Vibrio, an important human pathogen: new detection methods

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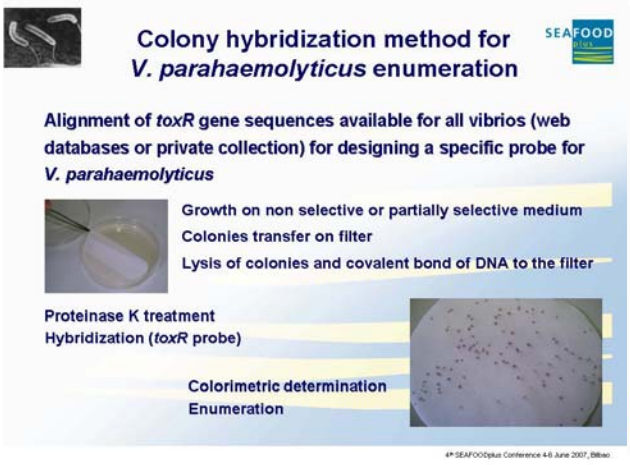

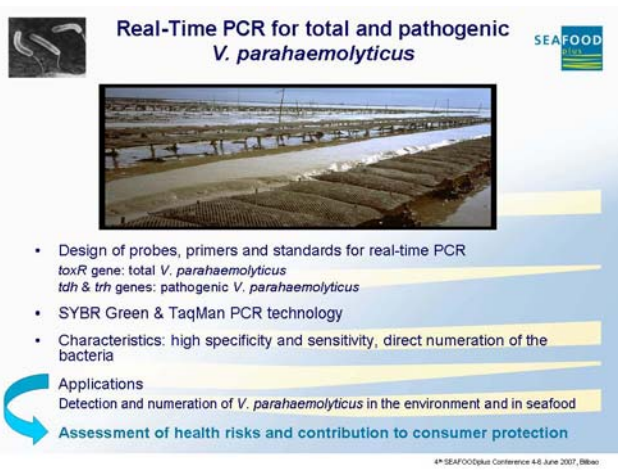

The *Vibrio* genus (*Vibrionaceae* family) comprises about 64 species of bacteria that are commonly found in aquatic environments. Some of the *Vibrio* species have been associated with human disease by consumption of contaminated seafood. Among these pathogenic species, *Vibrio parahaemolyticus* has been recognised as a common cause of gastroenteritis in Japan, Southeast Asia and the United States. In Europe cases are rarely reported, probably because this organism is seldom looked for and the disease is not included among those notifiable. Despite this, at least three medium-scale outbreaks (one in France, two in Spain) have been registered in the past decade in association with the consumption of seafood.

Currently, conventional presence/absence methods are used for testing seafood traded internationally, but they do not produce reliable results. In recent years, various molecular methods have been proposed for the detection, enumeration and characterization of pathogenic vibrios. The aim of the work carried out by the partners in the SEABAC project was the evaluation and the development of quantitative methods based on colony hybridisation (use of DNA probes to identify colonies growing on agar media) and Real-Time PCR for the determination of pathogenic vibrios. In particular the studies carried out for *V. parahaemolyticus* detection are reported.

For the development of the colony hybridization method a newly designed probe based on *toxR* gene has been considered. The experimental conditions such as hybridisation temperature and buffer composition have been optimised. Experiments performed under repeatability conditions using reference strains and recently collected environmental isolates have yielded good results. Specificity has been confirmed against a large panel of non-*parahaemolyticus Vibrio* spp. and non-*Vibrio* spp.

Real time PCR methods were developed for the detection and numeration of *V. parahaemolyticus* in the environment. TaqMan probes, primers and standards within species-specific sequence region (*toxR* gene) and/or region encoding for virulence genes (*tdh* and *trh*) were designed in order to discriminate pathogenic and non-pathogenic bacterial strains. These candidates are highly specific and sensitive. Because the time of analysis is considerably reduced, they are currently used in research to validate some experimental protocols (nucleic acid extraction) and will be used in routine analyses for the detection and numeration of *V. parahaemolyticus* in seawater and shellfish samples. These methods will contribute to the assessment of health risks and consumer protection.