so as to evaluate possible intrinsic limitations and validate the potential of such system techniques.

In brief, as a precautionary method when working with gnotobiotic organisms, one should always have in mind the combination of several appropriate techniques, instead of using a single methodology (Marques *et al.*, 2006a).

2.5.5. Breakthrough by Using Gnotobiotic Aquatic Animals

During the last 20 years, the use of aquatic animal models, for research purposes, has been increasing (DeTolla *et al.*, 1995), as a result of the importance aquaculture is achieving. As aquaculture is presently one of the most rapidly expanding new food industries, an increasing emphasis is placed on such laboratory organisms.

Aquatic animal models are, in many ways, very special in relation to the more traditional laboratory animals, with which they share many similarities (Obenschain, 2005). Since they are often easier to handle and require less space (as their small size allows large numbers to be kept in a relatively limited space). Also, their short life cycle provides the opportunity to examine multiple generations and gain information, in a very short period of time (DeVita, 1984 *in* DeTolla *et al.*, 1995).

Studies performed with aquatic gnotobiotes, are still scarce and mostly disperse. Findings may be separated under three major categories: (i) host organism nutritional requirements, (ii) host-microbe interactions and (iii) their metabolic functions (Marques, 2005).

Whereas, gnotobiotic aquatic organisms have proven to be useful models in diverse biological research areas (Table 2.2 to 2.4), results extrapolation to certain other organisms is not always feasible. Host microbial community (MC) dominant species, appear to be animal group specific (Vine *et al.*, 2004; Gerard *et al.*, 1994 *in* Marques *et al.*, 2006a). However, some major phylogenetic groups reveal, common beneficial and active effects on several distinct taxonomical groups (Table 2.5).

It should also be mentioned that present knowledge of aquatic organisms, in many areas, is significantly less than of the more traditional laboratory animals; something that should be taken into consideration, when reviewing some of the contributions that aquatic gnotobiology has made to our present conceptualization of the dynamic complex host-microbe interactions (Hem & Engh, 2001b).

2.5.5.1. Overview of Studies on Marine and Freshwater Fish

The study of how mutualistic relationships (symbiotic or commensal) are established between a microbe and its teleost host represents an emerging field (Olafsen, 2004).

Gnotobiotic marine fish models, which have significantly contributed to extend the understanding of host-microbial interactions, are rather scarce (Table 2.4). Hansen and Olafsen (1989), were among the first to perform research studies on host-microbe interactions, using gnotobiotic marine fish. Scanning electron microscopy, revealed that 2h after fertilization of *Gadus morhua* eggs shown substantial

bacterial growth, indicating a rapid primary colonization. *Caulobacter* and *Seliberia* spp., were observed attached to cod eggs, dissected from female ovaries under sterile conditions, thuespécies proving wrong the established dogma that gonads were considered sterile (Marques *et al.*, 2006a). Meanwhile, attempts to regulate the egg microflora by incubation of gnotobiotic eggs with defined antibiotic-producing strains, failed to prevent subsequent adherence colonization by the surrounding microbiota (Hansen & Olafsen, 1989).

Later, Munro *et al.* (1995) stated that when germ-free turbot (*Scophthalmus maximus*) larvae fed on gnotobiotic rotifers (*Brachionus plicatilis*), supplemented with known bacteria were challenged with an *Aeromonas* sp. isolate, shown no significant differences in the survival rates between control and challenging group.

More recently, gnotobiotic halibut (*H. hippoglossus*) larvae were used to evaluate several microbacterial isolates, from British halibut hatcheries. With the exception of *V.anguillarum*, most of the bacteria strains routinely isolated, were harmless to yolk sac larvae. Furthermore, "*in vitro*" tests that previously had shown to inhibit growth of pathogenic bacteria, failed when tested against *V. anguillarum* (Verner-Jeffreys, 2003).

Table 2.5. – List of studies realized by different authors, on the beneficial effects that specific bacteria phylogenetic groups had on several taxonomic animal-host groups.

Bacteria	Taxonomic animal-host group			
	Rotifers	Molluscs	Crustaceans	Fish larvae
Alteromonas sp.	Douillet (2000)	Douillet & Langdon (1993)*	-	-
Aeromonas sp.	Gibson *et al.* (1998) Martínez-Díaz *et al.* (2003)*	-	Verschuere *et al.* (1999) Verschuere *et al.* (2000b)	Hansen & Olafsen (1989) Munro *et al.* (1995) Irianto *et al.* (2003)
Bacillus sp.	-	Erasmus *et al.* (1997)	Moriarty (1998) Rengpipat *et al.* (1998) Verschuere *et al.* (2000b)	Gateoupe (1991)
Cytophaga sp.	Rombaut *et al*. (1999)	-	Marques *et al.* (2005)	-
Lactobacillus sp.	-	-	Venkat *et al.* (2004)	Gatesoupe (1994) Strøm & Ringø (1993)†
Moraxella sp.	Hagiwara *et al.* (1994)*	Erasmus *et al.* (1997)	Verschuere *et al.* (1999) Verschuere *et al.* (2000b)	-
Paracoccus sp.	Rombaut *et al*. (1999)	-	Marques *et al.* (2005)	-
Pseudomonas sp.	Hagiwara *et al.* (1994)*	Erasmus *et al.* (1997)	-	Skjermo & Vadstein (1999)
Roseobacter sp.	Rombaut *et al*. (1999)	Ruiz-Ponte *et al.* (1999)	Marques *et al.* (2005)	Hjelm *et al.* (2004b)†
Vibrio sp.	Martínez-Díaz *et al.* (2003)*	Riquelme *et al.* (1997)	Garriques & Arevalo (1995)§ Gomez-Gil *et al.* (1998) Verschuere *et al.* (2000b)	Ringø& Vadstein (1998) Makridis *et al.* (2000)

* Reference in Marques *et al.* (2006a); § Reference in Gomez-Gil *et al.* (2000); † Reference in Vine *et al.* (2006).

One particularly interesting and ground-breaking freshwater model was the study performed with zebrafish (*Danio danio*), which provided an opportunity to investigate the molecular mechanisms

underlying such interactions through genetic and chemical means during larval and juvenile stages from gnotobiotic and conventional zebrafish (Rawls *et al.*, 2004). Using DNA-microarray technology, this research study demonstrated that some genes were specifically expressed in response to certain microorganisms, indicating that early colonization of the GI tract by a particular microorganism can be responsible for a change in the larvae metabolism.

The same study provided consistent proof for the use of gnotobiotic wild-type or genetically manipulated zebrafish to help decipher the molecular foundations of symbiotic commensal host-microorganisms relationships in the vertebrate digestive tract (Olafsen, 2004).

Even though, no beneficial effects were found to positively affect host organisms (protecting them against pathogenic and opportunistic strains that may have caused diseases and other disturbances), gnotobiotic aquatic models have already proven to be an excellent research tool to analyze host-microbial interactions (Marques *et al.*, 2006a). Thus, future studies should consider all possible interactions, such as the host performance as a function of the quantitative and qualitative evolution of the microflora, and as a function of the differential gene expression. They should be extended to adult stages and validated on a wide range of target organisms (Marques, 2005).