Introduction

Many Gram-positive bacteria (GPC) have been described as being responsible for disease in a range of freshwater and marine fish species. The list of GPC pathogenic for fish has been expanding, and many of these bacteria display a high level of polymorphism by their ability to utilize or exclude compounds from a pre-defined panel of carbon sources. The system uses 95 enzymatically based biochemical reactions, 350 selective biochemical reactions, 5 dyed carbon source and a nitrate disc. When inoculated with a bacterial suspension in a gel-like inoculating fluid the tests are rehydrated and during incubation there is a burst of regeneration in the wells containing chemicals that can be oxidized. The bacterial cells then turn the dye a purple colour and playing a characteristic pattern of purple wells. Identification is achieved using Biolog Microlog software.

The identification of pathogenic GPC has always been considered to be difficult and has often led to misdiagnosis. Such methods depend upon the result of secondary tests, which are however expensive and time consuming and, although powerful, are not available in all fish diagnostic laboratories.

We have investigated the use of Biolog’s Microlog System (Biolog, USA) to identify and characterise some of the Gram-positive cocci listed above together with other GPC that may be markers of environmental contamination. Unlike other secondary tests, the Microlog System can recognize over 14,000 biochemical reactions allowing to identify bacteria by their metabolic profile.

Material and methods

Bacterial strains and culture conditions

The bacterial strains used in this study are listed in Table 1. All the isolates were stored at −20 °C in Brain Heart Infusion (BHI) broth (Oxoid) supplemented with glycerol (10%) and were used within 6 months of the date of collection. They were sub-cultured on Columbia sheep blood agar plates (Oxoid) at 37 °C for 24 hours. Bacteria were isolated from various sources and their species were identified by a combination of biochemical and molecular methods. All the isolates were kept in the collection of the Microbiology Department of the Instituto Zooprofilattico Sperimentale delle Venezie (Italy) and were described in previous studies.

RAPID ID 32 STREP test

The RAPID ID 32 STRIP is a standardised system for the identification of streptococci and related organisms. The system is based on the detection of 32 metabolic reactions and the metabolic profiles produced during incubation are read with a system that allows easy and rapid interpretation. The system is based on 32 colour-coded test strips, each containing 8 test wells. Each test well is inoculated with a suspension of the bacteria to be identified. The RAPID ID 32 STRIP test was performed according to the procedure of the manufacturer. The reactions were incubated at 35 °C for 48 hours. The results were recorded as a pattern of 32 red/brown discs on the strip. The results were then compared with the reference pattern recorded on the same strip.