

Isolation and Study of *Vibrio* -Specific Phages for Prevention and Treatment of Diseases in Mediterranean Fish

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Vibrios are ubiquitous and abundant in different aquatic environments, in the water column or attached to plankton and living on the surface and in tissues of various marine animals. *V. alginolyticus*, *V. damsela*, and *V. harvey* are important opportunistic bacterial pathogens for aquaculture causing high mortality in cultured fish and shellfish in early larval stages and leading sometimes to death of the entire population.

Bacterial strains under the interest were isolated from different water samples and from sick fish in the aquaculture facilities of the Aquaculture institute, Crete. Up to 50 bacterial strains were isolated from water samples, 25 strains – from sick fish tissues. Majority of isolates were attributed to *V. harvey* and *V. campbellii*- two closely related *Vibrio* species, opportunistic fish pathogens.

In total 40 primary phages lysates were obtained from different environmental sources using several fish pathogenic strains (standard strains and fresh isolates) of *Vibrio spp.* After standard passages through sensitive host and screening for lytic spectrum against set of selected strains 11 phages with broader host range were selected and subjected to further cloning/purification. In addition, 4 phages specific to *V. parahemolyticus* from EIBMV collection were checked for activity against newly isolated fish pathogenic strains. Fifteen selected phages were used in broader screening for determination of lytic spectrum on set of 26 *Vibrio spp.* isolates, mainly *V. harvey*. Different host range was revealed for the tested phages. Among them phages Mes5, Bt7, Df12, A07/11, R07/11 were shown to have broader lytic spectrum lysing in total up to 85% of tested strains. Phage nucleocapsid morphology for selected phage clones was studied by means of Transmission Electron Microscopy (Jeol 100 SX at the Eliava Institute). Myoviridae and Podoviridae type of virion morphology was revealed. For comparative analyses of phages, DNAs were isolated from 12 phages and DNA restriction was performed using different restriction endonucleases: HindIII, EcoRV, EcoRI, BamHI, SalI, PvuII. The comparative analysis of phage DNA restriction results allowed dividing phages into two groups by isolation time. 5 phages from the group I revealed similar restriction profiles, while three phages among seven in the group II appeared to be different. The selected phages will be used in the next step of studies - targeting composing of potential phage set to be used for therapy and prevention of diseases of cultured juvenile fish in aquaculture settings.