ECO-DIVERSITY OF AQUATIC BACTERIA AND VIRUSES ISOLATED FROM RIVER NILE AND DRAINAGE WATER IN EGYPT

By

MOHAMED IBRAHIM HASAN AZZAM

B.Sc. Agric. Cooperative Sc., Higher Institute for Agric. Cooperation, 2003

M.Sc. Agric. Sc. (Agricultural Viruses), Ain Shams University, 2010

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ABSTRACT

Mohamed Ibrahim Hasan Azzam: Eco-diversity of Aquatic Bacteria and Viruses Isolated from River Nile and Drainage Water in Egypt. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2015.

This study aims to determine the impact of five main drains as sources of pollution on the water quality of River Nile at Rosetta branch, concerning physicochemical and microbiological characteristics. Eco-diversity studies of bacterial isolates were carried out through: antibiotic sensitivity, virulence and genetic variability. Eco-diversity studies of viral isolates were throughout: plaque morphology, host range, particle size, shape and molecular weight of genome.

The results of physicochemical, bacteriological and virological analyses revealed that, all drains selected in the study were suffering from varying levels of pollution. There was a gradual decrease in pollution levels along Rosetta branch (from downstream El-Rahawy to downstream Tala drain). The pollution impact caused by drains on Rosetta branch was remarkable from El-Rahawy drain which was considered the main point source of pollution. Results of water quality index was very bad in El-Rahawy and Sabal drains outlet and bad for all sites taken in this study except for upstream El-Rahawy drain, it was evaluated as being of medium quality.

Identification of bacterial isolates was carried out according to Bergey‟ s Manual of Systematic Bacteriology and confirmed using the analytical profile index 20 E strip system. Results revealed that, out of 225 isolates, 212 were belonging to four main bacterial families. The results of antibiotics resistance patterns for different bacterial isolates showed that *Ecoli* isolates were resistant to about (75%), *Citrobacter freundii* isolates (45%), *Salmonella sp.* isolates (85%), *Proteus vulgaris* isolates (87.5%), *Pseudomonas aeruginosa* isolates (100%), *Enterococcus faecalis* isolates (82.5%) and *Staphylococcus aureus* isolates (77.5%) of the tested antibiotics. Most of isolates were multiple antibiotic resistant (MAR). Methicillin resistant *Staphylococcus aureus* (MRSA) was reported in four isolates from drains and thirteen isolates from Rosetta branch. While, vancomycin resistant isolates (VRSA) were recorded, three isolates from drains and four isolates from Rosetta branch. Results of virulence test for all isolated bacteria showed positive congo red test (100%) for *C.freundii* and *Salmonella sp*, followed by *P.aeruginosa* (98%), *P.vulgaris* (91%), *E.coli* (60%), *E.faecalis* (40%) and *S.aureus* (72.3%).

The three *P.aeruginosa* isolates selected from different sites were successfully amplified and sequenced using 16s rDNA gene. Data showed that partial nucleotide sequences were 1274, 1280 and 1286bp, respectively

and compared with species recorded on Genbank and identified as *P.aeruginosa*_1, *P.aeruginosa*_2 and *P.aeruginosa*_3, respectively.

Eight coliphage and *P.aeruginosa* phage isolates were isolated from water samples. The maximum of phage counts were recorded in River Nile and the minimum were detected in drainage water samples. Electron microscopy of the isolated *E.coli* and *P.aeruginosa* phages particles revealed that, phage particles had an isometric head and long-contractile tail and some particles appeared containing short tail with full heads.

Biological characteristics for isolated phages were determined by the spot test method. It was found that phages specific for *E.coli* could lyses *E.coli* strains 1, 3, B (ATCC) but failed to lyses *E.coli* strain 2. While, phages specific for *P.aeruginosa* could lyses *P.aeruginosa* strains B2 and 101 but failed to lyses *P.aeruginosa* strain 1, 2. On the other hand, coliphages had activity and were able to lyse their host at pH ranged from 6 to 10 and *P.aeruginosa* phages had activity and able to lyse their host at pH ranged from 6 to 9. Viral stability to acidity and alkalinity was also different from type of phage to another. Data of turbidity test showed that phage infection produced a drastic decrease of *E.coli* and *P.aeruginosa* cultures as compared to control and a constant increase in $O.D_{600}$ was seen after 16 and 18 h.

Restriction enzyme pattern of the eight isolated coliphages (C1 to C8) by *Eco*RI, *Hind*III and *Bam*HI showed the presence of dsDNA as well as heterogeneity among these phages. The results showed that *Eco*RI produced 8, 7, 5, 4, 4, 7, 9 and 5 fragments and *Hind*III produced 4, 3, 0, 0, 1, 6, 5 and 4 fragments while *Bam*HI produced only 1, 1, 1, 0, 0, 3, 0 and 1 unique fragment, for the eight phage isolates, respectively. Restriction enzyme pattern showed the DNA phage genomes diversity among eight phage isolates. Sixty four fragments appear specific amplified fragments represented (81%), one fragment appear common amplified fragment represented (10.13%). Also, the restriction enzyme pattern appear eight unique (genetic marker fragment) represented (10.13%).

Out of fifteen sites, two only (El-Rahawy and Sabal drains outlet) were found to be polluted with enteroviruses with rate of $3.6X10^4$ and $3.4X10^4$ genome copies per microliter, respectively using real time - quantitative reverse transcriptase – polymerase chain reaction (rt-qRT-PCR).

Key Words: River Nile, Drainage water, Pollution, Bacteria, Viruses, Bacteriophages, 16s rRNA gene, Restriction enzyme, rt-qRT-PCR.

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