Maize Journal (April 2023) 12(1): 1-9

RESEARCH PAPER

Rhamnolipid biosurfactant could be a green approach for the management of maydis leaf blight of maize

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Abstract: Southern corn leaf blight or maydis leaf blight (MLB) caused by *Cochliobolus heterostrophus* (Drechs.) [(anamorph *Bipolaris maydis* (Nisikado and Miyake) Shoemaker] is an important disease of maize. Current pest management strategy relies mostly on chemical pesticides becomes a public concern due to environmental pollution. Hence use of green compounds (GRAS) has been emphasized for pest management so as to achieve sustainable agricultural productivity. Recently, biosurfactants are receiving much attention and brought in to use for plant pathogen elimination and also for increasing the bioavailability of nutrients for beneficial plant associated microbes. Biosurfactants are a diverse group of surface active molecules produced mostly on microbial cell surfaces and they can be excreted extracellularly. The present study was carried out to evaluate the efficacy of rhamnolipid (RL) biosurfactant produced by newly isolated *Pseudomonas aeruginosa* strain SS14 as an antifungal agent against *B. maydis in Zea mays L. In vitro* experiment was conducted by adopting poison food technique at different concentrations (25, 50 and 100 ppm). The studies indicated that the biosurfactant produced by this bacterial strain inhibits the growth of *B. maydis* by 39 per cent at a concentration of 100 ppm. *In planta*, treatment of maize seeds with a RL concentration of 100 mg/lit with foliar spray of 100 mg/lit resulted in improved biomass compared to those of healthy control plants and complete suppression of characteristic disease symptoms of MLB disease. The results demonstrated the possibility to develop a sustainable and eco-friendly management measure against the necrotrophic fungal pathogen *B. maydis.*

Keywords: Maize · *Bipolaris maydis* · Maydis leaf blight (MLB) · Rhamnolipid (RL) · Biosurfactants · Management

Introduction

Maydis leaf blight (MLB) of maize, also known as southern corn leaf blight (SCLB) caused by ascomycetous fungus *Cochliobolus heterostrophus* (Drechs.) [(anamorph *Bipolaris maydis* (Nisikado and Miyake) Shoemaker] synonym *Helminthosporium maydis* (Nisikado) is common in tropical and subtropical regions (Singh and Singh, 1963; Singh and Srivastava, 2012). Symptoms are exhibited in the form of small diamond shaped spots (2-6 × 3-22 mm). The center of each lesion was straw colored to light brown, surrounded by a dark brown margin. These lesions elongate as they mature, although growth of the lesions is restricted by leaf veins. Symptoms may be confined to leaves or may develop on sheaths, stalks, husks, ears and cobs (Manamgoda *et al.*, 2014). The incidence of this disease was first time reported by Drechsler (1925) from United States and by Munjal and Kapoor (1960) from Maldah (West Bengal, India). The disease is presently prevalent in almost all the agro-climatic zones in India (Agarwal *et al.*, 2022). Payak and Renfro (1968) reported that disease epidemics at an early stage causing premature death of blighted leaves which lose their value as fodder.

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Received: 20 February 2023/ Accepted: 09 March 2023

The MLB pathogen found on seed and sporulates on seedlings from infected seeds (Singh *et al.,* 1974). White and Ellett (1971) concluded that severely infected seeds from MLB failed to germinate and loss in plant population is directly correlated with the percentage of infected seed. It is considered to be one of the most devastating diseases as it appears in sizeable form resulting in reduction of grain yield of maize by 28 to 91 per cent (Payak and Sharma, 1980). Severe losses in grain yield due to epiphytotics have been reported in several parts of India and these losses vary from 25 to 90 per cent depending upon the severity of the disease (Bisht, 2015). Cultivation practices favoring high humidity and moderate temperature conditions may influence the development of maydis blight. MLB disease management primarily relies on the screening, identification and use of resistant varieties (Vasmatkar *et al.*, 2022; Hooda *et al.*, 2012) and initially the use of fungicide was rare, but during the last few decades an increase in use of foliar fungicides has been noticed. A variety of fungicides was reported to use for management of this disease, but only few have crop specific label claim for maize in India (Bisht, 2015; ICAR-IIMR, 2016). Further, due to their (fungicides) environmental hazards, high cost and sometime unavailability in the local market, limits the widespread use of effective chemicals by the maize growers.

Several workers reported that apart from fungicides, some botanicals (Debnath *et al.*, 2020) and biocontrol measures may also play an important role in preventing the growth of the necrotrophic pathogen *B. maydis* (Deng *et al.,* 2014; Bisht, 2015). Fluorescent pseudomonads are widely used for the purpose of biocontrol, the antifungal properties of pseudomonads against many pathogens have been reported earlier by other researchers but still are not widely exploited to control MLB of maize. Pseudomonads produce many secondary metabolites which include antibiotics and biosurfactants that are inhibitory to plant pathogens (Haas and Defago, 2005). Various types of biosurfactants have been reported to produce by Pseudomonads based on their physico-chemical properties as glycolipids, lipopeptides, neutral lipids, phospholipids, fatty acids, and polymeric compounds (Cameotra *et al.*, 2010; Goswami *et al.,* 2015; Borah *et al.,* 2016). The biosurfactants exhibit antibacterial, antifungal, and antiviral activities, which make them relevant molecules for applications in combating many diseases of crop plants (Gudina *et al.,* 2010; Kiran *et al.,* 2015). Rhamnolipids (RLs) are one of the most widely studied biosurfactants over the years (Abdel-Mawgoud *et al.,* 2010; Randhawa and Rahman, 2014). RLs are having some antibacterial and antifungal properties which were demonstrated by many workers against a wide range of pathogens (Kiran *et al.,* 2015; Lahkar *et al.,* 2015; Borah *et al.,* 2016). In the present study, we evaluated the antifungal effect of RLs produced by *Pseudomonas aeruginosa* SS14 as a prospective antifungal molecule against MLB under laboratory and field conditions.

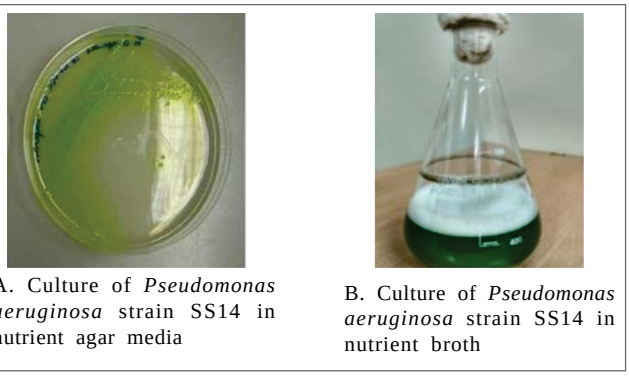
Materials and methods

Preparation of culture media

Mineral salt media (MSM) was prepared with the compositions (g/L) Na_4NO_3-4, KCl-0.1, KH_2PO_4-0.5, K_2HPO_4-1.0, $CaCl_2$ -0.01, $MgSO_4.7H_2O$ -0.5, $FeSO_4.7H_2O$ -0.01, yeast extract -0.01 with trace element solution (1%) containing (g/L) H_3BO_3 -0.26, $CuSO_4.5H_2O$ -0.5, $MnSO_4.H_2O$ -0.05, $(NH_4)_6Mo_7O_{24}.4H_2O$-0.06 and $ZnSO_4.7H_2O$-0.7 where glucose was used as the sole carbon source (Lahkar *et al.,* 2015). The chemicals used in preparation of MSM were purchased from HiMedia. The pH of the media was adjusted to 7.0±0.2 (Borah *et al.,* 2015).

Preparation of cell free supernatant (CFS)

The bacterial strain *P. aeruginosa* strain SS14 (GenBank accession no. KF031434, NCBI) was isolated from crude oil contaminated soil of upper Assam, India. A 5% inoculum from active culture of SS14 was transferred aseptically in 100 ml NB in a 250 ml Erlenmeyer flask (Figure 1) in shaking incubator (Scigenics Biotech, ORBITEK LJEIL, India) for 24 hrs at 150 rpm where temperature was maintained at 35±1°C. Fresh culture of SS14 from NB was inoculated (5%) in to mineral salt medium (MSM) under sterile condition and incubated in a shaker incubator at 150 rpm for 72 hrs at a temperature of 35±1°C (Borah *et al.,* 2016). After every 24 hrs till 72 hrs, surface tension (ST) of the bacterial cultures in MSM was measured in K1 tensiometer (Kruss, Germeny) using plate method and optical density (OD) was measured using an UV visible spectrophotometer. After 72 hrs, the bacterial culture was centrifuged for a period of 15



A. Culture of *Pseudomonas aeruginosa* strain SS14 in nutrient agar media

B. Culture of *Pseudomonas aeruginosa* strain SS14 in nutrient broth

Figure 1. Culture of *Pseudomonas aeruginosa* strain SS14 in different media

minutes at 10000 rpm at 4°C in a cooling centrifuge (Remi) and the cell free supernatant (CFS) was obtained. The ST of the CFS of all the bacterial cultures was measured.

Production of crude biosurfactant

For extraction of crude biosurfactant, the CFS of cultures of SS14 obtained by centrifugation was put to deproteinize at 10°C for 15 min followed by acidification with 6N HCl till pH of the CFS set at 2.0 (Lakhar *et al.,* 2015). The acidified CFS was kept at 4°C for overnight for precipitation of biosurfactant. After all the treatment, the precipitated biosurfactant was mixed with the solvent ethyl acetate (ACS) in a ratio 1:1 and shook vigorously. The solvent was then evaporated using rotary evaporator (Equitron 63R-D) at a temperature of 60°C and reduced pressure (Goswami *et al.,* 2016). The crude biosurfactant obtained so was collected in a glass beaker and dried to find out the yield in g/L unit. The production of crude biosurfactant is represented in Figure 2.

Purification of column purified biosurfactant

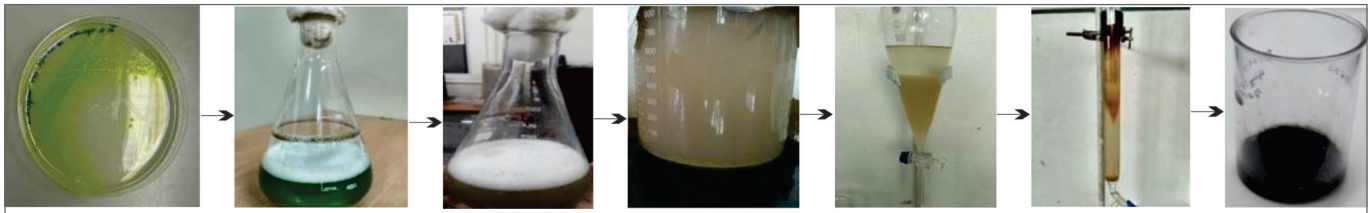
Silica gel (HiMedia) of mesh size 60-20 was dissolved in chloroform (ACS Mark) and a glass column of length 500 mm, diameter 25 mm was loaded with silica. For purification, 1g of crude biosurfactant was dissolved in 5 ml of methanol (ACS grade, Sigma Aldrich) and was allowed to elute through the glass column. A solvent system comprised of methanol and chloroform of the gradient 3:50, 5:50 and 50:50 was used for total elution of the biosurfactant. The eluted mixture was evaporated in rotary evaporator at 40°C under reduced pressure (Goswami *et al.,* 2015) and the extracted column purified biosurfactant was dried.

Isolation of Bipolaris maydis

The fungus *B. maydis* was isolated from leaf lesions obtained from maize field of the Division of Plant Pathology, ICAR-IARI, New Delhi using modified method of Sharma *et al.* (2005). Symptomatic tissues from leaves were cut into 5 mm pieces and surface-sterilized with 1% sodium hypochlorite (NaOCl) for 30–60 s. The tissues were then rinsed with three changes of sterile distilled water, dried on sterilized blotting paper and aseptically transferred to potato dextrose agar (PDA) medium in Petri plates with three pieces of tissues per plate. Plates were incubated at 25±2°C for 5 days. The mycelium growing out of the plant tissue was sub-cultured to PDA and incubated at 25±2°C again for 5 days. Purification was done by single spore isolation and identified the pathogen on the basis of cultural characters and morphological features like shapes and size of conidia and conidiophores.

In vitro evaluation of biosurfactant against Bipolaris maydis

Efficacy of the RL biosurfactant at 25, 50 and 100 ppm concentrations was studied against *B. maydis* by using poisoned food technique (Sharvelle, 1961). Crude extract of biosurfactant dissolved in acetone was determined as



Cultured *P. aeruginosa* in Petri plate | Cultured *P. aeruginosa* in broth for 24 hrs | Production of biosurfactant (BS) in MSM | Acidified cell free supernatant with precipitation | Production of crude BS | Column purification by silica gel chromatography | Extraction of BS using ethyl acetate

Figure 2. Production of crude rhamnolipid biosurfactant from *Pseudomonas aeruginosa* strain SS14

stock solution of 10000 ppm concentration. Required amount of the solution was added into 50 ml flask containing 50 ml of the sterilized molten PDA to get final concentrations of 25, 50 and 100 ppm. The culture medium was mixed thoroughly before plating and subsequently poured in three Petri plates. Media mixed with acetone was poured into separate Petri plates were kept as a negative control and plates with unamended media as positive control. After solidification of media a 2 mm mycelia disc of 10 days old culture of the test pathogen was cut out with sterile cork borer and placed at the centre of each Petri plate. The Petri plates were incubated at 25±2°C. Antagonistic activity was expressed in terms of percentage inhibition of mycelial growth (Vincent, 1947) measured after 10 days of incubation when the positive control plates were completely covered by the mycelia of *B. maydis* and the radial growth was compared with the test plates under observation.

Field evaluation of biosurfactant for efficacy against maydis leaf blight

Field preparation

Three field experiments were conducted to study the effect of RLs against MLB of maize for three years during *kharif* (summer), 2017-19 in the research field of ICAR-IARI, New Delhi. The experiments were laid out in randomised block design (RBD) maintaining plot size 3 m x 3 m with spacing of 20 cm and replicated thrice. One MLB susceptible cultivar of sweet corn was used for the study. The package of practices standardized by ICAR-Indian Institute of Maize Research (Parihar *et al.*, 2011) was followed during the cropping period.

Inoculum preparation, inoculation and field experimentation

Inoculum of *B. maydis* was prepared by mass multiplication of its pure culture on sorghum seeds by following the method of Ahuja and Payak (1978). Little quantity of the inoculum powder was taken in between fingers and placed into the central whorl of 30 days old (4-6 leaf stages) maize plants in the evening to allow successful infection when moisture and ambient temperature are optimum (Payak and Sharma, 1983). After 8-10 days, the plants were inoculated for the second time to avoid failure of disease establishment.

Three treatments were applied for the management of MLB of maize, *viz.*, seed treatment with biosurfactant (100 mg/kg) (T1), biosurfactant seed treatment along with foliar spray (100 mg/l) (T2), biosurfactant foliar spray (100 mg/l) (T3), Mancozeb foliar spray (2.5 g/l) (T4) and untreated control (T5). The seed treatment was given for overnight and foliar spray was performed one week after the appearance of first lesions. The per cent disease progress was recorded using blight assessment keys of the standard 1-5 scale and percent disease index (PDI) was calculated (Payak and Sharma, 1983). The observation of the effect of different treatments of RL on germination and grain yield (q/ha) were also recorded.

Statistical analysis

The data represented arithmetical averages of three replicates and the error bars indicate the standard deviations. Statistical analyses were performed with PASW-18 software (SPSS Inc.). Pearson's correlation was used to analyze the correlation between biosurfactant production and percentage inhibition ($p<0.05$). Results for the effect of RL on germination per cent, percent disease index and yield were analyzed using one-way ANOVA with *PostHoc* pair wise Least Significant Difference (LSD) comparison and Duccan Multiple Range Test (DMRT) at a significance level of 0.05.

Results

In vitro efficacy of rhamnolipids against Bipolaris maydis

The antagonistic effect of different concentrations of RL biosurfactant on mycelial growth of *B. maydis* was evaluated by calculating percentage inhibition in comparison to the control on tenth day of inoculation of the fungus (Figure 3). The assessment of antifungal activity of RL biosurfactant produced by newly isolated *P. aeruginosa* strain SS14 against *B. maydis* revealed significant inhibition in mycelial growth ($p<0.05$, $df_{2,6}$; F 24.9) at all the concentrations. The RL biosurfactant exhibited 39 per cent inhibition of *B. maydis* at the concentration of 100 ppm which was followed by 33% at 50 ppm and 21% at 25 ppm (Table 1). The percentage of inhibition of the mycelium growth was gradually increased with the increase in concentration of RL biosurfactant (Figure 4).

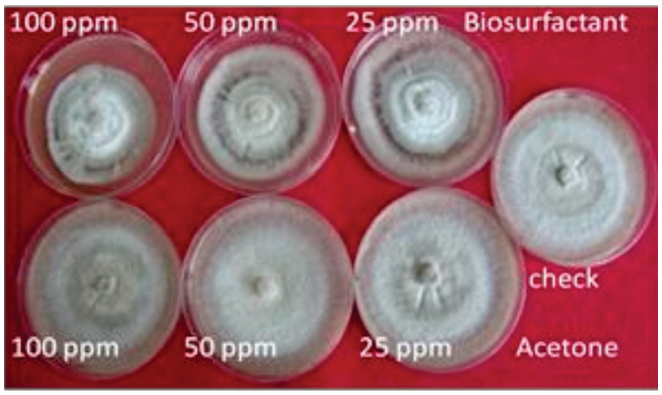


Figure 3. Inhibition of mycelial growth of *Bipolaris maydis* at different concentrations of rhamnolipid biosurfactant

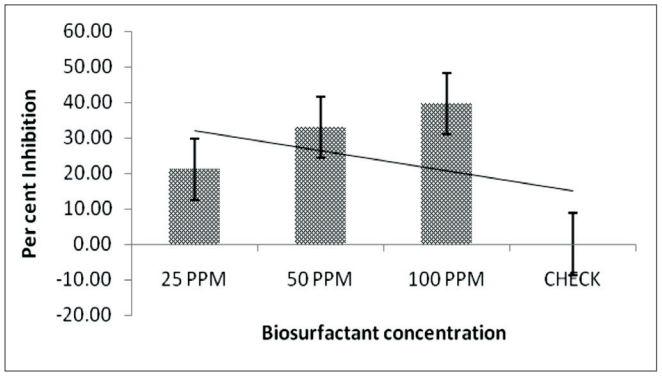


Figure 4. Percent inhibition of mycelial growth of *Bipolaris maydis* at different concentrations of biosurfactant

Table 1. Effect of biosurfactant on mycelial growth of *Bipolaris maydis* at different concentrations

Concentrations of biosurfactant	Mycelial growth (mm)*	Inhibition (%)*
25 ppm	7.1±0.17^{c}	21±1.7^{a}
50 ppm	6.0±0.45^{b}	33±5.0^{b}
100 ppm	5.4±0.21^{a}	39±2.1^{c}
Control	9±0.0^{d}	-

* After 10 days of incubation; "mm"; values of mycelial growth (MG) and % inhibition is mean of three replications; ± values represent standard deviations (SD); Different letters within each column indicates significantly different values according to LSD at alpha = 0.05.

Field evaluation of biosurfactant for efficacy against maydis leaf blight

The RL biosurfactant was found effective in improving germination percent of sweet corn variety of maize (Table 2; Figure 5). As per observation, the highest 58.3 percent germination was recorded in the maize seeds treated with a concentration of 100 mg/l of biosurfactant and 53.6 per cent germination was in seed treatment (100 mg/l) coupled later with foliar spray (100 mg/l) treatment. Seed treatment with RL biosurfactant exhibited similar results

Table 2. Effect of rhamnolipid biosurfactant on seed germination, percent disease index of maydis leaf blight and grain yield of maize during *kharif* (summer) 2017-2019

Rhamnolipid biosurfactant	Germination (%)				PDI (%)				Yield (Q/ha)			
	2017	2018	2019	Pooled*	2017	2018	2019	Pooled*	2017	2018	2019	Pooled*
Seed treatment (100 mg/l) (T1)	65.0	50.0	60.0	58.3±7.6^{a}	36.4	67.5	65.5	56.4±17.4^{a}	15.9	35.0	34.5	28.5±10.8^{a}
Seed treatment (100 mg/l) + Foliar spray (100 mg/l) (T2)	62.0	55.0	44.4	53.6±9.0^{a}	34.9	49.8	50.1	44.9±8.6^{a}	18.4	46.9	42.5	35.9±15.3^{a}
Foliar spray (100 mg/l) (T3)	60.3	46.7	40.3	48.8±10.3^{a}	45.3	67.5	64.5	59.1±12.0^{a}	14.4	34.8	37.1	28.8±12.3^{a}
Mancozeb (2.5 g/l) (T4)	66.7	40.0	30.6	45.7±18.7^{a}	32.4	47.3	50.1	43.2±9.5^{a}	20.8	48.9	44.4	38.0±15.0^{a}
Control (water) (T5)	60.0	45.0	33.3	46.1± 13.3^{a}	47.8	86.7	75.6	70.0 ±20.0^{a}	11.1	33.1	40.0	28.0 ±15.9^{a}

*Data are means of three replicates ± SD. Different letters within each column indicate significantly different values according to LSD at alpha = 0.05. ANOVA was performed for germination percent ($F_{4,9}$ = 0.47), PDI ($F_{4,10}$ = 1.7), Yield ($F_{4,10}$ = 0.32)

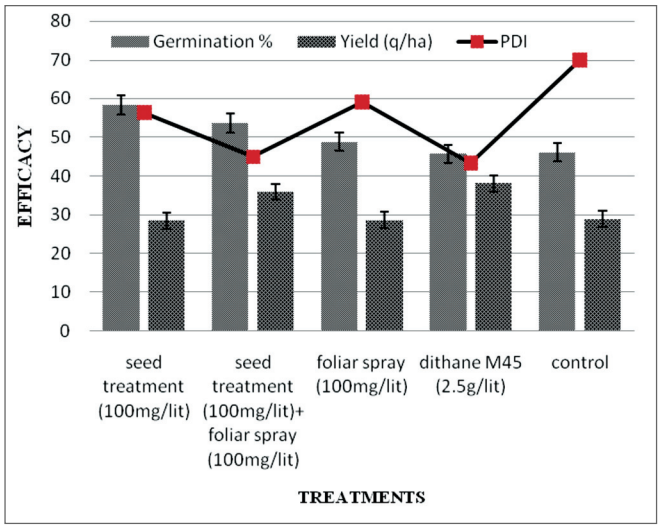


Figure 5. Effect of rhamnolipid biosurfactant on seed germination, percent disease index of maydis leaf blight and grain yield of maize during *Kharif* (summer) 2017-2019

($F_{4,9}$ = 0.47, $p<0.05$) with that of mancozeb. It is interesting to note that, both seed treatments (T1 and T2) with RL biosurfactant induced better germination of the maize seeds than the rest treatments (T3, T4 & T5).

Reduction in the percent disease index (PDI) of MLB was achieved due to application of RL biosurfactant (Figure 5). Seed treatment (100 mg/l) combined with foliar spray (100 mg/l) of biosurfactant (T2) exhibited lowest PDI (approx. 45%, $F_{4,10}$ = 1.7, $p<0.05$) which was at par with mancozeb spray (43.2%). The three years data showed significant difference among all the treatments in comparison to plain water spray ($p<0.05$).

Among all the treatments, maize grain yield (q/ha) was maximum (35.9 q/ha) in seed treatment (100 mg/kg) combined with foliar spray (100 mg/l) of biosurfactant (T2) which exhibited comparable result ($F_{4,10}$ = 0.32, $p<0.05$) with that of the fungicide spray (38.0 q/ha). It was followed by the treatment of foliar spray (100 mg/l) which contributed grain yield nearing about 29 q/ha. In case of seed germination percent and yield of consecutive three years, no significant difference was found among the treatments in comparison to the recommended fungicide mancozeb application, but significant difference was observed among all the treatments in comparison to the control (water) (LSD $p<0.05$).

Discussion

Biosurfactants are reported to produce by bacteria, yeasts, and fungi which can serve as green surfactants. The rhamnolipid biosurfactants are considered as less toxic and eco-friendly, and thus several types of biosurfactants encompass the potential to produce commercially for extensive applications in pharmaceuticals, cosmetics, and food industries (Cardoso *et al.*, 2010; Saikia *et al.*, 2012). Such biosurfactants synthesized by microorganisms also show evidence of promising role in the agricultural industry (Canova *et al.*, 2010). Several reports had highlighted the role of biosurfactants for plant pathogen elimination and for increasing the bioavailability of nutrients to the beneficial plant associated microbes (Sachdev and Cameotra, 2013; Goswami *et al.*, 2015).

In the present study, management of maydis leaf blight disease in a sweet corn variety of maize was studied by using biosurfactant. The biosurfactant was produced by newly isolated *P. aeruginosa* strain SS14 using mineral salt media. The crude biosurfactant was identified as rhamnolipids (Borah *et al.*, 2016; Goswami *et al.*, 2014). The rhamnolipid (RL) biosurfactant exhibited inhibition of 39 per cent of *B. maydis* at concentration of 100 ppm which was followed by 33% inhibition at 50 ppm and 21% at 25 ppm. The percentage inhibition in mycelium growth was gradually increased with the increase in concentration of RL biosurfactant. The results of the present study were truly anticipated as the reports of previous workers opined that rhamnolipids can be a potential antifungal agent to control various diseases of several crop plants (Borah *et al.*, 2016; Goswami *et al.*, 2015; Yang *et al.*, 2014). The unique analogues present in the RLs produced by the strain SS14 seemed to enhance the antifungal activity as reported earlier (Benincasa and Accorsini 2008; Borah *et al.*, 2016). Biological control with bacteria and the biosurfactant produced by them offers a simple and cost-effective strategy for managing various phytopathogens (Pacwa *et al.*, 2011; Sachdev and Cameotra, 2013). The biosurfactant was very effective in inhibiting the mycelial growth of the fungus and suppressing the disease symptoms as well as found highly effective in increasing crop growth (Ngullie, 2010; Gamalero and Glick, 2011).

Commonly used surfactants are divided on the basis of polar and non-polar groups into synthetic surfactants and biosurfactants (Banat *et al.*, 2010; Zhong *et al.*, 2016). The mechanism of RL action against microorganisms is not yet elucidated, but, it is supposed that the cell membrane is the target. Some studies showed that biosurfactants can increase the permeability of

microbial cells (Sotirova *et al.,* 2008), cell surface charge and CSH (Kaczorek *et al.,* 2010; Kim *et al.,* 2015). Liu *et al.* (2012) observed that rhamnolipids can modify the CSH and the surface charge of *Penicillium simplicissimum* due to the change of the cell surface functional groups (increased the hydrophobic functional group) and element concentrations. This will alter the membrane structure and character, which will amend the major membrane functions (such as matter transport, energy generation and membrane permeability, and cell surface properties (Sotirova *et al.,* 2008; Banat *et al.,* 2010). Sotirova *et al.* (2009) observed that RLs increased the membrane permeability of *Bacillus subtilis* and *Pseudomonas aeruginosa*, resulting in metabolite leakage leading to the change of cell surface properties. It was also reported that surfactants can induce increase of membrane lipids and led to the decrease of membrane fluidity of *Arthrobacter* sp. strain Sphe3 (Kallimanis *et al.,* 2007). The effects of RLs on cell surface properties depend on the concentration and types of rhamnolipids, the species of microorganism, and environmental conditions etc. (Yuan *et al.,* 2007). However, no correlation with the antimicrobial activity was demonstrated.

The RL biosurfactant was found successful in improving germination and grain yield of maize. The maize seeds treated with RL biosurfactant showed better rate of germination which exhibited comparable results with the recommended fungicide. Foliar spray of this RL biosurfactant affected expression of the disease symptom on the leaves. The external symptoms were appeared in the form of small diamond shaped spots (2-6 × 3-12 mm). The center of each lesion was straw colored to light brown, surrounded by a dark brown margin and restricted only on the affected leaves. Otherwise, diseased lesions appear on the entire leaf lamina under severe condition and also may develop on the sheaths, stalks, husks, ears and cobs (Manamgoda *et al.,* 2014). Keeping consistency with this, the percent disease index in the present study was evaluated in terms of symptomatic leaf infections using blight assessment keys (Payak and Sharma, 1983). Further, yield (q/h) was evaluated to monitor the effect of disease on plant growth and health and ultimately on the cobs. The use of RL biosurfactants for plant growth promotion was also well documented owing to their detrimental effect on pathogens (Gamalero and Glick, 2011). On account of the same reason, RL biosurfactant could effectively reduce intensity of MLB disease which was comparable with the performance of mancozeb, a fungicide recommended in the package of practice of maize cultivation. Among the entire treatments considerably higher yield (q/ha) was obtained by practicing seed treatment and subsequent foliar spray with RL biosurfactant @ 100mg/l. The enhanced grain yield resulted from dual application of RL biosurfactant has indicated about its role in encouragement of better plant health and suppression of the MLB diseases more effectively.

Conclusion

In conclusion, the results of the present investigation reveal that the RL biosurfactants produced by the bacterial strain *P. aeruginosa* strain SS14 have strong antifungal property against the necrotrophic fungal pathogen *B. maydis*. The performance of biosurfactant was similar with the recommended fungicide mancozeb with respect to MLB disease control. However, its mode of action against the target pathogen needs to be determined. Biosurfactants as a whole are highly biodegradable under natural environmental conditions. They are non-toxic and safe for the environment, which may offer a possibility of their application as an alternative to the synthetic fungicides to control the fungal diseases of crops like maydis leaf blight of maize.

Acknowledgements

Authors are thankful to the Department of Biotechnology, Ministry of Science & Technology, GoI, India for funding research project under Twinning Project for the North East, 2017. Authors also duly acknowledge the Head, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi and the Director, Institute of Advanced. Study in Science & Technology, DST, Guwahati, Assam for the logistics and other supports.

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