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Evaluation of the Genetic Diversity of *Renibacterium salmoninarum*

Bacterial kidney disease (BKD), which results in significant mortality among wild and hatchery-reared salmonid fishes, is caused by the bacterium *Renibacterium salmoninarum*. Studies of this pathogenic bacterium include determination of the extent of genetic diversity over numerous strains within the species, which has important implications for disease treatment, taxonomic identification, serodiagnostics and vaccine development. The purpose of this study was to use multilocus enzyme electrophoresis (MEE) to determine the presence and mobility of enzyme activities for a representative number of *R. salmoninarum* isolates. The level of genetic diversity for the species could then be calculated.

Previous serological studies have shown strains of *R. salmoninarum* to be similar both antigenically and in their lack of extracellular biological activity. Other researchers have demonstrated the existence of two distinct phena by evaluation of biochemical, serological, and physiological studies. More recently, based on quantitative slide agglutination and Western blot assays, it has been suggested that there are two antigenically distinct groups.

Multilocus Enzyme Electrophoresis

Multilocus enzyme electrophoresis is a technique that combines electrophoresis in a starch gel matrix with specific enzyme staining. With MEE, variations in functional enzymes (allozymes) are detected in stained starch gels following electrophoresis. Positive activity is visually detected as bands in the gels. Presence or absence (null alleles) of activity is scored, as is the mobility of those bands present. Mobility differences among strains of bacteria are due to a difference in net charge of the enzyme, which is a result of the sum total of the amino acids that comprise the enzyme. After a number of enzymes are evaluated, a bacterial strain profile (electrophoretic type) is realized.

Using MEE, numerous bacterial isolates can be studied at relatively low cost. Furthermore, results are easily obtained for slow-growing or fastidious organisms such as *R. salmoninarum*, whereas other more conventional methods are exceedingly difficult. Results with MEE correlate highly with DNA/DNA hybridization, another well recognized method that requires more expensive equipment.

Diversity Of Isolate Origins Determined

The 40 isolates of *R. salmoninarum* used in this study represent five groups based on host and geographic location of origin: (1) various hosts from the Pacific northwest (PNW); (2) chinook salmon (*Oncorhynchus tshawytscha*) from the Manistee Weir, Michigan (MCK); (3) coho salmon (*O.*

kisutch) from the Manistee Weir, Michigan (MCO); (4) chinook salmon from the Kewanee Weir, Wisconsin (A strains); and (5) chinook salmon from Strawberry Creek, Wisconsin (B strains). Each isolate was grown by inoculation in 500 mL KDM2 broth medium supplemented with 1% filter-sterilized ATCC 33209 metabolite. Cultures were incubated on a shaker (100 rpm) at 15 C for 2–3 weeks. Cells were collected by centrifugation and were suspended in 15 mL of a lysate buffer (10 mm Tris, 1 mm EDTA, pH 6.8). They were again centrifuged and resuspended in 5 mL of fresh lysate buffer. While in an ice bath, cells were sonicated with 1-min bursts (five total). The supernatant (whole cell lysate) was placed into five vials and was frozen at -70 C until used.

Starch gel electrophoresis was conducted using 11%–11.5 % hydrolyzed starch. Two buffer systems were used to evaluate the isolates for each enzyme, a pH 6.5 amine-citrate and a pH 8.0 buffer. An isolate (B58) was used on all gels as a control (100 allele) to adjust the gel to gel mobility differences. Forty-four enzyme-staining systems were evaluated, representing hydrolases, isomerases, lyases, oxidoreductases, and transferases. Overall mean genetic diversity (H_T) was calculated from the resulting isolate profiles, as was genetic diversity for each locus (h_T) and for each isolate origin group at each locus (h).

Renibacterium salmoninarum Exhibits Relatively Low Genetic Diversity

Enzyme activity was detected among the isolates with 24 of the enzyme-staining systems. Two zones of activity (presumptive loci) were observed for the enzymes glycyl-leucine peptidase and esterase; therefore, genetic diversity was determined over 26 loci. No monomorphic loci were found and there was an average of only 2.65 different scores (electromorphs) per locus. The number of electromorphs per locus, by group, was: PNW (1.23), MCO (1.77), MCK (2.12), A (1.27), and B (1.15).

Mean genetic diversity (H_T) of all isolates was 0.161. This relatively low genetic diversity is comparable to that for other genetically homogeneous bacteria. The percentage of the mean genetic diversity calculated to be due to variability among isolate origin groups was minimal: G_{st} = 8.1% (i.e., 91.9% of the total genetic diversity was contributed by differences among individual isolates). Genetic diversity by group was highest for those isolates from Michigan Weir origin, MCO (0.298) and MCK (0.270); for isolates from the two Wisconsin origins, genetic diversity was A (0.067) and B (0.051), and 0.058 for those isolates from the PNW group. There were 21 electrophoretic types; however, 17 were represented by single isolates and many of these differed at only one locus, as indicated by the low mean genetic diversity relative to the electrophoretic type/isolate ratio (21/40).

The highest locus of genetic diversity was recorded for the slow locus of esterase (h_T = 0.587). Twenty-three of the 40 isolates displayed the 100 electromorph at this locus and 9 of 10 PNW isolates had an electromorph that was 35% faster. Consequently, the proportion of genetic diversity explained by variation among groups (G_{st}) was higher, 36.5%. Other loci with relatively high genetic diversity were succinate dehydrogenase (0.385), aconitase (0.311), and cytochrome oxidase (0.273).

The activity of cytochrome oxidase was noteworthy. It was previously reported that *R. salmoninarum* is negative for cytochrome oxidase, an important enzyme for taxonomic identification of bacteria when the standard biochemical method is used. This contrast in results could be explained by differences in preparation of the whole cell lysates for the MEE method. With MEE, cells are disrupted by sonication, perhaps releasing the enzyme into the lysate solution. Using the standard biochemical method, which employs colonial growth, the cells remain intact.

Conclusion

The results presented here indicate that strains of *R. salmoninarum* of North American origin are genetically relatively homogeneous. This finding does not corroborate the results of some previous studies describing diversity; however, it is noted that the different approaches used to evaluate variability did not use the same set of strains. Furthermore, the other studies included strains that were not of North American origin with those that were.

For further information, contact:

Clifford E. Starliper
USGS Biological Resources
National Fish Health Research Laboratory
1700 Leetown Road
Kearneysville, West Virginia 25430
(304)725-8461
Cliff_Starliper@usgs.gov
