

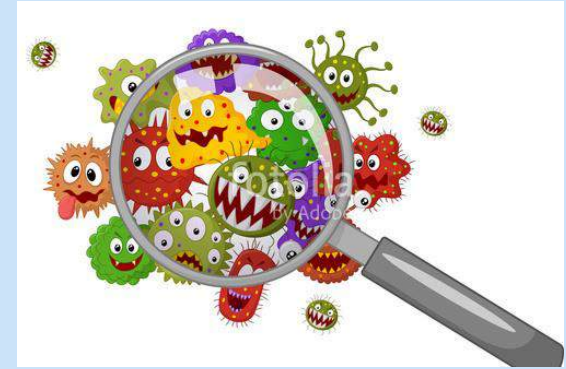


BSUN Master Courses on Blue Growth: Microbiology and Genetic Engineering, April 7–8, 2021

Black Sea Microbial Water Quality Assessment via Next Generation Sequencing-Based Approaches

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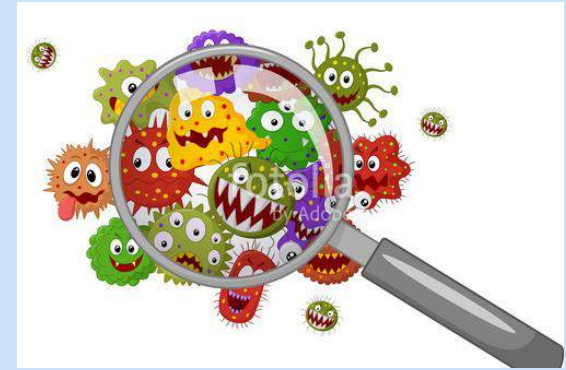
Marine bacterial community

Non-pathogenic
species

Pathogenic
species:

- indigenous
- externally introduced

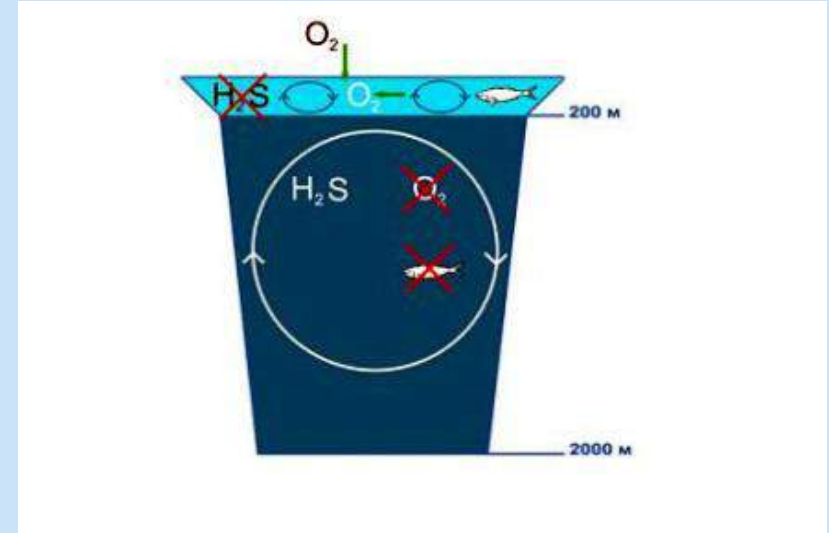
Marine pathogenic bacteria



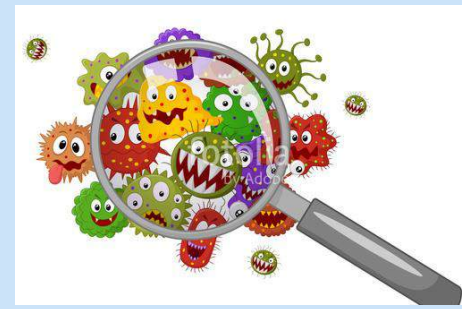
- most are found in coastal marine habitats
- free-floating through the seawater column (planktonic) and attached to different surfaces (marine animals, phytoplankton, zooplankton, sediments and detritus)
- can cause a broad spectrum of human and animal infectious diseases
- affect marine ecosystem functioning and health status
- are detected by both cultivation-based and cultivation-independent (metagenomics) approaches

Microbial contamination in coastal marine environment is a worldwide phenomenon not yet well understood ...

The Black Sea ...



- **the most isolated sea from the World Ocean** (connected to the Oceans via the Mediterranean Sea and the Sea of Azov)
- **very vulnerable to pressure from land based human activity**
- **its health is equally dependent from the coastal and non-coastal states of its basin**
- **the thin upper layer of marine water (up to 150 m) supports the unique biological life** in this sea
- **provides specific environment** (*unique geomorphological structure and specific hydrochemical conditions*) **for many species of non-pathogenic and pathogenic bacteria**

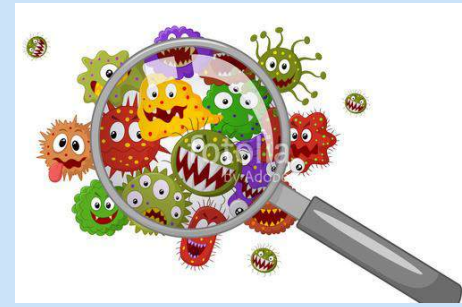


The Black Sea pathogenic microbes

The current data available on the microbial pathogens in the Black Sea region *is still limited*

For decades, the assessments of quality of the Black Sea marine environment is performed at *national level monitoring programs* under the Bathing Water Directives 2006/7/EC, 76/160 EEC and the Shellfish Water Directive 2006/113/EC, in most of the countries in the region

Intestinal enterococci and *Escherichia coli* for bathing waters, as well as total fecal coliforms, *Escherichia coli* and *Salmonella spp.* for designated shellfish growing areas are determined, on *culture-based enumeration and detection of fecal indicator bacteria (FIB)*



The Black Sea pathogenic microbes

FIB Limitations:

- monitoring for FIB does not provide insights on the possible occurrence of other microbial contaminants (e.g. pathogens).
- FIB culture-based methods are prone to false-negative results that arise from the failure to resuscitate viable but non-cultivable cells

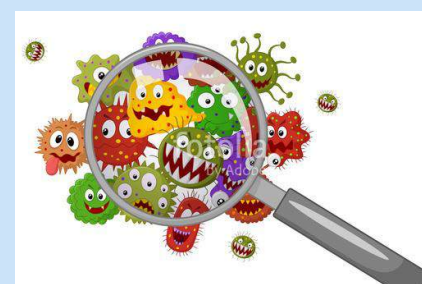
The Black Sea pathogenic microbes

Cultivation – independent molecular biology tools



PCR-based molecular methods

- cultivation-independent molecular methods based on direct detection of nucleic acids or amplification of targeted genes using polymerase chain reaction (PCR) were developed to avoid some problems associated with culture-based methods (FIBs)
- permit detection and quantification of specific pathogenic bacteria
- such methods target DNA extracted from environmental samples which is subsequently subjected to analysis for the presence or abundance of genes from indicator species or pathogens of interest.
- allow for more fine-scale taxonomic identification by using the small subunit ribosomal RNA gene (e.g. 16S rRNA gene for bacteria) – one of the most frequently used target genes for molecular analysis, due to its ubiquity in all organisms and sequence structure
- PCR-based detection and quantification of organisms in environmental DNA has proven useful for enumeration of sewage indicators and one DNA-based method is approved for quantification of the fecal indicator *Enterococcus* by the US Environmental Protection Agency (USEPA, 2009, 2013)



The Black Sea pathogenic microbes

Existing data generated by PCR-based molecular methods

Literature review indicates data on *microbial pathogens of the most predominant cultured fish species* at the Black Sea obtained in recent years by using a *combination of classical cultivation methods and traditional molecular techniques* (PCR technique and Sanger sequencing)

Iran, J Vet Res. 2017 Winter; 18(1): 18-24 PNCID: PNC5447134

Bacterial pathogens and health problems of *Acipenser gueldenstaedtii* and *Acipenser baeri* sturgeons reared in the eastern Black Sea region of Turkey

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Abstract

Farming sturgeons is an economically important practice in a number of Asian and European countries. However, since it is not widely implemented in Turkey, fertilized eggs necessary for research and industrial activities are imported from Germany. Due to the interest of several fish farms in culturing sturgeon in Turkey and the lack of relevant data, this study investigated bacteria related health problems of two different sturgeon species, the diamond sturgeon (*Acipenser gueldenstaedtii*) and the Siberian sturgeon (*Acipenser baeri*). The fungal, parasitic and bacterial pathogens found in these fish were investigated until the fish reached about 3 kg of weight (3+ years). A number of bacterial disease pathogens (*Aeromonas hydrophila*, *Vibrio cholerae*, *Yersinia ruckeri*, *Aeromonas salmonicida*, *Photobacterium damela*, *Photobacterium anguillarum* and *Aeromonas caviae*), and fungus *Saprolegnia* sp. were identified in the fish. Both phenotypic and molecular characterizations of the isolated bacteria were performed. Furthermore, swim bladder and spinal problems, cannibalism, tumor growth and mechanical injuries on the external surface of the fish were observed during the study period.

Key Words: Bacteria, Health problems, Sturgeons, Turkey

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REVIEW

Bacterial and Viral Fish Diseases in Turkey

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Abstract

This review summarizes the state of knowledge about the major bacterial and viral pathogens of fish found in Turkey. It also considers disease prevention and treatment. In this study, peer reviewed scientific articles, theses and dissertations, symposium proceedings, government records as well as recent books, which published between 1976 and 2013 were used as a source to compile dispersed literature. Bacterial and viral disease problems were investigated during this period in Turkey. Total of 48 pathogenic bacteria and 5 virus species have been reported in Turkey. It does mean that all the bacteria and viruses present in fish have been covered since every year new disease agents have been isolated. The highest outbreaks occurred in larval and juvenile stages of the fish. This article focused on geographical distribution, host range, and occurrence year of pathogenic bacteria and virus species. Yersinia, Furunculosis, Motile Aeromonas Septicemia, Yersiniosis, Photobacteriosis and Flavobacteriosis are among the most frequently reported fish diseases. Menhaden, *Yersiniosis salmoninarum*, *Renibacterium salmoninarum*, *Piscirickettsia salmonis* and *Pseudomonas latidens* are rarely encountered pathogens and might be emerging disease problems. Finally, the current status in fish diseases prevention and their treatment strategies are also addressed.

Letters in Applied Microbiology

Original Article

Molecular characterizations of *Vibrio parahaemolyticus* in seafood from the Black Sea, Turkey

G. Teroz Günel¹, J. Martínez-Urtaza

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Cited by (CrossRef): 1 article

Abstract

Vibrio parahaemolyticus is a marine bacterium that is considered as one of the major causes of bacterial food-borne outbreaks at a global scale. A total of 114 samples including mussel ($n = 42$), seawater ($n = 22$) and fish ($n = 50$) samples were collected and subjected to investigation. *Vibrio parahaemolyticus* was detected in 45 (39%) of 114 samples with an occurrence in mussel, seawater and fish samples of 75, 40.9 and 8% respectively. A total of 96 isolates were positive for the species-specific genes *toxR* and *tdh* and confirmed as *V. parahaemolyticus*. Presence of the virulence marker gene *trh* was not identified in any of the strains investigated; however, four of strains were positive for the *trh* gene. Serological analysis of eight randomly selected *trh*-negative isolates identified three different serotypes: O4:K:untypable (RUT), O2:K:U1, O3:K:U1. Conversely, all four *trh*-positive strains belonged to a single serotype (O1:K1) and share an undistinguishable genetic profile by PFGE analysis, suggesting the existence of a dominant clone for the *trh*-positive strains in the region.



The Black Sea pathogenic microbes

High-throughput sequencing approach

- also known as next-generation sequencing (NGS)
- is a powerful new tool for obtaining information about nucleic acids (allows massively parallel analysis of DNA/RNA sequence information from PCR amplicons or environmental nucleic acids)
- allows faster and more accurate species identification
- decreases dependence on cultivation and morphological taxonomic expertise
- includes the earlier 454 pyrosequencing (Roche), the better optimized Illumina (Solexa), the Ion Torrent and single molecule real-time sequencing (SMRT) technology, and the recent improved Illumina MiSeq



The Black Sea pathogenic microbes

High-throughput sequencing approach – FINDINGS:

- Information derived from DNA NGS sequencing *is not included yet* in the current Black Sea status assessment programs
- There is widespread recognition of the importance of this approach, and an *increasing number of regional projects generate sequence-based bacterial community diversity inventory (e.g. MARCY-BS-ERA NET, EMBLAS I, II, Plus – UN/UNDP)*



The Black Sea pathogenic microbes

NGS sequencing approach – FINDINGS:

The *first study to assess the presence and diversity of the pathogenic bacterial community in the inshore and offshore Black Sea waters* by means of a mining DNA next-generation-sequencing dataset approach **shown an unprecedented diversity of pathogenic bacteria dominated by Actinobacteria, alpha-proteobacteria and gamma-proteobacteria**, which varied in relative abundance between species and regions (Stoica et al., 2018).

The screenshot displays the CIESM website interface. At the top, the CIESM logo and name 'THE MEDITERRANEAN SCIENCE COMMISSION' are visible, along with a star icon and the date '7 April 2021'. Below this is a navigation bar with 'ON-LINE RESOURCES' and links to 'Workshop Monographs', 'CIESM Archives', 'Exotic Species Atlas', and 'Coastal Institutes Guide'. The main content area features the cover of the workshop monograph 'Searching for Bacterial Pathogens in the Digital Ocean', which includes a blue and white abstract image of a wave. To the right of the cover, the text reads: 'CIESM Workshop Monographs, n°49', 'Searching for Bacterial Pathogens in the Digital Ocean', 'To be cited as: CIESM 2017. Searching for Bacterial Pathogens in the Digital Ocean. CIESM Workshop Monograph n° 49 [F. Briand, ed.], 158 p., CIESM Publisher, Monaco.', 'This volume, fully illustrated and rich in references, is available in print from our on-line bookstore.', 'You may also download the Executive Summary if based in a CIESM Member State.', and 'This book is protected by international copyright law.'



The Black Sea pathogenic microbes

NGS sequencing approach – FINDINGS:

Black Sea pathogenic bacterial diversity at different trophic conditions :

Among the major bacterial phyla containing pathogenic species, *Actinobacteria* and *alpha-proteobacteria* were the most common in estuarine waters, whereas *gamma-proteobacteria* dominated in the coastal Black Sea (Figure 2). *Mycobacterium* and *Erythrobacter*, containing pathogenic species, formed the most abundant genera of microbial assemblage from the Danube estuarine waters .

Vibrio and *Aeromonas*, formed a diverse and dynamic genera of pathogenic microbial assemblages from the Romanian coastal waters. Two gamma-proteobacterial pathogenic strains related to *Aeromonas* (*Aeromonas spp.* and *Aeromonas veronii* *bv. veronii*) were found to constitute a relatively large percentage of the total bacterial rRNA contribution





The Black Sea pathogenic microbes

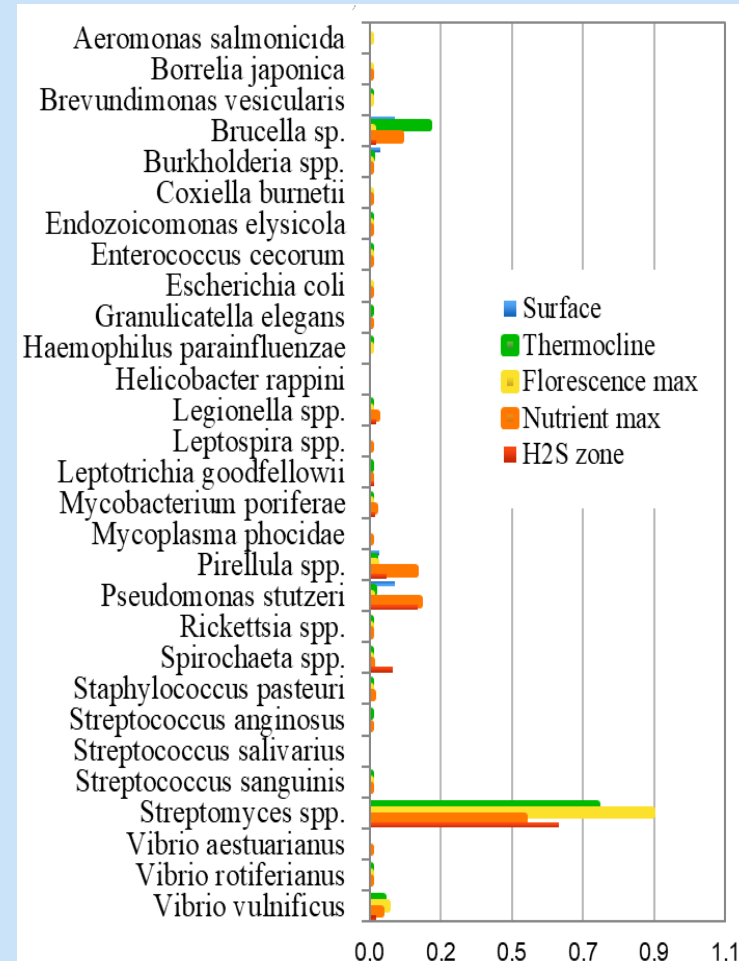
NGS sequencing approach – FINDINGS:

Occurrence of pathogens in offshore Black Sea :

NGS sequences matching pathogenic species were surprisingly found in the offshore waters collected during EMBLAS project in late May – early June 2016.

A total of 29 taxa harboring potentially pathogenic species were identified at different water column depths ranging from the surface to the anoxic H₂S zone of the Black Sea.

The pathogenic bacterial communities observed in the offshore waters were dominated by *Streptomyces spp.*, *Brucella sp.*, and *Pseudomonas stutzeri*. These microorganisms had previously reported in the various inshore regions of the world seas, mostly in water samples taken closest to the coast. No information is available on the occurrence of pathogenic bacteria in open marine waters.





The Black Sea pathogenic microbes

High-throughput sequencing approach – FINDINGS:

- The bacterial OTUs obtained by NGS Illumina sequencing showed that high-throughput tools are needed to decipher the pathogen bacterial diversity of the Black Sea.
- In order for NGS to become a useful tool for Black Sea water quality monitoring purposes, long term sequence data collection and management will be crucial in establishing databases that are important for inter-laboratory data comparison and comparative metagenomics studies.