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Stanols as a tool to track the origin of microbial contamination of oysters, Crassostrea gigas, in shellfish areas.

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Runoff of cattle manures (cows, pigs, sheeps) or discharge of effluent from wastewater treatment plants (WWTP) into aquatic ecosystems can lead to microbiological contamination of waters and living organisms. In coastal ecosystems and particularly in shellfish harvesting areas, the presence of pathogen microorganisms in waters induces fecal contamination of filter feeding bivalves (oysters, mussels, scallops...), therefore leading to human health risks associated to the consumption of these contaminated organisms. Watershed management plans that aim at limiting these risks require the development of tools able to identify fecal contamination sources.
The fecal indicator bacteria used in the regulations to determine fecal contamination are not source specific since they are found in the feces of most warm-blooded animals. Thus, microbiological biomarkers have been developed in association with chemical biomarkers as Microbial Source Tracking (MST) methods. Fecal stanols, by-products of sterols obtained by human and animal microbial gut flora, are found in considerable amounts in feces with different relative proportions depending on their animal or human source. Recently, in association with microbiological biomarkers, the stanol fingerprint of contaminated waters has been successfully used to determine the main source of fecal contamination (cow, pig or human sources) in rural watersheds (Brittany, France).
Up to now, the use of the stanol fingerprint to track the fecal contamination in shellfish tissues, especially bivalves, has been limited to the analysis of coprostanol, a stanol commonly associated to human contamination. Therefore, whether the stanol fingerprint can be used as a MST method in bivalves or not is still unknown.
The first aim of this study was to compare several organic extraction procedures of stanols in the oyster Crassostrea gigas to determine a reliable method for stanol fingerprint analysis in bivalves. Solvent extraction and purification steps have been carried out with attention as they are critical for stanol quantification.
Secondly, the evolution of the stanol fingerprint of oysters with time was evaluated during 6 days by artificially contaminating microcosms with two concentrations of a WWTP effluent. In the microcosms, the fingerprint of stanols as a chemical biomarkers of fecal (human) contamination was compared to counts of Escherichia coli, a commonly used microbial indicator.
In association with microbial markers, the method developed from the two previous steps will be applied at the watershed scale in order to identify sources of fecal contamination in Brittany and Normandy (France).