INFLUENCE OF MARINE COASTAL SEDIMENT TYPE ON PERSISTENCE OF WATERBORNE PATHOGEN BACTERIA

I. Boukef Ben Omrane [1]*, N. Zabboub [2], M. Martins [3], I. Lahiba [1], R. Mraouna [1] and M. El Bour [1]
[1] Laboratoire de Microbiologie Institut National des Sciences et Technologies de la Mer - imen.boukef@yahoo.fr
[2] Laboratoire du Milieu Marin, Institut National Des Sciences et Technologies de la Mer
[3] University of Aveiro – Portugal

Abstract

In present study, we assessed the effect of sediment characteristics (particle grain size and organic matter contents) on waterborne pathogen bacteria survival. The survival test was conducted *in vitro* in microcosms where the two pathogen species *Escherichia coli* (O55B5) and *Vibrio parahaemolyticus* (ATCC 17802) were separately incubated on lagoon sediments and brackish water brought from different coastal areas of Bizerte lagoon (Northern Tunisia).. The results obtained revealed (1) extended bacterial survival in sediments, (2) higher growth and lower decay rates of the two strains in sediments with high levels of organic matter and small grain size sediment, and (3) longer survival of *Vibrio paraheamolyticus* compared with *Escherichia coli*.

Keywords: Bacteria, Sediments, Organic matter, Mediterranean Ridge

Introduction:

Extended persistence of waterborne pathogen bacteria in coastal sediments and their potential remobilization during natural turbulence may induce an increased risk of human infections [1]. In aquatic ecosystems the sediments represent the main reservoir for almost heterotrophic bacteria due to high levels in nutrients and organic matter combined with different grain size that should influence microbial populations and their persistence [2] [3] [4]. Pathogen bacterial survival in sediments and their possible re-suspension constitute high threat of pollution with human or animal risk during recreational or faming activities principally in coastal shoreline [5]. The present study highlight the influence of coastal sediments on the survival of two waterborne pathogen species of bacteria: *Escherichia coli* and *Vibrio parahaemolyticus* assessed *in vitro* experiments using microcosms filled with three types of coastal sediments from Bizerte lagoon in Tunisia.

Material and Methods:

Microcosms consisted of Erlenmeyer flasks of 500ml and filled with sterilized sediment (200g, wet weight) collected from three different coastal stations, located in front of discharge areas in Bizerte lagoon. All microcosms were then filled with brackish water (300ml) inoculated separately with *Escherichia coli* enteroagregative and *Vibrio parahaemolyticus* strains (three microcosms for each species of bacteria for final concentration of 10^7cfu/ml). Three Microcosms filled each with one type of sterile sediment and brackish water were used as negative control. Suitable dissolved oxygen was provided for each microcosm carefully without disturbing the upper layer of sediment using aquarium air pumps. All microcosms were kept in dark at a temperature of 20±2°C. During experimentation, microcosm sediments were sampled for bacterial analysis (culturable and total cells count). Bacteria were detached from sediments aliquots by vigorous shaking for 120 s [6] and *E.coli* were selected on DL agar (incubated at 37°C after 24h) whereas. *Vibrio* were selected on TCBS agar (at 22°C for 24 h).. The total cells number (TC) were counted using the DAPI (4', 6-diamide-2-phenylindole-Sigma).

Results and discussion:

Sediments characteristics including particle grain size and organic matter and nutrient contents were measured at the beginning and the end of the experimentation. Sediments varied with a proportion of 81% of sand (>63µm) for sediment S1 to approximately 53% and 37% for S2 and S3 respectively. The results demonstrated that organic matter decreased during experimentation and bacterial concentration (10^7cfu/g) remained quite constant during the first ten days of the experiment for the two species of bacteria. Extended bacterial survival in sediment may be attributed to the greater content of organic matter provided than in seawater [2] [4]. The present results suggest that indicator and pathogen bacteria released into the coastal environment should accumulate in sediment, leading to increased persistence and therefore, their survival may induce an increased risk of human infection due to the re-suspension and release of these pathogens during natural turbulence.

References

1 - Chahinian N., C. Bancon-Montigny, Caro A., Got P., Perrin J.L., Rosain D., Rodier C., Picot B., Tournoud M.G., 2012. The role of river sediments in contamination storage downstream of a waste water treatment plant in low flow conditions: Organotins, faecal indicator bacteria and nutrients. Estuar. Coast. Shelf Sci., 114 : 70-81.

2 - Craig et *al*., 2004; D.L. Craig, H.J. Fallowfield and N.J. Cromar., 2004. Use of microcosms to determine persistence of *Escherichia coli* in recreational coastal water and sediment and validation with in situ measurements. J. Appl. Microbiol., 96 : 922–930.

3 - Czajkowska D., Witkowska-Gwiazdowska A., Sikorska I., Boszczyk-Maleszak H. and Horoc M., 2005. Survival of *Escherichia coli* serotype O157:H7 in water and in bottom-shore sediments. Poli. J. of Enviro. Studi., 14 : 423-430.

4 - Haller L., Amedegnato E., Poté J. and Wildi W., 2009. Influence of Freshwater Sediment Characteristics on Persistence of Fecal Indicator Bacteria. Wat. Air Soil Pollut., 203: 217- 227.

5 - Luna G. M., Vignaroli C., Rinaldi C., Pusceddu A., Nicoletti L., Gabellini M., Danovaro R., and Biavasco F., 2010. Extraintestinal *Escherichia coli* Carrying Virulence Genes in Coastal Marine Sediments. Appl. Environ. Microbiol., 76 : 5659–5668

6 - Mika K.B., Imamura G., Chang C., Conway V., Fernandez G., Griffith J.F., Kampalath R.A., Lee C.M., Lin C.-C., Moreno R., Thompson S., Whitman R.L. and Jay J.A., 2004. Pilot- and bench-scale testing of faecal indicator bacteria survival in marine beach sand near point sources. J. Appl. Microbiol., 107: 72-84.