
Molecular Requirements for the Colonization of *Hirudo medicinalis* by *Aeromonas veronii*

Joerg Graf

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Introduction

A fundamental feature of symbioses is the colonization of the host by specific microorganisms. The degree of specificity is reflected both in the number of microbial species associated with the host and in the frequency of detecting any given microbe in the host. Some associations such as the light organ symbiosis of *Vibrio fischeri* and the squid *Euprymna scolopes* are highly specific; involving one microbial species that is always detectable in the functional symbiotic association to the exclusion of all other microorganisms (McFall-Ngai and Ruby 1991). This association has been well studied and revealed multiple layers of molecular communication between the partners that allow the precise development of the association (McFall-Ngai and Ruby 1991; Visick and McFall-Ngai 2000; Chap. 13). Such two-member associations are probably an exception, especially among extracellular associations.

The microbiota in most digestive tracts consists of a large number of different species and could be considered to be less specific (Savage 1977; Moore and Moore 1995; Lilburn et al. 1999). Inside the host, these symbionts find a predictable source of nutrients and relatively constant environmental conditions that are presumably favorable for their growth. For a single host species, the composition of the microbial community inside individual animals can vary. For example, some bacterial species are regularly found in association with the host while other species are present in only some host specimen or occur only transiently, possibly depending on the age of the animal, its health, food or geographic location (Savage 1977; Moore and Moore 1995; Broderick et al. 2004). Factors such as the introduction of new microorganisms with each food consumption and changes in diet represent special challenges for the maintenance of specific microbial communities in the digestive tract (Savage 1977). Four hundred bacterial species can be cultured from the human digestive tract and between 800 and 1,200 operational taxonomic units of uncultured bacteria are present (Suau et al.

J. Graf (e-mail: joerg.graf@uconn.edu)

University of Connecticut, Department of Molecular and Cell Biology,
91 N. Eagleville Rd. U-3125, Storrs, CT 06268, USA

1999; Hayashi et al. 2002). This decreased specificity in the association does not necessarily imply that there is a lower degree of molecular communication between the symbionts and the host. Indeed, the communication may be more complex because more microbial partners need to be controlled by the host and because the microorganisms interact with each other. In this chapter, I will begin with an introduction of a relatively new model for digestive-tract associations, the digestive-tract symbiosis of *Aeromonas veronii* biovar *sobria* with *Hirudo medicinalis*, the medicinal leech, and present first insights into the molecular requirements for bacteria to colonize the digestive tract of the leech.

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The Digestive Tract Symbiosis of *Hirudo medicinalis*

In contrast to the digestive tract communities residing in most animals (Savage 1977; Demaio et al. 1996; Lilburn et al. 1999), the digestive tract of *Hirudo medicinalis* appears to be colonized by a relatively simple microbial community (Lehmensick 1941; Büsing et al. 1953; Graf 2000). Only one symbiont, an *Aeromonas* sp., was consistently cultured in several investigations and culture independent approaches suggest the presence of only a few additional taxa (described below). This apparent simplicity is intriguing and suggests that several mechanisms are involved in establishing and maintaining this simplicity.

The biology of the leech is important for identifying possible factors that may contribute to the specificity of this symbiosis and I want to begin by briefly introducing this fascinating parasite that was used extensively for bloodletting in the 1800s (the biology of *H. medicinalis* is reviewed in the monograph by Sawyer (1986)). The medicinal leech feeds exclusively on blood. In a single blood meal, *H. medicinalis* can consume over five times its body weight. During the feeding, the leech releases powerful anticoagulants and vasodilators. Their activity is so potent that the blood continues to flow for about 15 min from the wound after the engorged leech falls off. This “blood-letting” ability has been utilized for centuries and the heavy collection in conjunction with habitat destruction has led to the near extinction of the medicinal leech in Western Europe (Graf 2000; Carter 2001). The medicinal use of leeches has made a recent revival to rescue tissue with venous congestion after microvascular plastic surgery (Henderson et al. 1983; Whitlock et al. 1983; de Chalain 1996). Interestingly for microbiologists, in up to 30% of these patients, wound infections with the digestive tract symbiont, *Aeromonas* sp., were reported (Whitlock et al. 1983; Dickson et al. 1984; Abrutyn 1988; de Chalain 1996;