.1</td>
<td>$5 \times 10^3$</td>
<td>3/5 60</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>0.1</td>
<td>$5 \times 10^2$</td>
<td>0/5 0</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0.1</td>
<td>$5 \times 10^1$</td>
<td>0/5 0</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0.1</td>
<td>5</td>
<td>0/5 0</td>
</tr>
</tbody>
</table>

Table 5

Table 5. Differential reactions of Vibrios

<table>
<thead>
<tr>
<th>Gelatin</th>
<th>H₂S</th>
<th>Citrate</th>
<th>M. R.</th>
<th>V. P.</th>
<th>Cholera-red</th>
<th>Indole</th>
<th>NO₃</th>
<th>Milk</th>
<th>Potato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present species</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>yellow</td>
<td>+</td>
<td>+</td>
<td>no change</td>
<td>yellowish, smooth, luster</td>
</tr>
<tr>
<td>V. comma</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>red</td>
<td>+</td>
<td>+</td>
<td>no change</td>
<td>whitish, moist, spreading growth</td>
</tr>
<tr>
<td>V. anguillarum</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>clot proteolysis reduction</td>
<td>smooth, shiny yellow</td>
</tr>
<tr>
<td>V. piscium</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>soft coag. pept. alkaline</td>
<td>brownish red streak</td>
</tr>
</tbody>
</table>

Reactions to various sugars

Present species
- acid, no gas — arabinose, glucose, fructose, maltose, glycogen, starch, mannit, galactose, mannose, trehalose, sucrose, lactose, dextrin.
- no acid, no gas — inositol, raffinose, rhamnose, xylose, cellulbiose, inulin, salcin, dulcitol, adonit, glycerin, sorbit, sorbose.

V. comma
- acid, no gas — glucose, galactose, maltose, fructose, sucrose, mannitol, glycerol.
- no acid, no gas — lactose, inulin, dulcitol.

V. anguillarum
- acid, no gas — glucose, fructose, sucrose, maltose, trehalose, starch, dextrin glycogen, mannitol, sorbitol.
- slight acid, no gas — galactose.
- no acid, no gas — arabinose, rhamnose, xylose, lactose cellulbiose, raffinose, inulin, glycerol, dulcitol, inositol, amygdalin, salcin.

V. piscium
- no action in sugar media
Table 6. Inhibition of bacterial growth by nitrofuran derivatives. (ppm)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nitrofurazone</th>
<th>Furazolidone</th>
<th>α-furan</th>
<th>AF₂</th>
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<tbody>
<tr>
<td>M—1 (virulent)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.1</td>
</tr>
<tr>
<td>M—2</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
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<tr>
<td>M—3</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
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<tr>
<td>M—4</td>
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<td>&gt; 100</td>
<td>&gt; 100</td>
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<tr>
<td>M—5 (virulent)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.1</td>
</tr>
<tr>
<td>I—1 (virulent)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.1</td>
</tr>
<tr>
<td>K—2</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>8.5</td>
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Table 7. Resistance of rainbow trout which had been fed with a diet containing furazolidone against a compulsory Vibrio infection.

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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td>Number of fish</td>
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<tr>
<td>Dilution of inoculated strain (M—1)</td>
<td>$10^{-3}$</td>
<td>$10^{-2}$</td>
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<td>$10^{-3}$</td>
<td>$10^{-2}$</td>
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<td>Time after inoculation (day)</td>
<td>Number of dead fish</td>
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<tr>
<td>Total number Killed by infection</td>
<td>5</td>
<td>3</td>
<td>5</td>
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<td>0</td>
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<td>2</td>
<td>4</td>
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<td>Mortality %</td>
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<td>60</td>
<td>100</td>
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<td>20</td>
<td>40</td>
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