Aeromonas salmonicida subsp. salmonicida Type Strain Does Not Possess a Type III Secretion System

A study by Chacón et al. examined the presence of type III secretion genes in Aeromonas species of clinical significance. The authors demonstrated, by dot blot hybridization at 50°C, that the prevalence of these genes was high in clinical strains of A. hydrophila and A. veronii, particularly those isolated from extraintestinal infections. However, the positive control used in this study was A. salmonicida subsp. salmonicida CECT 8941. This raises concerns, as type III secretion genes are absent in an isoform of this strain, A. salmonicida subsp. salmonicida ATCC 33658, due to a lack of the plasmid that carries the type III secretion locus (1, 4).

We carried out a dot blot analysis of commonly used isoforms of the A. salmonicida subsp. salmonicida type strain using a probe against the gene ascV (5). Approximately 100 ng total DNA was denatured in 0.4 M NaOH, 10 mM EDTA and spotted onto positively charged nylon membranes. Hybridization was carried out overnight at 50 or 65°C using a digoxigenin-11-dUTP (DIG)-labeled probe for the ascV gene of the pathogenic strain A. salmonicida subsp. salmonicida JF2267. Reactions were detected using anti-DIG antibodies and the chemiluminescent reagent CPD-Star (Roche). When hybridization was carried out at 50°C, all A. salmonicida strains tested gave a positive result (Fig. 1A). However, A. hydrophila ATCC 79665 also gave a positive signal under these conditions. This is significant as the genome sequence of this strain has been published, and it does not contain type III secretion genes (3). The apparently false-positive signal obtained with A. hydrophila ATCC 79665 was eliminated by increasing the hybridization temperature to 65°C (Fig. 1B). Under these conditions, all A. salmonicida type strain isoforms were also negative for the ascV gene. However, A. salmonicida subsp. salmonicida JF2267, which served as our positive control, and a human A. hydrophila isolate, CDC 1271-82, both gave positive signals under these conditions.

We next performed a PCR for the ascV gene using primers specific for A. salmonicida subsp. salmonicida JF2267. All A. salmonicida subsp. salmonicida type strain isoforms gave a negative result, as did A. hydrophila ATCC 79665 (Fig. 1C). However, we were able to amplify the ascV gene of A. hydrophila CDC 1271-82 and A. salmonicida subsp. salmonicida JF2267. Sequencing and BLAST analysis of the resulting PCR product indicated that the ascV fragment of A. hydrophila CDC 1271-82 shares 87% nucleotide identity with A. salmonicida subsp. salmonicida JF2267. The fact that we could amplify this gene from A. hydrophila CDC 1271-82 indicates that the PCR conditions we used were not too stringent.

As a further control, we carried out a PCR for a second type III secretion gene, ascR. Again, the results indicate that this gene is absent in all the A. salmonicida subsp. salmonicida type strain isoforms tested yet was present in A. salmonicida subsp. salmonicida JF2267 and A. hydrophila CDC 1271-82 (Fig. 1D). Finally, as false-negative PCR results can be obtained if samples contain inhibiting substances, we also tested all A. salmonicida subsp. salmonicida strains for the presence of the aexT gene. The amplification was successful in all strains, thereby verifying the quality of our DNA (results not shown).

Our findings indicate that type III secretion genes are not present in the A. salmonicida subsp. salmonicida type strain and demonstrate that false-positive results can be obtained by carrying out DNA-DNA hybridization at low temperatures. We recommend that future studies into the prevalence of type III secretion genes in the genus Aeromonas carry out DNA-DNA hybridization experiments at 65°C and include appropriate positive and negative controls.

This work is supported by the Swiss National Science Foundation, grant no. 3100A0-101595.

REFERENCES


Sarah E. Burr
Joachim Frey*
Institute of Veterinary Bacteriology
Universität Bern
CH-3001 Bern, Switzerland

*Phone: 41 31 6312 414
Fax: 41 31 6312 634
E-mail: joachim.frey@vbi.unibe.ch

* Published ahead of print on 24 June 2009.