ROUTINE ISOLATION OF NOVEL LEGIONELLA SPECIES FROM DIFFERENT HABITATS

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1. Objectives

The genus *Legionella* currently includes 63 validly published species, but there are other candidate novel species deposited by authors at the *mip*-gene sequences database in the UK, and GenBank. A wide range of both public health and commercial samples have been examined in our laboratory routine since 1986. Over the 36 years a small collection of atypical isolates have been gathered. Their phylogenetic analysis has been performed based on *mip*-gene sequencing.

2. Material and methods

Samples of potting mixes, garden soils and spa peloids were plated directly at 36^0C and 30^0C, and after the enrichment for six weeks at 30^0 C. The same approach was used for sewage, sludge and grey waters. Cold drinking waters from private wells were incubated for 3-6 weeks at 30^0C and 22^0C. Atypical isolates were identified by serology and *mip*-gene sequencing. Strains dissimilar to existing species by more than 5% based on *mip*-gene sequence were further confirmed by 16S rRNA sequencing.

3. Results

About 31 confirmed and 11 putative novel *Legionella* species have been revealed. Modifications to the culture method appear to be a key factor for their isolation. Only 6 species cultured on direct plating at 36^0C, but after enrichment at 30^0C, 14 species were detected in waste water samples. 15.8% well water samples were positive without modification but 46.2% were positive following enrichment at 30^0 and 22^0C. The majority of the isolates grew at 30^0C but 4/35 grew only at 22^0C. *L. quateirensis* was the predominant strain isolated in cold well waters. Novel species made up 59.2% of the cold water isolates. A similar picture was confirmed for the garden soils and waste waters. Four phylogenetic dendrograms from different environments will be presented.

4. Conclusion

The study has confirmed that cold well waters, industrial and waste waters, garden substrates and soil provide ideal conditions for *Legionella* growth, including the occurence of novel species. Their successful isolation requires a modification to culture conditions which better reflects the conditions in their natural habitats. All the environments examined are inhabited by specialist legionella communities consisting of a group of various species capable of living in such conditions. Their role in such settings and possible health risks remain unknown.

Literature

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