

Biochemical, serological, and genetic characterisation of *Renibacterium salmoninarum* isolates recovered from salmonids in Chile

J Bethke^{1,2}, J Quezada^{1,2}, M Poblete-Morales^{1,2}, R Irgang^{1,2},
A Yáñez^{2,3}, C Oliver^{1,2} and R. Avendaño-Herrera^{1,2,4*}

¹Universidad Andres Bello. Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuicola, Facultad de Ciencias Biológicas, Viña del Mar, Chile; ²Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile; ³Facultad de Ciencias, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile; ⁴Centro de Investigación Marina Quintay (CIMARQ), Quintay, Chile

Abstract

The Gram-positive *Renibacterium salmoninarum* causes bacterial kidney disease, a serious threat to Chilean salmon farming. To aid in vaccine development, this study used biochemical, antigenic, and genetic techniques to characterise 39 *R. salmoninarum* isolates from diseased *Salmo salar* and *Oncorhynchus kisutch*. Regardless of host, *R. salmoninarum* isolates were highly homogeneous phenotypically. However, the isolates presented varied hydrophobicity (10-90%), as found by hydrocarbon adhesions, and distinct siderophores production, as evaluated on chrome azurol S agar. Serological assays established antigenic homogeneity among isolates, which can facilitate vaccine development. While western-blot profiles (using antiserum against iron-limited *R. salmoninarum*) differed, all isolates belonged to the same serogroup. Finally, RAPD, ERIC-PCR, and REP-PCR supported genetic *R. salmoninarum* homogeneity, suggesting clonal relationships. In conclusion, high homogeneity might facilitate vaccine development, but *in vivo* studies are needed to clarify the relationship of *R. salmoninarum* virulence with hydrophobicity/siderophores synthesis.

Introduction

Due to exponential growth over 20 years, the Chilean salmon industry is the second largest worldwide. Unfortunately, rapid productive gains directly impact sanitation, thus increasing the chance for bacterial infections. Bacterial kidney disease (BKD), caused by the Gram-positive *Renibacterium salmoninarum*, is a serious threat to salmonids globally, causing up to 80% mortality (Evenden et al., 1993). In Chile, this pathogen was initially isolated from chum

salmon (*Oncorhynchus keta*, Walbaum) reared in seawater cages (Sanders and Barros, 1986), but it has since been reported in Atlantic salmon (*Salmo salar* L.) and Coho salmon (*Oncorhynchus kisutch*, Walbaum) cultured in different Chilean regions.

Health screenings in 2015 found *R. salmoninarum* in 19% of samples, ranking second only to *Piscirickettsia salmonis*. Despite the impact

* Corresponding author's e-mail: ravendano@unab.cl; reavendano@yahoo.com