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Green approach to phytopathogen: Characterization of lytic bacteriophages of *Pseudomonas* sp., an etiology of the bacterial blight of pomegranate

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Authors are dedicating this research article to Late. Prof. Hans-Wolfgang Ackermann for his remarkable contribution to bacteriophage taxonomy and electron microscopy of bacteriophages

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ABSTRACT

Two morphologically different bacteriophages were isolated from the river and soil samples from various locations of Maharashtra, India against the phytopathogen *Pseudomonas* sp. that was recently reported to cause a new bacterial blight of pomegranate. Both the phages belonged to the order *Caudovirales* representing the families *Siphoviridae* (vB_Psp.S_PRϕL2) and *Myoviridae* (vB_Psp.M_SSϕL8). The multiplicity of infection ranged from 0.01 to 0.1, phage adsorption rate from 39% to 66%, latent period from 10 to 20 min with a burst size of 24–85 phage particles per infected host cell. The genome size of phages PRϕL2 and SSϕL8 was approximately 25.403 kb and 29.877 kb respectively. Restriction digestion pattern of phage genomic DNA was carried out for phage PRϕL2, *Eco* RI resulted in two bands and *Hind* III resulted in three bands while for phage SSϕL8, both *Eco* RI and *Hind* III each resulted in three bands. SDS-PAGE protein profile showed six bands for PRϕL2 and nine bands for SSϕL8 of different proteins. Phages showed high pH stability over a range of 4–9, temperature stability over a range of 4–50 °C and UV radiation showed a reduction up to 89.36% for PRϕL2 and 96% for SSϕL8. In short, the present research work discusses for the first time in-detailed characterization of phages of a phytopathogen *Pseudomonas* sp. from Maharashtra, India, which can be further efficiently used for biological control of the causative agent of a new bacterial blight disease of pomegranate.

1. Introduction

Pomegranate scientifically known as *Punica granatum* L. is also called as 'Fruit of Paradise' or 'Super food'. Pomegranate as whole fruit or in the form of processed pomegranate products has enormous economic value. Traditionally, pomegranate has various health benefits due to its nutritional and medicinal properties (da Silva et al., 2013). Pomegranate has a major contribution in several industrial food products such as pomegranate juice, wine, fruit concentrate, beverages, syrup, jelly, pomegranate jam, anardana, etc (Singh and Singh, 2004).

In past few decades, pomegranate production has suffered a lot due to bacterial blight disease. Bacterial blight disease of pomegranate is known to be caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Mehta, 1952; Hingorani and Singh, 1960). Later, the blight disease was reported often throughout India. The production of pomegranate fruit was depleted which resulted in 60–80% reduction of yield thus leading to a great economic loss. Various management strategies failed to overcome the blight disease which mainly included traditional cultural practices, use of antibiotics and agrochemicals (Kumar et al., 2009; Mondal and Mani, 2009) etc. Recently, a new bacterial blight of pomegranate in various regions of Maharashtra, India caused by *Pseudomonas* sp. has been reported (Jagdale et al., 2018). This yellow-pigmented bacterium showed bacterial blight symptoms and the agrochemicals used for the management of this blight along with the traditional agricultural practices were unsuccessful in controlling the disease.

The genus *Pseudomonas* is a Gram negative bacterium categorized as highly pathogenic phytopathogen known to cause a wide variety of plant diseases, which includes blight, spots, streaks, stripes etc. There are numerous pathovars of genus *Pseudomonas* known to cause diseases in economically important plants/crops and which in turn have resulted in massive loss throughout the world (Bradbury, 1986; Höfte and De Vos, 2007). Initially traditional cultural practices were used to control plant infections from spreading from one region to other (Cooksey, 1990; Gašić et al., 2011; Gent and Schwartz, 2005; McManus et al., 2002) but later, the chemical approach was used which included use of antibiotics, copper containing agrochemicals and other pesticides (Cooksey, 1990; McManus et al., 2002). Due to excessive utilization of

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