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Original Article

Induced systemic resistance against *Cucumber mosaic cucumovirus* and promotion of cucumber growth by some plant growth-promoting rhizobacteria

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β-1,3-Glucanase pathogen related protein

Abstract During the investigation *Cucumber mosaic cucumovirus* (CMV) was isolated from cucumber plants showing virus like symptoms depending on indirect enzyme linked immunosorbant assay (I-ELISA) and *Chenopodium quinoa* as local lesion host. Three isolates from the predominant plant growth-promoting rhizobacteria (PGPR) were isolated from cucumber plants rhizosphere, and identified morphologically and physiologically to be related to *Bacillus subtilis*, *Pseudomonas fluorescens* and *Azotobacter chroococcum* species. The bacterial liquid crude cultures (72 h of age) and their supernatants were tested for their ability to induce systemic resistance within cucumber plants (*Cucumis sativus* L. cultiver Beit Alpha) against CMV infection. Two types of treatment were carried out: (1) spraying of healthy cucumber plants (carrying 4–5 leaves) and challenging by mechanical CMV inoculation at time intervals (5–10 days), (2) irrigation, as healthy cucumber seeds were irrigated with 200 ml from each bacterial culture or their supernatants and inoculated with CMV 15 days post treatment. Data proved that best results were obtained by treatment of irrigation with the *Azotobacter* crude culture, as the number of symptomless plants were 11 out of 30 plants inoculated, followed by *Pseudomonas* treated plants which gave eight asymptomatic plants. The induced resistance was tested using I-ELISA and immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) for the detection of CMV coat protein gene (*cp*), which proved that the mentioned symptomless plants were virus-free or with a low level of virus infection. *Azotobacter* treated plants giving virus-free results revealed the higher peroxidase and β-1,3-glucanase enzyme activities, 7 U/gm and 500 nktal/gm, respectively. Using gel electrophoresis and in comparison with control plants, a new protein band was detected in the protected cucumber plant extracts (molecular weight of about 30 KDa), assuming to be a plant pathogen related protein. Increase in growth

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measures was observed for *Azotobacter* and *Pseudomonas* treated cucumber plants, as the higher plant dry weights were 16.1 and 13.8 gm, respectively. Statistical lowest significant differences test (LSD) showed significant differences between *Azotobacter* and *Pseudomonas* results for biological data of plant dry weights.

Introduction

Cucumber (*Cucumis sativus* L.) is the main field and greenhouse vegetable crop of the coastal areas of the Mediterranean Basin and Middle East (Tognoni and Serra, 2003). Family cucurbitaceae contains approximately about 90 genera, but three are widely grown in Egypt, i.e., *Cucumis* spp. (melon and cucumber), *Citrullus* spp. (watermelon) and *Cucurbita* spp. (Squash and Pumpkin). In Egypt, cucumber is among the most important Cucurbitaceous crops and the leading export vegetable. Unfortunately, cucumber is infected with several pathogens and *Cucumber mosaic cucumovirus* (CMV) is considered as the major virus infecting such plants in Egypt.

CMV, genus: *Cucumovirus*, family: Bromoviridae, is one of the most widespread plant viruses with an extensive host range infecting about 1000 species including cereals, fruits, vegetables and ornamentals (Soleimani et al., 2011). The virus is readily transmitted in a non-persistent manner by more than 75 species of aphids (Palukaitis et al., 1992). CMV is a multicomponent virus with a single stranded positive sense RNA. RNAs 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein. Numerous strains of CMV have been classified into two major subgroups (subgroups I and II) on the basis of serological properties and nucleotide sequence homology (Palukaitis et al., 1992; Madhubala et al., 2005). The subgroup I has been further divided into two groups (IA and IB) by phylogenetic analysis (Roossinck, 1999).

Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. However, increasing use of chemical inputs causes several negative effects (Gerhardson, 2002). Furthermore, the growing cost of pesticides and the consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of fastidious diseases, mainly viral and viroid diseases, for which chemical solutions are ineffective (Shehata and El-Borollosy, 2008). Plant growth-promoting bacteria (PGPB) are associated with many, if not all, plant species and are commonly present in many environments. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces (Saharan and Nehra, 2011), i.e., *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bacillus*, *Pseudomonas* and *Serratia* (Compant et al., 2005).

Systemic resistance for virus infections can be induced in plants treated with certain bacteria or with bacterial products, and also by treatment with some chemicals which may be more risky when compared with bacteria (Bakker et al., 2003; Shoman et al., 2003). The role of such induced systemic resistance described by the enhancement of the production of plant antioxidant protective enzyme, peroxidase (Shoman et al., 2003), besides the activation of some plant defense genes producing pathogenic related proteins (PR-Ps), is not well studied yet for its mode of action (Shehata and El-Borollosy, 2007).

Many investigators study the effect of PGPR on controlling plant virus infection, i.e., Bergstorm et al. (1982) showed that resistance in cucumber against cucumber mosaic virus (CMV) could be induced by previous treating of plants with *Colletotrichum orbiculare* or *Pseudomonas syringae*. Maurhofer et al. (1994) studied successfully the effect of *Pseudomonas fluorescens* on the resistance of tobacco against tobacco necrosis virus (TNV). De Meyer et al. (1999) enhanced the resistance of tobacco plants against tobacco mosaic virus (TMV) using *Pseudomonas aeruginosa* and Ryu et al. (2004) protected *Arabidopsis thaliana* plants against CMV infection using *Serattia marcescens*.

Therefore, the objective of this investigation is to study the effect of treating cucumber plants with some isolates of PGPR, on controlling CMV infection and enhancement of plant growth, study the enhancement of peroxidase and β-1,3-glucanase production as an antioxidant plant protecting enzyme, and the induction of PR-Ps plant genes for protein production.

Materials and methods

Isolation of virus and bacteria

Cucumber mosaic cucumovirus (CMV) was isolated from cucumber plants showing virus like symptoms (collected from the open fields of Faculty of Agriculture, Ain Shams University, Cairo, Egypt). Isolation was performed depending on I-ELISA (Koenig, 1981) using specific polyclonal antibodies (Agdia Inc., USA), and *Chenopodium quinoa* as local lesion host. Virus was maintained on *Nicotiana tabacum* cv. White Burley under greenhouse conditions (28 °C ± 2).

On the other hand, three of the predominant rhizobacteria were isolated from the mentioned cucumber plant rhizosphere. Media used for isolation were nutrient agar (Thiery and Francon, 1997), King's medium B (KMB) (King et al., 1954) and nitrogen-free Jensen's medium (Jensen, 1942) (Sigma–Aldrich Inc., USA) depending on the agar plate dilution method (Black, 1996).

Bacterial cultures

Three isolates of the most predominant cucumber rhizosphere bacteria were selected and isolated as pure cultures. Bacterial colonial properties were determined, and cells were observed under microscope after proper staining (Gram and spore staining). Essential biochemical tests were carried out according to Collins and Lyne (1984). Finally bacteria were identified according to Bergey's Manual (Sneath, 1984; Holt et al., 1994; Ahmad et al., 2005).

Bacteria were grown separately in nutrient broth or Ashby's mannitol media (Jones, 1913) for 72 h/30 °C on a