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<p style="text-align: center;">TITLE</p> <p style="text-align: center;">Identification and characterization of extracellular proteases from T4 environmental isolates of <i>Acanthamoeba</i></p>	<p style="text-align: center;">*BehrozMahdavi Poor¹, Abdolhossein Dalimi¹, Fatemeh Ghafarifar¹, Fariba Khoshzaban², Jalal Abdolalizadeh³</p> <ol style="list-style-type: none">1. Department of Parasitology, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran2. Department of Parasitology and Mycology, Faculty of Medical Sciences, Shabed University, Tehran, IR Iran.3. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran <p style="text-align: center;">* Corresponding author: b.mahdavi@modares.ac.ir</p>
<p>Background: <i>Acanthamoebais</i> an omnipresent organism which could be classified as an opportunistic parasite in immunocompromised individuals. The amoeba also causes keratitis, a painful disease of the cornea, in healthy people particularly those wearing contact lenses. Since all <i>Acanthamoeba</i> strains cannot cause diseases, it is essential to discriminate between pathogenic and nonpathogenic strains from environmental sources.</p> <p>Methods: Eight environmental isolates belonged to T4 genotype were axenically cultured in PYG medium. To determine the pathogenic potential of the isolates, thermotolerance and osmotolerance assays were utilized. For extracellular proteases identification, <i>Acanthamoeba</i> conditioned medium (ACM) was prepared by incubation of trophozoites from log phase in PYG for 24 hours. Then, ACM was applied to gelatin zymography analysis. For proteases characterization, samples were pretreated with idoacetamide and phenylmethylsulfonyl fluoride as cysteine and serine protease inhibitors, respectively.</p> <p>Results: Out of 8 samples, 2 isolates showed high, 2 showed low, and 4 showed nopathogenicity in growth assays. Furthermore, all evaluated isolates showed different extracellular banding patterns in zymography analysis. However, the majority of protease activity was related to serine proteases. While, protease activity patterns of ACM were not found sufficient for discriminating of pathogenic and non-pathogenic isolates.</p> <p>Conclusion: The extracellular protease patterns of different isolates of <i>Acanthamoeba</i> could be more complicated than previously described.</p>	