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Short communication

The fish pathogen *Renibacterium salmoninarum*: Growth in a microaerophilic atmosphere

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Abstract

Renibacterium salmoninarum is the etiologic agent of bacterial kidney disease (BKD) occurring worldwide in salmonid fish. This bacterium has previously been regarded as a strict aerobic species. However, in this study it is shown that *R. salmoninarum* grows well in microaerophilic atmosphere, the colony size being larger and the colonies being more mucoid than in aerobic conditions. Microaerophilic cultivation might be one possibility to increase the sensitivity of the cultivation method for the detection of this slowly growing pathogen.

Keywords: *Renibacterium salmoninarum*; Microaerophilic growth; Salmonid fish

1. Introduction

Renibacterium salmoninarum is the etiologic agent of bacterial kidney disease (BKD) in salmonid fish, a chronic, systemic infection characterized by granulomatous lesions in the kidney and other organs (Fryer and Sanders, 1981; Fryer and Lannan, 1993). This bacterium is widespread in nearly all countries where salmonid fish are cultured and it also affects wild fish populations (Fryer and Sanders, 1981; Klontz, 1983). It was characterized in terms of the G + C% content of the DNA, the cell wall sugar composition and the amino acid composition of the peptidoglycan cell wall layer and was recognised as forming a single species belonging to a new genus, *Renibacterium* (Sanders and Fryer, 1980).

R. salmoninarum is a Gram-positive short rod occurring frequently in pairs. It is aerobic, catalase positive, cytochrome oxidase negative, non-acid fast and non-motile, does not produce acid from sugars and does not form endospores. All strains require cysteine for growth. Optimal growth occurs at 15–18 °C, and growth is very slow at 5 or 22 °C and non-existent at 37 °C. Growth on all media is slow, often requiring several weeks of incubation (Sanders and Fryer, 1980; Benediktsdottir et al., 1991). *R. salmoninarum* strains isolated from different regions form a very homogeneous group in terms of both their morphological, biochemical and physiological (Goodfellow et al.,

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1985; Bruno and Munro, 1986) and also their antigenic properties (Bullock et al., 1974; Getchell et al., 1985; Daly and Stevenson, 1990). The bacterium is a highly conserved genospieces, and studies on isolates from different parts of the world indicate relatively low genetic diversity (Starliper, 1996; Grayson et al., 1999; Rhodes et al., 2000).

R. salmoninarum has been described as an aerobic bacterial species (Sanders and Fryer, 1980). In BKD there are often large granulomatous lesions in the internal organs of the diseased fish, pointing to an ability to survive and proliferate in circumstances involving a decrease in oxygen concentration. In this study we tested the growth of *R. salmoninarum* under aerobic, microaerophilic and anaerobic conditions.

2. Materials and methods

2.1. Trial 1

Three strains of *R. salmoninarum* were used: type strain of *R. salmoninarum* (ATCC 33209) and two strains, which had been isolated from rainbow trout in northern Finland in the years 1997 and 2002 through the fish health control programme, using the Icelandic cultivation method (Benediktsdottir et al., 1991). All the strains had been kept frozen at −80 °C prior to the experiment. The bacterial population obtained on KDM2 agar (Evelyn, 1977) after 14 days was suspended in 0.1% peptone–saline to OD 0.08 (615 nm). Tenfold serial dilutions were prepared using peptone–saline, and 0.1 ml of each test dilution was streaked onto two parallel KDM2 agar plates. One of the parallel plates was incubated aerobically and the other under microaerophilic conditions, both at 16 ± 1 °C. The microaerophilic atmosphere containing 5–8% oxygen and 12–15% carbon dioxide was generated in sealed jars using the CampyGen™ equipment (Oxoid, Hants, U.K.), and was tested using *Campylobacter fetus* subsp. *fetus* (ATCC 27374) as a control strain. The KDM2 plates were observed for growth after 5 and 12 weeks.

One microaerophilic subculture per strain used in Trial 1 was identified as *R. salmoninarum* (Table 1), based on the observation of small Gram-positive rods occurring in pairs in Gram staining, a negative oxidase test (Spot test, Difco/BBL), a positive catalase test, a positive result in fluorescent antibody (FA) staining, typical results with API ZYM test strips (bioMérieux, France) and little or no growth on TSA agar. A polymerase chain reaction (PCR) was also used as a confirmatory test. In the FA staining a fixed smear of bacterial culture was covered with polyclonal rabbit anti-*R. salmoninarum* serum, incubated in a moist chamber at 37 ± 1 °C for 30 min, rinsed with PBS and covered with fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit IgG (Nordic Immunological Laboratories, the Netherlands). It was then incubated as above, rinsed and covered with phosphate-buffered

Table 1
The number of bacterial colonies on KDM2 agar plate of three strains of *Renibacterium salmoninarum* after incubation in an aerobic or microaerophilic atmosphere for 12 weeks

Dilution	*R. salmoninarum*: type strain		Isolate 1		Isolate 2	
	Aer[a]	CO_2[b]	Aer	CO_2	Aer	CO_2
10^{-4}	>200[c]	>200	>200	>200	>200	>200
	>200	>200	>200	>200	>200	>200
10^{-5}	>200	>200	>200	66	>200	>200
	>200	>200	>200	66	>200	>200
10^{-6}	45	27	84	8	>200	44
	45	27	84	15	>200	174
10^{-7}	5	8[d]	70	2[d]	114	30[d]
	5	8	74	20	114	40
10^{-8}	0	0	35	0	42	0
	0	0	35	12	47	5

[a] Incubation in an aerobic atmosphere.
[b] Incubation in a microaerophilic atmosphere.
[c] The number of colonies could not be counted exactly because of dense growth.
[d] Microaerophilic growth identified as *R. salmoninarum*.