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Isolation, characterization of glycolipid type biosurfactant from endophytic *Acinetobacter* sp. ACMS25 and evaluation of its biocontrol efficiency against *Xanthomonas oryzae*

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Abstract

Acinetobacter sp. ACMS25, which showed inhibitory effect on *Xanthomonas oryzae* pv. *oryzae* XAV24 was tested for biosurfactant production. Preliminary characterization showed that this strain was positive for hemolytic and oil spreading activity and was able to reduce the surface tension of water from 71.9 to 37.6 mN/m. The biosurfactant produced by this strain was characterized as a 'glycolipid-type' based on thin layer chromatography (TLC), fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (¹HNMR) analysis. Inhibition studies showed that this biosurfactant was able to reduce the specific growth rate of *X. oryzae* by 38.4% and spermosphere population by 43.5%. Glycolipid biosurfactant treatment improved the germination and vigour index, when challenge inoculated with *X. oryzae* and this treatment was able to offer 76.9% disease protection efficiency against the rice blight disease in rice.

Introduction

Endophytic bacteria after successful survival in the spermosphere and rhizosphere are known to colonize different plant parts such as stem, leaf and other reproductive organs (Rosenblueth and Martínez-Romero, 2006, Compant et al., 2011, Reinhold-Hurek and Hurek, 2011). During this period of colonization they form metabolic association with the host plants (Compant et al., 2005) to enable them to selectively adapt to different ecological niches (Gray and Smith, 2005, Hallmann et al., 1997). These endophytic bacterial association with the host plant can play a major role in improving plant growth and offering protection against various pathogens (Bent and Chanway, 1998, Chanway, 1997).

To offer protection endophytic bacteria are known to synthesize several metabolites with antagonistic properties such as siderophores, antibiotics and biosurfactants (Brader et al., 2014, Joe et al., 2012). Among these antimicrobials, the use of biosurfactants against various plant pathogenic microorganisms as an alternative to the synthetic chemicals and antibiotics is gaining momentum (Nihorimbere et al., 2011, Sachdev and Cameotra, 2013). These biosurfactants, are low molecular weight surface-active amphiphilic biomolecules produced by a diverse group of microorganisms, that can reduce the surface tension either at the air/water interfaces or the interfacial tension at oil/water interfaces (Banat et al., 2010).

Taking this into account, the present study was conducted to evaluate the biosurfactant production of *Acinetobacter* sp. ACMS25, which showed inhibitory effect against *X. oryzae* p.v. *oryzae*. This was followed by purification and characterization of biosurfactant. In vitro and greenhouse studies were carried out to evaluate the efficiency of biosurfactant against *X. oryzae*.

X. oryzae is one of the most devastating diseases in rice, throughout the world and in particular to Asia (Ou, 1985). Being a seed borne pathogen and due to its high epidemic potential, it has been considered as a threat to all rice cultivars throughout the world. Though the use of pesticides and other antibiotics has been recommended against this pathogen, residual toxicity and development of antibiotic resistance has made researchers look for other viable alternatives.

Section snippets

Bacterial strains growth and maintenance

Acinetobacter xylosoxidans used in the present study was isolated from a medicinal plant *Catharanthus roseus* (Karthikeyan et al., 2012), this strain was selected after preliminary screening for antagonistic activity from numerous strains of our lab collection. The bacterial strains were maintained at -20°C as glycerol stocks. Prior to use they were grown overnight in nutrient broth at $28 \pm 2^{\circ}\text{C}$, 120rpm or in nutrient agar medium at $28 \pm 2^{\circ}\text{C}$ for 24–36h. Strain *X. oryzae* p.v. *oryzae* XAV24 was

Screening for antagonistic activity against *X. oryzae*

Among twenty four Plant growth-promoting Rhizobacteria (PGPR) strains from our lab collection tested for their in vitro growth inhibition activity against *X. oryzae* p.v. *oryzae*, seven isolates showed antagonistic effect and four isolates were also found to be positive for biosurfactant production (Data not shown). Among these four isolates evaluated *Acinetobacter* sp. ACMS 25, which showed the highest antagonist activity against *X. oryzae* was selected for further study. Though reports regarding

Acknowledgements

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2020, Journal of Biotechnology

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...Surfactants are chemical compounds distinguished by the occurrence of amphipathic molecules consisting of hydrophobic and hydrophilic moieties, which favors separation at the interface between fluids phases with different degrees of polarity and hydrogen bonds. Thus they can solubilize hydrocarbons by the formation of microemulsions and by reduction of surface and interfacial tension (Shalini et al., 2017). The surfactants have polar heads with ionic, non-ionic or amphoteric moieties...

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2018, Scientia Horticulturae

Citation Excerpt :

...Plant debris, seeds, and weeds are the most important sources of inocula for black rot (Soudi et al., 2011; Krauthausen et al., 2017). Different management strategies have been developed against phytopathogenic bacteria that include the use of microbial-free seeds and seedlings, inherently resistant cultivars (Luiz et al., 2016), antibiotics (Stockwell and Duffy, 2012; Dunegan, 1954) antimicrobial chemicals (Sayler and Kirkpatrick, 2003; Behlau et al., 2008) and biocontrol agents (Wulff et al., 2002; Massomo et al., 2004; El-hendawy et al., 2005; Newman et al., 2008; Mishra and Arora, 2012; Ghazalibiglar et al., 2016; Shalini et al., 2017). However, these strategies are not always effective, especially when the pathogenic bacteria are widely disseminated, the plants are heavily affected and the environmental conditions are optimal for disease emergence...

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