USGS Biological Resources

Biological Information & Technology Notes

No. 97-008

Susceptibility of Salmonid Fishes to *Renibacterium salmoninarum*

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is a chronic disease that affects the salmonid fishes. Typical manifestations of BKD range from asymptomatic in carrier fish to internal and external clinical signs and mortality. The range of clinical signs may vary from one infected population to another or among individuals within the same infected population. Factors that might contribute to this range of disease expression are varying susceptibility of the hosts and differential pathogenicity or virulence of strains of the bacterium.

The purpose of this study was to evaluate the susceptibility of eight different salmonid hosts to various isolates collected from Lake Michigan and the Pacific Northwest, two geographic regions that are continually plagued with clinical BKD.

A total of 23 strains of *R. salmoninarum* were used in this study, representing five groups based on host and geographic site of origin. Four groups were from Lake Michigan: chinook salmon (*Oncorhynchus tshawytscha*) from Manistee Weir, Michigan (MCK); coho salmon (*O. kisutch*), also from Manistee Weir (MCO); chinook salmon from Kewanee Weir, Wisconsin (A strains); and chinook salmon from Strawberry Creek Weir, Wisconsin (B strains). Isolates from the fifth group were from chinook and coho salmon originating in the Pacific Northwest (PNW). The species type strain, ATCC 33209, was also used in the challenge studies.

Fish Exposed By Injection Challenge

Growth of each isolate used to challenge fish was achieved by inoculation into KDM2+M broth medium, incubated at 15 °C on a shaker (100 rpm) for 2-3 weeks. Serial 10-fold dilutions were then prepared in KDM2 broth. Viable cell counts were done to quantify the challenge dose. Groups of 9-15 fish were challenged with 0.1 mL of each dilution by intraperitoneal injection. The number of bacteria challenged was reported as colony-forming units per mL (cfu/mL). Eight different species of salmonids were challened: brook trout (*Salvelinus fontinalis*), rainbow trout (*O. mykiss*), chinook salmon (*O. tshawytscha*), lake trout (*Salvelinus namaycush*), brown trout (*Salmo trutta*), coho salmon (*O. kisutch*), and both Atlantic salmon smolts and domestic *Salmo salar*.

A group of controls for each host was injected with KDM2 broth. Prior to challenge, representative numbers of fish from each population were determined to be BKD-free by the fluorescent antibody test (FAT) of kidney tissues. Following challenge, the fish were observed for 70 days, mortalities were recorded, and BKD was diagnosed by presence of numerous *R. salmoninarum* cells in kidney tissues evaluated by FAT. Experimental fish were supplied with spring water (1.5-3 L/min; 12.5 °C; 249 mg/L [$CaCO_3$] hardness).

Experiments

Two sets of experiments were carried out. In the first set of experiments, all *R. salmoninarum* isolates were used to challenge groups of brook trout only. This was done to determine if isolate virulence differences exist and if they could be related to the isolate origins. Virulence of individual isolates (LD_{50} values) was determined, and the effect of isolate origin was assessed by an analysis of variance. Results of this analysis revealed no significant difference ($F = 1.502$; $P = 0.243$) of virulence among the five isolate origins. The mean LD_{50} for all 23 isolates in brook trout was $1.087\ 10^6$ cfu/mL ($s = 2.022\ 10^6$), and the values ranged from $8.457\ 10^6$ to $2.227\ 10^4$ cfu/mL. BKD was diagnosed in all dead fish, and there were no mortalities in any control fish injected with cell diluent alone.

Based on the results determined from the challenges in brook trout, one isolate representing each of the five groups (MCK4M, MCO4M, A34, B26, 384) was selected in addition to ATCC 33209, and these six isolates then were used in the second set of experiments, which challenged the seven other hosts. For each host-isolate challenge combination, logistic regression was used to relate the probability of surviving the 70-day trial to isolate origin with challenge dilution (cfu/mL expressed in a log base 10 scale) included as a covariate. The effect of isolate origin on survival was tested by likelihood ratio tests using PROC LOGISTIC in the SAS/STAT software. Based on best fitting logistic regression models, LD_{50} values and 95% confidence limits were estimated.

Domestic Atlantic, Chinook, and Coho Salmon Were Most Susceptible

Kidney tissues of experimentally exposed fish mortalities examined by FAT showed that all had positive *R. salmoninarum* cells and most had numerous cells in each microscopic field. In addition, these fish showed some or all of the following clinical signs: abdomens were distended due to excess ascitic fluid, kidneys were swollen and liquified with a pale grayish color, and livers were pale red in color. Externally, there were no open lesions; however, petechial hemorrhaging was present on some chinook and coho salmon mortalities. Clinical signs were present in nearly all dead fish, regardless of dilution of the challenge isolate; however, length of time to death was highly dependent upon--and increased with dilution of--the cell challenge. Another characteristic of many fish was that they appeared healthy until 2-3 days prior to death, during which time they suffered appetite loss and became lethargic.

Differences in virulence among the isolates depended upon which host species was being challenged. Isolates were similar in their virulence when used to challenge the more resistant hosts, such as brook trout. More variation was noted among the more susceptible hosts. Had we used one of our more susceptible hosts to evaluate the relationship of group origin to virulence, there might have been a statistically significant difference among those groups.

In general, the isolates MCO4M, B26, and A34 tended to be more virulent; MCK4M, 384, and ATCC 33209 were less virulent. Overall, there was a wide range in LD_{50} values among the host-isolate challenge combinations, from as low as $2.94\ 10^8$ cfu/mL for ATCC 33209 in lake trout to the highest calculated virulence of $9.00\ 10^{-1}$ cfu/mL for MCO4M in chinook salmon. Three host species were relatively resistant (lake, rainbow, and brook trout); three hosts were relatively susceptible (coho, domestic Atlantic, and chinook salmon); and two were intermediate in their susceptibility to *R. salmoninarum*, Atlantic salmon smolts and brown trout. Of particular note were the differences in susceptibilities of the Atlantic salmon smolts and domestic Atlantic salmon for *R.*

salmoninarum isolates MCO4M, B26, and A34, the more virulent bacteria. In one comparison, the calculated LD_{50} of B26 was 5.34 10^5 cfu/mL for the smolts and 6.37 10^1 cfu/mL for the domestics. Along with the physiological changes associated with smoltification, other reasons for the differences in susceptibilities of these two populations might be size and age, and the potential for natural pathogen exposure of previous generations of the Atlantic salmon smolts. The cell challenge was not adjusted in relation to size of the host; however, for the B26 comparison, the number of viable cells used for challenge per gram of fish was actually higher for the Atlantic salmon smolts (5.50 10^5 cfu/g) compared to the domestic population (4.40 10^5 cfu/g).

Conclusions

Virulence of *R. salmoninarum* and death to the host in experimentally infected fish appears to be a result of the bacterium achieving a threshold number of cells within the fish. Mortality and time to death were, for many of the host-isolate combinations, highly dependent upon the 10-fold dilution (the number of challenge organisms) injected. With decreasing numbers of challenge cells, mortality tended to decrease and time to mortality increased, implying that a substantial infection must be established within the fish to cause death. Furthermore, a direct relationship to the quantity of cells used for challenge was established. Perhaps more interesting was an apparent relationship to the in vivo production of metabolite (extracellular growth product that enhances the bacterium's growth); with decreasing numbers of cells, more time is required for initial bacterial growth and subsequent metabolite production.

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Last Updated: Monday, 17-Nov-97 14:03:24 MST