VIRULENCE FACTORS OF GRAM NEGATIVE BACTERIA ISOLATED FROM NATURAL AQUATIC ECOSYSTEMS

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Abstract. Natural environments contain a variety of microorganisms, of which, a limited number are able to develop in association with higher organisms and of these, a small number are pathogenic bacteria. The ability of pathogenic bacteria to cause disease in a susceptible host is determined by multiple virulence factors acting individually or together at different stages of infection. The main goal of the present paper was to perform microbiological investigations to phenotypically emphasise the virulence factors of Gram negative bacteria isolated from aquatic ecosystem Danube Delta. The experimental study was performed in 2013, on St. Gheorghe Branch, where some areas are used as raw surface water for drinking, representing a risk to human health. The surface water samples were monthly collected from 11 control sections and they were quantitatively assessed for the microbiological contamination with fecal bacteria. Bacteriological technique used for fecal bacteria monitoring was by Membrane Filtration Method according with SR EN ISO 9308-1:2004/AC/2009. The Gram negative strains were isolated and tested for virulence factors. During the experimental study, an increase of the density of fecal bacteria was revealed in July and August 2013. The presence of enzymes that determined virulence factors for bacterial strains from the surface water samples was identified: DNase, amylase, caseinase, gelatinase, esculinase, lipase, haemolysin and lecitinase.

Keywords: virulence factors, pathogenicity, enzymes.

AIMS AND BACKGROUND

The human body is constantly influenced by a lot of external factors that create an equilibrium cell between organism and environment which is a prerequisite misbalance occurrence of diseases. Under the conditions of tumultuous urbanisation, the contemporary civilisation is characterised by an alarming process of ecologic balance deterioration and water resources pollution[1]. The aquatic ecosystems are dynamic systems that keep their stability in the conditions of permanent fluctuations of biotic and nonbiotic parameters[2]. Through its complex work is included in all major ecosystems of the ecosphere, human society development strategy

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consisting in achieving maximum productivity with the increasing exploitation of natural resources. Thus, human impact on the environment is often unlimited and uncontrolled[3,4]. Free-living of planktonic mode of growth of microorganisms is usually observed in laboratory cultures. But this growth mode is infrequent in the natural environment and bacteria may seek out advantageous niches[5]. The rivers play a major role in shaping the ecological status of the areas they cover and control the global water cycle and the hydrological cycle, being the most dynamic transport factors[6].

Surface water supports microbiological communities including potential pathogenic microorganisms. In order to protect public health, quantifying the changes and sources of these microbes in source water for drinking is important for an effective water treatment. Many kinds of pathogens may present in surface water and various detection methods for identification were required. As a result it is unfeasible to test all of them in water. Number of coliform bacteria is widely used as microbiological parameter indicating fecal pollution[7].

Of the large number of microorganisms existing in nature, limited can be developed in association with other organisms and a small number of these are pathogenic bacteria. Pathogenic bacteria have two defining proprieties: pathogenicity and virulence[8].

The ability of bacteria to cause disease depends to a large extent on the expression of virulence factors which help them to invade the host, produce pathological effects and evade host defenses. The study of these factors is essential for development of new chemotherapeutic agents to fight the bacterial infections since the development of antibiotic resistance by bacteria has led to these diseases becoming one of the major problems in the sector.

Production of extracellular proteolytic enzymes is property shared by non-pathogenic and pathogenic microorganisms. These enzymes are indispensable factors in their life cycles and may be lethal to the host when produced by pathogenic bacteria. The role of proteases in pathogenesis is not clear, but it seems that they are involved in colonisation and invasion during host-pathogen interaction apart from providing nutrients for the microorganisms. Bacterial haemolysins are cytolytic exotoxins, generally considered as important virulence factors. These toxins cause damage to erythrocytes and other cell types by two different action models which involve a pore-forming protein or a phospholipase enzyme[9].

The virulence factors concept has been a powerful engine in driving research and the intellectual flow in the fields of microbial pathogenesis and infectious diseases. The microbial attributes that confer the potential for virulence fall primarily within several categories, including the ability to enter a host, to evade host defenses, to grow in a host environment, to counteract host immune responses, to acquire iron and nutrients from the environment and to sense the environmental changes.

Enzymes that digest host tissue damage the host generate nutrients and can promote entry and mechanisms that permit a microbe to evade phagocytosis enable survival in a host. Numerous enzymes have been implicated in microbial virulence. Although the number of enzymes in this category is vast we will discuss several examples to illustrate their mechanisms of action[10].

Discovery of virulence factors is important to understanding the pathogenesis of bacteria and their interactions with the host. Essentially, the capacity of pathogenic bacteria to elicit pathology in a susceptible host is determined by multiple virulence factors acting alone or together in a various stages of infection. These are often involved in direct interaction with the host tissues[11].

EXPERIMENTAL

The analyses were monthly performed in 2013. There were established 11 control sections to cover all route of St. Gheorghe branch. The sampling points are illustrated in Fig. 1.

Fig. 1. Sampling points on the St. Gheorghe branch (S1 – Iscaccea, S2 – upstream Tulcea, S3 – downstream Tulcea, S4 – Nufaru, S5 – Balteni, S6 – Mahmudia, S7 – Murighiol, S8 – Uzlina, S9 – Ivancea, S10 – St. Gheorghe port, S11 – Black Sea confluence)

The surface water samples were assessed for microbiological contamination level with Gram negative bacteria and their virulence factors. The quantitative analyses was based on membrane filtration method according to SR EN ISO 9308-1:2004/AC/2009. The culture media with different enzymatic substrates were used to emphasise the virulence factors for some strains identified with API system.

RESULTS AND DISCUSSION

Numerous studies have shown that individual bacterial populations are highly dynamic and can strongly differ in their response to resource availability. Others have shown that shifts in microbial community structure can be related to seasonal cycles in the source water and dissolved organic matter. Similarity, seasonal shifts in water column stability and water temperature may demonstrate an annual pattern of bacterial community variability[12].

The major sources of pollution in the Danube Delta Biosphere reservation are represented by the economical agents from the nearby area, and also by the naval transportation[13].

The quantitative analyses of coliform bacteria density showed the highest values in the summer of 2013 compared to other months of this year. Results are presented in Fig. 2.
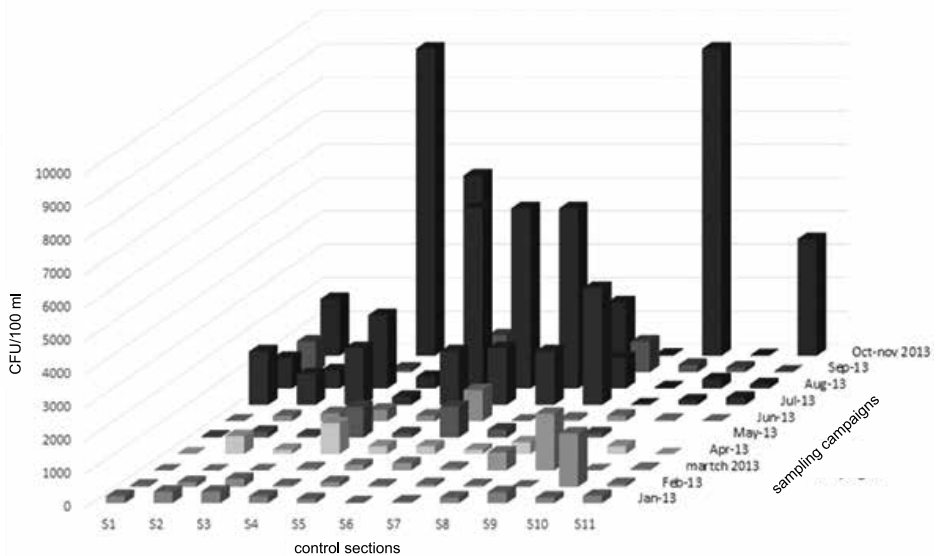

Fig. 2. Spatial and temporal variation of coliform bacteria in the surface water from St. Gheorghe branch during 2013; measure units are CFU/100 ml; the chart analyses the coliforms densities obtained in the St. Gheorghe branch control sections monthly monitored during 2013

The density of total coliforms ranged from 0 to 9200 CFU/100 ml during 2013, with an average of 785 CFU/100 ml and a standard deviation of 845 CFU/100 ml. The sampling point with the highest density was S3-downstream Tulcea in the last sampling campaign.

Microorganisms produce a variety of enzymes, mostly in small amounts to catalyse the metabolic process, but some strains however produce a large amount of enzymes which are excreted in the environment. In natural environments, extracel-

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lular enzymes hydrolyse nutritive macromolecules and monomers and oligomers are transported into the cell. In addition to enzymes, microorganisms produce some esterases that act as toxins to destabilise the membrane pore-forming; they have a role of virulence factors.

Some strains from the sampling points with high microbiological contamination levels were isolated and they were seeded on different enzymatic substrates to emphasise the virulence factors. There were identified bacterial strains of: *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Citrobacter freundii*, *Serratia odorifera*, *Enterobacter cloacae* and *Pasteurella pneumotropica.* The presence of DNase, amylase, gelatinase, hemolisin, lipase, caseinase, lecitinase and the metabolism of esculin were tested like in Fig. 3.
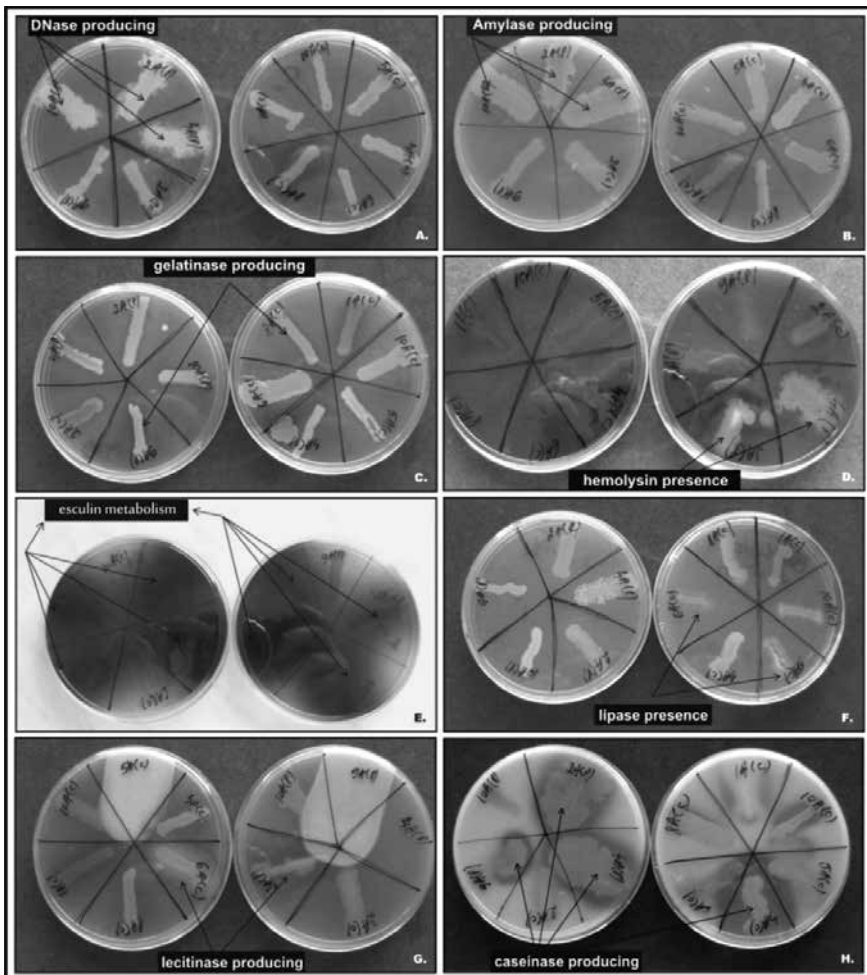
Fig. 3. Emphasising of virulence factors at Gram negative bacteria isolated from surface water of St. Gheorghe branch in 2013

Interpretation of positive results after sowing are made as follows: DNase – emphasising a white and irregular halo, Amylase – producing a white halo around the groove, Caseinase – producing a halo over a large area around the groove, Gelatinase – bacterial growth on a regular groove and around it, Esculinase – transfer culture medium colour to black-brown, Lipase – producing a transparent halo around the bacterial culture developed on the groove, Lecitinase – intense bacterial growth and dispersion halo density in the area, and Hemolysin – transfer culture medium colour from red to yellow.

CONCLUSIONS

Microbiological analyses determining the coliform bacteria density revealed the highest values in the summer months of 2013 with a maximum value of 9200 CFU/100 ml at S3-downstream Tulcea. ANOVA test on all values from 2013 indicates a *P*-value of 0.9 which means that the difference is irrelevant to significance threshold of 95%.

Bacterial pathogenicity is considered as biochemical mechanism by which microorganisms determine the appearance of pathology. Not all pathogens have this capability as a multifunctional complex. The presence of enzymes involved in the detection of the integrity of tissue invasiveness and gelatinase, pore-forming toxins in all strains analysed, may explain their ability to invade eukaryotic cells, to escape from fagosomi and to outsource in infected cells, thereby producing systemic infections.

Characterisation of virulence factors involved in adhesion and progression of infection at isolated strains demonstrate different virulence spectra according to taxonomic affiliation of these bacteria and the pathogenicity degree.

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