Isolation of *Pantoea agglomerans* from Brown Trout (*Salmo trutta*) from Gilchrist Creek, Michigan, USA

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Abstract

*Pantoea agglomerans* is a gram-negative environmental epiphyte that is associated with plants, found in soil, and is emerging as a source of localized and systemic infections in humans. To date, there have been two reports of *P. agglomerans* infecting fish, none of which have occurred within the Great Lakes basin. Herein, we report on the isolation of *P. agglomerans* from the kidney of an infected feral brown trout (*Salmo trutta*) caught from the Gilchrist Creek, Lake Huron watershed, Michigan, USA.

Introduction

*Pantoea agglomerans* (Gavini et al., 1989), formerly known as *Erwinia herbicola* or *Enterobacter agglomerans*, is a ubiquitous epiphytic gram-negative bacterium belonging to the family Enterobacteriaceae (Gavini et al., 1989). *Pantoea agglomerans* is found primarily on the surfaces of plants (Monier and Lindow, 2005; Dye 1969); however, it has also been isolated from the gut of a number of insects (Hogg & Lehane, 2001; DeMaio et al., 1996; Pidiyar et al., 2004) and the cephalothorax of the mangrove crab, *Ucides cordatus* (Vieira et al., 2004). In fish, *P. agglomerans* has been recovered from a mortality event in hatchery-raised dolphin fish (*Coryphaena hippurus*) in Bermuda (Hansen et al., 1990). It is unknown, however, if *P. agglomerans* was the etiologic agent behind the mortality event. In another instance, *P. agglomerans* was isolated from the heart blood and surface-disinfected non-fertilized eggs of spawning chinook salmon (*Oncorhyncus tshawytscha*) from the Abernathy Salmon Culture Technology Center in Longview, Washington, USA (Sauter et al., 1987). With the exception of poor egg quality from one *P. agglomerans*-infected chinook salmon, no lesions or adverse health effects were reported. In humans, this epiphyte is one of the most common organisms transmitted through plant thorn injuries (Flatauer & Khan, 1978; De Champs et al., 2000; Kratz et al., 2003). Most recently, a number of studies connected *P. agglomerans* to serious localized and systemic infections in humans, such as neonatal pneumonia (Van Rostenberghe et al., 2006), peritonitis (Lim et al., 2006), arthritis (Ulloa-Gutierrez et al., 2004), bacteremia (Ulloa-Gutierrez et al., 2004), sepsis (Cicchetti et al., 2006), as well as osteomyelitis, urinary tract infections, and abscesses (Cruz et al., 2007). The prevalence or potential pathogenicity of *Pantoea agglomerans* to other animal species is currently lacking.

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Herein, we report the isolation of *P. agglomerans* from a brown trout (*Salmo trutta*) collected from Gilchrist Creek, Lake Huron watershed, Michigan, USA.

**Materials and methods**

A total of sixty brown trout were collected via electro-shocker from two different counties bordering Gilchrist Creek (GPS coordinates N 44.8404; W 84.00155) and transported to the Aquatic Animal Health Laboratory (AAHL) at Michigan State University. Immediately upon arrival, the fish were necropsied and samples aseptically obtained from the kidneys. Tissues sampled were streaked onto trypticase soy agar (TSA, Remel Inc., Lenexa, KS) and incubated at 22 °C for 72 hours. Subcultures of individually picked colonies were incubated for 18-24 hours at 22°C before testing for morphological, cultural, and biochemical characteristics.

Gram reaction, motility testing (at 22°C and 37°C), and biochemical reactions were performed according to standard bacteriological methodologies (MacFaddin, 2000). Biochemical reactions tested included: cytochrome oxidase, catalase reaction (3% H₂O₂), indole production, hydrogen sulfide production, oxidation/fermentation reactions (O/F Basal medium; BD, Sparks, MD), mixed acid production (methyl red test), 2,3-butanediol production from glucose (Voges-Proskauer test), nitrate reduction, citrate utilization, triple sugar iron reaction, ONPG (β-galactosidase), lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, hemolysis reaction on sheep blood agar (SBA), phenylalanine deaminase (BD), and esculin hydrolysis. Production of acid from the following carbohydrates was examined in phenol red broth base at a final concentration of 1%: arabinose, galactose, inositol, lactose, malonate, maltose, mannitol, mannose, rhamnose, salicin, sucrose, trehalose, and xylose. All biochemical tests were incubated at 22°C. Unless otherwise described, all ingredients were purchased from Remel Inc. Lenexa, KS. Results were followed for seven days, with the following exceptions: methyl red, Voges-Proskauer, indole production, phenylalanine deaminase, and TSI reactions were read at 2 days.

**Results/Discussion**

Primary bacterial isolation from one brown trout yielded a pure culture of yellow-pigmented, undulate, translucent colonies that exhibited umbonate surfaces with slightly concave centers. The colonies measured 5 mm in diameter, and a colony count estimated the presence of $2.2 \times 10^3$ colony forming units/g kidney tissues. The bacterium was a gram-negative, straight rod (0.5 × 1.25 mm) arranged in palisade-like structures and was motile at 22°C, but not at 37°C. The isolate partially hemolyzed sheep red blood cells (α-hemolytic). Other biochemical reactions are listed in Table 1.

The phenotypic characteristics observed strongly agree with those characteristic of *P. agglomerans* described in the Bergey’s Manual of Determinative Bacteriology (Holt et al., 2000; Table 1) and reported by Gavini et al. (1989), Iimura and Hosono (1996), Stiles and Lai-King (1981), and MacFaddin (2000). The production of phenylalanine deaminase, the hydrolysis of esculin, the reduction of nitrate, acid production from salicin, and the
utilization of malonate allowed for the
differentiation between *P. agglomerans*
and *P. dispersa* (Holt et al., 2000). In addition, the
production of yellow pigment by our isolate
allowed for the elimination of other possible
bacterial species lacking pigment production.

In the same context, other yellow-pigment
producing members of the family
Enterobacteriaceae were ruled out by vast differences in their phenotypic and biochemical characteristics (Holt et al., 2000). Minor differences in biochemical phenotype, namely mixed acid production (methyl red test), were observed when comparing the isolate recovered in this study to those recovered from dolphin fish by Hansen and colleagues (1990; Table 1). These differences do not preclude the fact that the two isolates are strains of the same species.

Although the prevalence of \textit{P. agglomerans} in this sample was less than 2%, the presence of this facultative pathogen in the kidney of the infected fish, as well as the relatively high number of cfus present \((2.2 \times 10^3 \text{ cfu/g})\) indicated that the infection was of systemic nature. No external clinical signs were evident, but internally, a dark, mottled liver and moderate splenomegaly were observed. It is currently unknown whether the \textit{P. agglomerans} strain recovered in this study is truly pathogenic to fish, but its very presence within this host species and geographic locale is noteworthy due to its zoonotic potentials.

This is considered the first reported isolation of \textit{Pantoea agglomerans} from a teleost host within the Great Lakes basin. A possible mode of infection is the ingestion of infected insects (e.g., mosquitoes), which harbor this bacterium (DeMaio et al., 1996; Pidiyar et al., 2004). The impact of \textit{P. agglomerans} infection in the brown trout remains to be elucidated.

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\textbf{References}


