Microbiota and environmental stress: how pollution affects microbial communities in Manila clams

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ARTICLE INFO

ABSTRACT

Given the crucial role of microbiota in host development, health, and environmental interactions, genomic analyses focusing on host-microbiota interactions should certainly be considered in the investigation of the adaptive mechanisms to environmental stress. Recently, several studies suggested that microbiota associated to digestive tract is a key, although still not fully understood, player that must be considered to assess the toxicity of environmental contaminants. Bacteria-dependent metabolism of xenobiotics may indeed modulate the host toxicity. Conversely, environmental variables (including pollution) may alter the microbial community and/or its metabolic activity leading to host physiological alterations that may contribute to their toxicity. Here, 16s rRNA gene amplicon sequencing has been applied to characterize the hepatopancreas microbiota composition of the Manila clam, *Ruditapes philippinarum*. The animals were collected in the Venice lagoon area, which is subject to different anthropogenic pressures, mainly represented by the industrial activities of Porto Marghera (PM). Seasonal and geographic differences in clam microbiotas were explored and linked to host response to chemical stress identified in a previous study at the transcriptome level, establishing potential interactions among hosts, microbes, and environmental parameters. The obtained results showed the recurrent presence of putatively detoxifying bacterial taxa in PM clams during winter and over-representation of several metabolic pathways involved in xenobiotic degradation, which suggested the potential for host-microbial synergistic detoxifying actions. Strong interaction between seasonal and chemically-induced responses was also observed, which partially obscured such potentially synergistic actions. Seasonal variables and exposure to toxicants were therefore shown to interact and substantially affect clam microbiota, which appeared to mirror host response to environmental variation. It is clear that understanding how animals respond to chemical stress cannot ignore a key component of such response, the microbiota.

1. Introduction

In the last two decades, symbiotic relationships between bacteria and eukaryotes have been extensively explored, showing that host microbiota (i.e. microbial communities associated with a host) has a vital importance in host development and health, as well as in its responses to environmental perturbations (e.g. Gootenberg and Turnbaugh, 2011; Proctor, 2011; McFall-Ngai et al., 2013).

To date, at least 40 drug substances have been identified for gastrointestinal (GI) microbiota, suggesting a potential to metabolise xenobiotics and to modulate the toxicity for the host at least at comparable level to that of the liver (Claus et al., 2016; Scheline, 1973).

Conversely, environmental variables (including pollution) may alter the microbial community and/or its metabolic activity leading to physiological alterations of the host that may contribute to their toxicity. Overall, although increasing evidence indicates that GI microbiota is a key player in the toxicity of environmental pollution, the mechanisms of interaction between digestive tract microbiota and endogenous enzymes involved in xenobiotic detoxification pathways still need to be elucidated (Claus et al., 2016).

The beneficial role of microbiota in bivalve species, and in particular the ability to protect against pathogens and environmental stressors, has recently been investigated (Green and Barnes, 2010; Lokmer and Wegner 2015; Meisterhans et al., 2016). However, the role
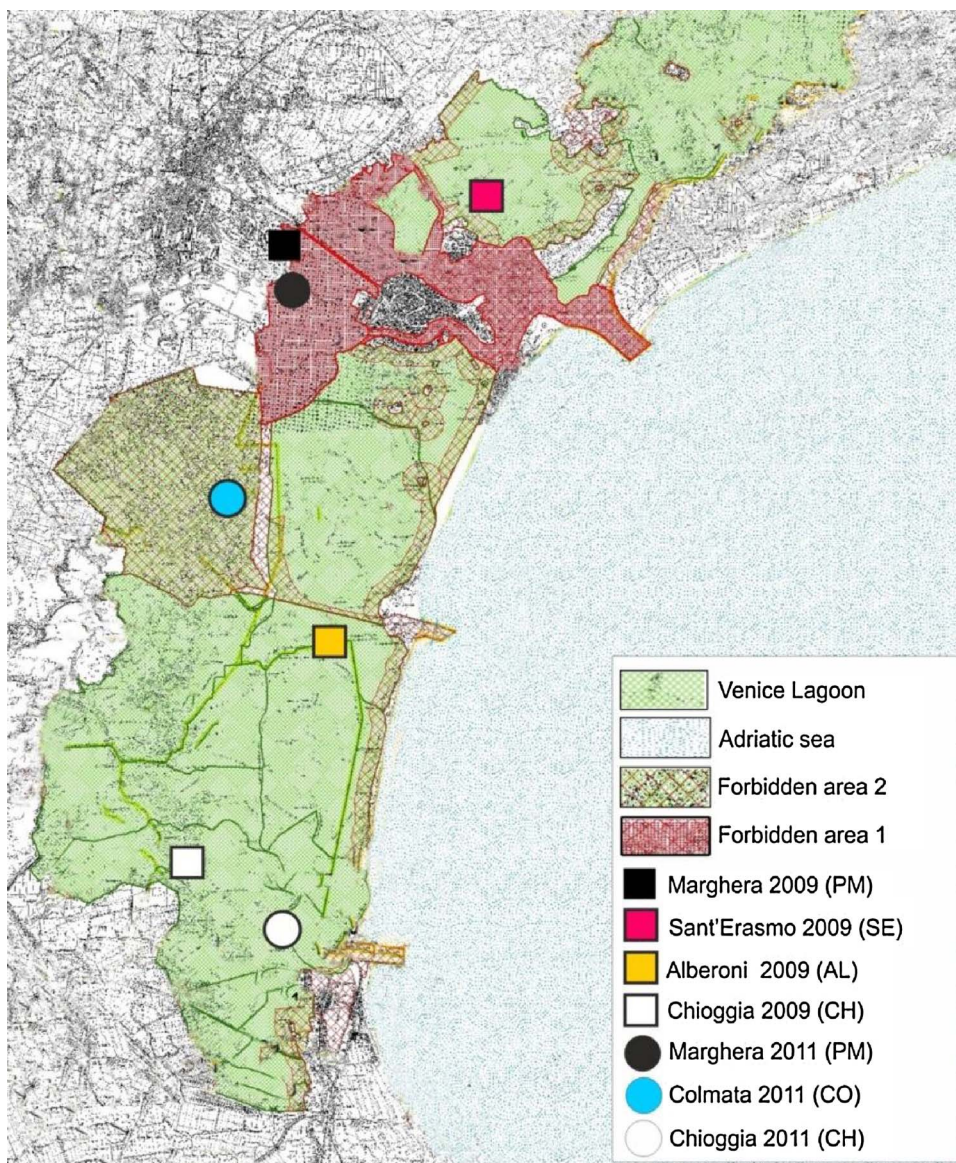
Fig. 1. Map of the Venice lagoon indicating the Manila clam sampling sites. In 2009, Manila clams were collected in June and December in all sites, while 2011, clams were collected in January. To ensure that seafood from lagoon areas is absolutely safe for the consumer, one third of the Venice lagoon is classified as not suitable for shellfish harvesting due to the high-level of chemical contamination (DGR3366/2004). Despite clam harvesting for human consumption is strictly forbidden in both CO and PM areas, the different classification as "forbidden area 1" and "forbidden area 2" refers to seed recruitment, that is allowed only in "forbidden area 2" (CO).

of resident bacteria in the host response to chemical contamination remains largely unexplored. The Manila clam, *Ruditapes philippinarum*, is a bivalve mollusk with a worldwide distribution that inhabits sandy-mud bottoms. While this species was originally from Asia, because of its rapid growth rate and resistance to different environmental conditions it has been introduced to other continents. Following its introduction in France (1973) from the Pacific coast of North America, *R. philipppinarum* has rapidly spread along the European coasts becoming the most important species for commercial clam landings in Europe and one of the major cultured species in the world (FAO data). Beside its commercial production, the Manila clam has been employed as a sentinel species to evaluate environmental quality, especially in the Venice Lagoon, where it is widely distributed and represents the most important commercial seafood species (e.g. Losso and Ghirardini 2010; Matozzo et al., 2005; Matozzo et al., 2010).

The Venice lagoon constitutes an excellent example of ecosystem subjected to heavy anthropogenic pressures, mainly represented by the industrial activities of Porto Marghera (PM) that led to severe chemical contamination of soil, groundwater, and sediments (e.g. Apitz et al., 2007; Matozzo et al., 2010). The high concentrations of dioxins (PCDDs), polychlorinated biphenyl (PCB), and heavy metals (Hg, Pb, and Cd) detected in the water column, sediment, and organisms living

in this area (e.g. *Mytilus galloprovincialis*, *Ruditapes philippinarum*) have led local authorities to classify one third of the lagoon as not suitable for shellfish harvesting (DGR3366/2004). Genome-wide gene expression analysis of hepatopancreas in Manila clams collected in different areas demonstrated that individuals sampled from the contaminated sites (PM) showed a distinctive transcriptomic signature centered on a few key biological processes, such as drug metabolism and detoxification processes, response to oxidative stress, protein turnover, energy metabolism, apoptosis regulation, and immune-response (Milan et al., 2011, 2013, 2015). Most of these changes persist after removal from the contaminated site and affect the clam response in a controlled chemical stress challenge (Milan et al., 2016).

Here, 16s rRNA gene amplicon sequencing was used to characterize the hepatopancreas microbiome of the same Manila clam that had been previously analysed at the transcriptome level through a species-specific DNA microarray (Milan et al., 2013, 2015). Extending the analysis to resident microbial communities offers a unique opportunity to understand how host and resident bacteria altogether respond to chemical contaminations. The hepatopancreas, or digestive gland, represents the ideal target organ for transcriptome and microbiome analysis because of its major role in digestion, absorption, storage of nutrients, as well as detoxification of xenobiotics (e.g. Wang et al., 2014; Rodrigo and Costa,