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### ASSESSMENT OF AGRICULTURALLY IMPORTANT METABOLITES FROM THE ENTOMOPATHOGENIC BACTERIUM, *Photorhabdus temperata* M1021

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#### KEYWORDS

Plant microbe interaction

*Photorhabdus temperate*

Plant growth promotion

Insecticidal activity

*Galleria mellonella*

#### ABSTRACT

*Photorhabdus temperata* M1021 is an entomopathogenic enterobacterium, producing an array of metabolites in the culture extract. *Photorhabdus* spp. produced various classes of toxic proteins and secondary metabolites, which generate mortality in a wide range of insects. Aim of present study was to assess the effects these metabolites towards the insect and plants. Intra-hemocoel injections of whole cell and soluble extract caused 100% mortality in *Galleria mellonella* larvae within 48 h as compare to control. However, the culture filtrate (CF) took 72 h to kill 100 % larvae. In addition, there was no significant ( $P<0.05$ ) decrease in the rate of larval mortality, injected with CF, treated at 70°C for 30 min as well as with 50 mg/l proteinase-K. Gas chromatography mass spectrometry analysis revealed 23 compounds, among which “*trans*-Cinnamic acid” and “indole” were the most important for the insecticidal activities and plant growth promotion. The auxin contents quantified through Salkowski’s assay were 28 µg/ml to 30 µg/ml, insignificantly varied the by the addition of L-tryptophan ranged from 0 g/l to 0.5 g/l. The optimum pH for the auxin production was determined to be pH 7.0. The CF treatment increases significantly ( $P<0.05$ ) plant growth that attributes e.g. total plant length, chlorophyll content and biomass of both Dongjin-beyo and Waito-Crice plants, in a comparison with control. The findings of this study suggest that the *P. temperata* M1021 could be an ideal candidate, to be exploited in agriculture as biocontrol agent.

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## 1 Introduction

*Photorhabdus temperata* a gram negative bacterium used as a bio-pesticide in different countries of the world. It belongs to the family *Enterobacteriaceae*, which is a member of the genus *Photorhabdus* (Boemare et al., 1993), having three species: *P. asymbiotica*, *P. temperate* and *P. luminescens* (Akhurst, 1980). The infective juvenile (IJ) nematode of the family *Heterorhabditidae*, when encounters the potential insect prey, it penetrates the outer cuticle of the insect and, upon accessing the hemolymph, releases bacteria into the insect's hemolymph (Akhurst, 1982). The bacteria multiply rapidly, killing the insect within 48 h (Poinar, 1975). These lethal effects are attributed to the potential of *Photorhabdus* spp. to produce various classes of secondary metabolites such as stilbene derivatives, anthraquinone derivatives, genistine, a furan derivative, and a phenol derivative which generate mortality in a wide range of insects, especially at their larval stage (Park et al., 2013) including, *Galleria mellonella*, *Manduca sexta* and *Plutella xylostella* and many other insects (Duchaud et al., 2003). However, *Photorhabdus* bacteria can be grown as free living bacteria on artificial medium and their potential of secondary metabolites production can be exploited in agriculture to control insect pests (Jang et al., 2012). Moreover, toxins, extracted from the *Xenorhabdus* and *Photorhabdus* spp. have been successfully applied against the *Dothiorella* sps. that causes disease in guava plants (Isaacson & Webster 2002; San-Blas et al., 2013).

Despite the high class of insecticidal activity associated with toxins produced by *P. temperata*, it has yet to be utilized as a bio-control agent in the agriculture field, as the relationship of *P. temperata* with plants has been poorly elaborated. It is pivotal to thoroughly investigate the effects of *P. temperata* on plant growth and development prior to suggesting it as bio-control agent (Ullah et al., 2014). Chemical control has been used frequently in many parts of the world (Smith et al., 1999). Although they minimize the harmful effects of insects and phytopathogenic microorganisms, the use of agrochemicals poses severe environmental hazards, such as the leaching of nitrates and other undesirable compounds into groundwater (Hallmann et al., 1999). These agrochemicals are often non-degradable and bio-accumulated, thus posing health risks to consumers (Hu & Webster, 2000). The utilization of biological control agents as a substitute to chemical pesticide has been considered as sustainable solution to avoid agricultural based pollutions (Leach et al., 2008). Plant disease and pest control via bio-control agent such as entomopathogenic microbes is an alternative strategy that may contribute to reduce the usage of agrochemicals in the field of agriculture.

It is an ideal strategy to combat various environmental and crop production threats by achieving dual impact by using Microbes (Ullah et al., 2014). It opens new dynamics in the field of Agriculture biotechnology by exploring such beneficial microorganisms that has relationship with crop plants. Depending on the mode of action and effects of beneficial symbiotic microbes like *P. temperata*, these can be used as bio-control, plant strengtheners, phyto-stimulators and bio-pesticides (Ullah et al., 2013). The present study is a first step in this regard to assess the insecticidal and plant growth promoting effects of *P. temperata* M1021. During the recent study, the toxicity pattern of the proteinous and non-proteinous metabolites was assessed through intra-hemocoel injection in the *G.mellonella* larvae.

## 2 Materials and Methods

### 2.1 Maintenance for *P. temperate* M1021

Entomopathogenic nematodes of family *Heterorhabditidae* were isolated from the soil samples through the insect baiting method and *P. temperata* M1021, were isolated and characterise as described by Jang et al. (2012). The strain *P. temperata* M1021 was re cultured at 30 °C for 24 h in Luria-Bertani (LB) broth (0.5% yeast extract, 1% NaCl, 1% tryptone). The pre-culture was used to inoculate the culture medium, beginning with an initial O.D of 0.65 at 600nm.

### 2.2 Toxin extraction and bioassay

Bacterial strain grown at  $28 \pm 2^\circ\text{C}$  for 48 h was used for the toxin extraction and bioassay. *P. temperata* M1021 was cultured at  $28 \pm 2^\circ\text{C}$  for 48 h and centrifuged at  $10,000 \times g$  for 20 mins to partition the supernatant and cell pellets (Jang et al., 2012). Supernatant, containing insecticidal metabolites was filtered and was used as injection sample. Obtained pellet was re-suspended in 1 ml, 0.5% NaCl solution and a portion of this suspension was used as whole cell injection sample and the rest was subjected to sonication. Followed by sonication, the suspension was centrifuged at  $10,000 \times g$  for 20 mins at  $4^\circ\text{C}$  and supernatant was used as a soluble injection sample. Simple LB media was used as control. Using the Bradford protein assay the protein concentrations of all the injection samples were determined (Bradford, 1976). The protein contents of the toxin in three injection samples (whole cells, soluble and supernatant) were evaluated using a 10% resolving gel for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the Laemmli method (Laemmli, 1970).

In order to analyse the insecticidal toxicity of non-proteinous metabolites in the extracellular extract, the CF was treated at  $70^\circ\text{C}$  for 30 min. Furthermore, extract was treated proteinase in order to confirm the non-protein nature of the toxin. The CF was treated

with 50 mg/l concentrations of proteinase-K at incubated at 37°C for 60 min. After treatments samples were injected into hemocoel of *G. mellonella* larvae. The CF, without heat and proteinase-K treatments was used as reference (control). To examine the post injection effects on larvae, they were put under the observation in a Petri plate, incubated at 25 ± 2°C with 50 ± 5% relative humidity. Rate of mortality was monitored at 12 h of interval. Fifteen larvae were used per replication and three replications were used, the assay was performed three times.

### 2.3 GC-MS analysis of the culture extract

In order to list the important secondary metabolites produced by *P. temperata* M1021, the CF was analysed through GC-MS, according to procedure of Ullah et al. (2013). The CF was acidified to pH 2.5 ± 0.2 and partitioned through solvent-solvent extraction and organic layers were vacuum dried in rotary evaporator. Sample were eluted with EtOAc:hexane (v/v) and saturated using formic acid. The fraction was dried under vacuum, and the residues were resuspended in 2 ml of 100% MeOH. Evaporation of the fraction was done using a Savant Automatic Environmental Speed-Vac. The residue was dissolved in diazomethane and Methanol that leads to Methyl esters fractions. Oxygen-free nitrogen gas was used in order to remove the excess amount of diazomethane. Finally remaining residues were dissolved in Dichloromethane (HPLC grade). The oven was programmed with a starting temperature of 60°C, 70 eV ionization energy, 30 kPa head pressure and a final one of 285°C. Helium gas was used as carrier in this method. On the basis of retention time, compounds were identified after align with present online NIST MS spectra.

### 2.4 Auxin Detection and Purification in Culture Broth

Total auxin contents in the culture broth were detected by Salkowski's assay, using spectrophotometer. The strain was cultured in LB broth with a gradient of L-tryptophan ranging from (0.1–0.5 g/l) at incubated at 28 ± 2°C with 200 rpm. Cultures were centrifuged at for 10 mins at 10,000× g and supernatant was filtered using cellulose acetate filter (0.45 µm). Since *P. temperata* M1021 produces brick-red pigmentation in the culture broth therefore, the CF was passed through a series of purifications to eliminate the pigmentation. The CF was acidified to pH 2.5 ± 0.2 by 1N HCl, and partitioned using ethyl acetate solvent-solvent extraction. The organic layers were harvested and evaporated to dryness at 45°C. The dried extracts were re-suspended in 3 ml of Ethanol (100%) and kept at 4°C. The presence of auxin contents were confirmed by Salkowski test (Ullah et al., 2013). About 2 mL Salkowski's reagent was added to one ml of supernatant (12 g FeCl<sub>3</sub> L<sup>-1</sup> of 7.9 M H<sub>2</sub>SO<sub>4</sub>) that was incubated for 30 min in dark condition. The samples were then analysed after 30 min of incubation at 535 nm using spectrophotometer.

### 2.5 Auxin Production Profile

Auxin profile, under different time intervals of incubation and pH was determined by incubating 20 mL of *P. temperata* M1021 at 28 ± 2°C in shaking incubator at 200 ± 20 rpm. Approximately 5 ml samples were aliquoted at a day interval for up to 10 days, purified and analysed for auxins production. Similarly, pH range 4–8 was examined for its effect on auxin biosynthesis by *P. temperata* M1021 in the culture broth. The initial pH of the media was adjusted by 1 N HCl or 1 N NaOH and then the cultures were incubated at 28 ± 2°C, 200 ± 20 rpm for 72 h. approximately 5 ml sample from each pH was aliquoted, purified and assayed for auxin production by Salkowski's method.

### 2.6 Seed Germination and Plant Growth Dynamics

The two rice varieties, Dongjin (phenotypically normal rice plant) and *Waito-C* (phenotypically dwarf variety, mutant gibberellin production) were selected for plant growth assay. The seeds were sterilized using 75% ethanol for 2 min and subsequently washed 5–7 times with ddH<sub>2</sub>O. The surface sterilized seeds were germinated on pre-soaked filter paper (soaked with 1.5 mL double distilled water) in a Petri-dish, incubated at 25°C for 48 h. The germinated seeds were transferred into plastic pots (22 × 15 × 7 cm) filled with 40 mL of 0.8% agar.

In order to determine the effect of bacterial strain on plant growth, the rice seedlings were inoculated with CF of *P. temperata* M1021. The bacterial strain was cultured on 1L LB broth and incubated at 28 ± 2°C for 7 days. The culture was centrifuged for 20 mins at 10,000× g and the cell free culture was filtered through 0.4 µm pore size filter. Approximately, 5 mL of supernatant was applied to both rice varieties i.e. Dongjin and *Waito-C*, at two leaf stage. The plants were grown in chamber with control environment viz., day cycle of 13 h at 30°C with light intensity of 1,600 lux, and a night cycle of 11 h at 25°C. The plants were harvested after 14 days.

The effects of *P. temperata* M1021 treated rice varieties (*Waito-C* and Dongjin) were analysed against the control (without bacteria) rice plants. Shoot and root lengths were measured, while chlorophyll contents of the fully expanded leaves were examined by a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). The plant fresh bio-mass was measured immediately after harvesting while the bio-mass was measured after drying the plants at 70°C for 72 h in an oven.

### 2.6 Statistical Analysis

Statistical analysis was performed by using EXCEL software (Microsoft) and Mean values were compared with Duncan's multiple range test at a *P* value of 0.05 (analysis of variance; SAS release 9.1; SAS, Cary, NC, USA).

### 3 Results

#### 3.1 Insecticidal bioassay of culture extracts of *P. temperata* M1021

Five micro-liters of injection samples i.e. whole cell, soluble, and CF were injected into the larvae through intra-hemocoel injection. The results (Figure 1A) of study revealed up to 100% mortality in *G. mellonella* by whole cell and soluble injections with in 36 h and 48 h, respectively with reference to larvae injected with control. However, up to 60% larval mortality was noticed in the result of CF injection within 48 h, which reached to 100 after 72 h. The paralysis symptoms were appeared in larvae within first hour of injection.

Heat treatment was applied to eliminate the proteinous insecticidal factor and focus on the non-proteinous metabolites causing toxicity in insects. For this purpose, the CF was treated at 70°C for 30 min. Results (Figure 1B) revealed there was no significant ( $P < 0.05$ ) change in the larvae mortality and the activity was remained comparable with control. Besides the heat-treatments, Proteinase-K treatments at the concentration of 50 mg/l were applied, which confirmed the non-proteinous status of toxin extracted present in the CF of the *P. temperate* M1021. Results (Figure 1B) revealed that there was no significant ( $P < 0.05$ ) effect of proteinase-K treatment on the toxin. More than 90% of the insecticidal activity was preserved after application of proteinase-K as compare to control.

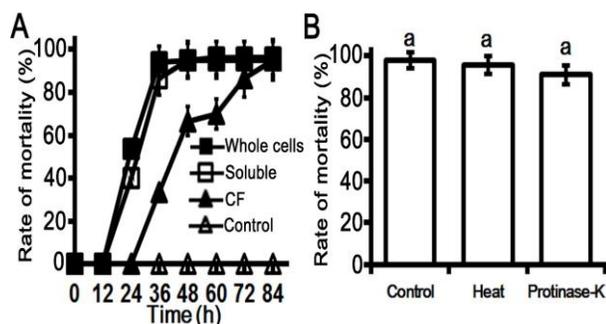


Figure 1 Toxicity pattern of the proteinous and non-proteinous metabolite, extracted from *Photorhabdus temperata* M1021 in *Galleria mellonella* larvae. Precisely 5  $\mu$ L of each injection sample was injected into the hemocoel of larvae. The resultant values are averages of three replications; error bars represent standard deviations. Different letters over the error bars indicate significant differences at  $P < 0.05$  levels as estimated by Duncan's multiple range test (DMRT). (A) Rate of mortality in *G. mellonella* larvae due to the intra-hemocoel injections of different proteinous fraction extracted from *P. temperata* M1021. Luria-Bertani media was used as a control. (■) whole cells, (□) soluble, (▲) culture filtrate (Δ), represents control. (B) Mortality in larvae caused by the intra-hemocoel injection of heat (70 °C) and proteinase-K (50 mg/l) treated CF of *P. temperata* M1021. The CF without heat and proteinase-K was used as control.

#### 3.2 GC-MS profiling of metabolites present in CF

The culture extract was subjected to gas chromatography–mass spectrometry, selected ion monitoring (GC-MS; SIM) analysis for determination of constituent compound. The chemical composition of EtOAc extract of CF was analysed by GC and GC-MS. Twenty-three compounds, summarized in table 1 were detected in *P. temperata* M1021. These compounds have vital consequence in the entomopathogenic bacteria, for the insecticidal, antifungal, antibacterial and antioxidant activities.

Table 1 Chemical composition of the culture filtrate of *P. temperata* M1021

S.No.	Constituents	RT	RA(%)
1	Styrene oxide	7.951	0.33
2	1H-Indole	13.055	0.44
3	dl-Ornithine	13.257	0.46
4	1-Alpha-amino-epsilon-caprolactum	15.073	2.87
5	trans-Cinnamic acid	15.514	0.24
6	2-Propenoic acid, 3-phenyl-	15.523	0.58
7	L-Isoleucine	16.625	9.18
8	L-Leucine	18.247	16.33
9	L-Norleucine	18.432	10.43
10	L-Leucine, ethyl ester	19.305	15.91
11	(3R,8aS)-3-Methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	20.433	2.02
12	DL-Phenylalanine	20.830	1.41
13	Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-	21.156	2.10
14	Cyclo(-L-Pro-L-Val-)	22.046	6.97
15	Phenol,3,5-dimethoxy-,acetate	22.434	1.44
16	L-Proline, N-pivaloyl, ethyl ester	23.448	6.77
17	4,8-Dihydroxy-2-(1'-hydroxyheptyl)-3,4,5,6,7,8-hexahydro-2H-[1]-benzopyran-5	23.695	7.71
18	3,9-DIAZATRICYCLO[7.3.0.0(3,7)]DODECAN-2,8-DIONE	23.880	4.78
19	2-ethoxy-3,4-dihydro-5-methyl-3-methylene-2H-pyran	27.759	1.19
20	5-Methyl-2-phenyl-4,6-diisopropylpyrimidine	27.935	1.44
21	Dihydroergotamine	28.993	1.34
22	3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0] nonane	29.530	5.03
23	4,6-Di-t-butyl-1H,3H-thieno[3,4-c] thiophene	31.540	1.03

RT = indicates the retention time of the compound; RA% = Relative Peak area).

Therefore, presence of these valuable and effectual compounds in *P. temperata* M1021 may play multifunctional role in the agriculture. The list indicated metabolites with proven insecticidal effects such as trans-Cinnamic acid and Cyclo-(L-Pro-L-Val) and indole. However, indole also has a role in the plant growth and developments therefore, it was further elaborated by Salkowski's assay and its effects were assessed on plant growth.

### 3.3 Detection of Auxin Production and Effect of L-Tryptophan

Auxins are common indole compounds and well known plant growth regulators. Having detection of indole through GC-MS, the auxin production was quantified through Salkowski's assay. The analysis revealed that *P. temperata* M1021 produced auxin in the culture medium (Figure 2). The concentration of auxin produced by *P. temperata* M1021 was about 30 µg/ml augmented with g/l of L-tryptophan. Similarly, the addition of L-tryptophan (0.1–0.5 g/l) in the bacterial inoculated culture broth exhibited no significant increase ( $P < 0.05$ ) in the auxin production. The concentration of auxin produced by *P. temperata* M1021 in the presence of L-tryptophan was ranged from 28 to 30 µg/ml.

*P. temperata* M1021 was cultured in LB media at  $28 \pm 2^\circ\text{C}$  for 7 days and the growth curve was found to approach the stationary phase at day seven of the incubation period after a long exponential phase lasting for six days (Figure 3A). In addition, to investigate the effect of incubation period on auxin production, the samples were harvested from the production media at a day interval for up to 10 days. The data obtained suggest growth-associated auxin production, and maximum production was observed on day seven of the incubation period (Figure 3A). The auxin production was reached to the peak at day seven and afterward the production gradually decreased. Furthermore, effect of the pH on auxin production was determined and the results

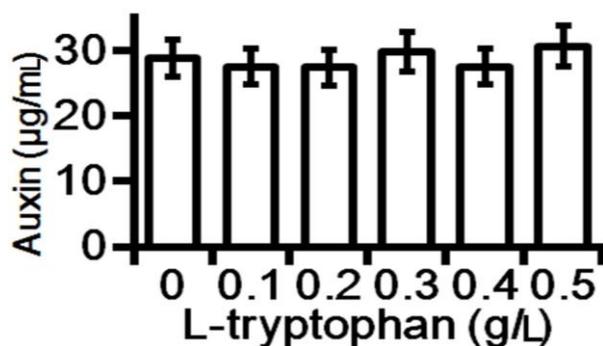


Figure 2 Production of auxin by *Photorhabdus temperata* M1021 in the culture broth. The culture broth was supplemented with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 g L<sup>-1</sup> of L-tryptophan. Auxin contents were determined by Salkowski's assay. The resultant values are averages of three replications; error bars represent standard deviations.

revealed that pH 7 was the optimum for maximum production of auxin in CF *P. temperata* M1021 (Figure 3B).

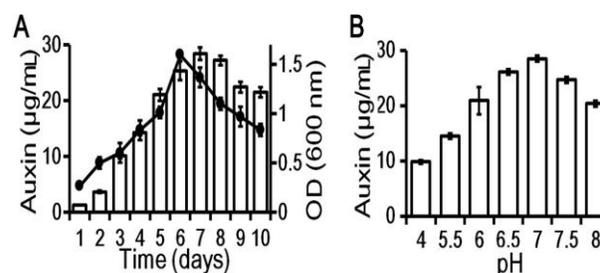


Figure 3 Auxin production profile of *Photorhabdus temperata* M1021 in LB broth. (A) The effect of incubation period on auxin with respect to growth cycle. The exponential phase was lasting for six days, however, maximum auxin production was obtained at day seven, just beginning of the stationary phase. (B) Effect of pH on auxin production of *P. temperata* M1021. The pH 7.0 was the optimum pH for the higher auxin production. The resultant values are averages of three replications; error bars represent standard deviations.

### 3.4 Effects of *P. temperata* M1021 on Plant Growth Attributes

Subsequent to the detection of the auxin in the CF of *P. temperata* M1021, Dongjin" and "Waito-C" rice plants were selected for the bioassay to confirm the presence of auxin. Results summarized in Table 2, indicated a relationship between treatment of CF and plant growth attributes. Plants of the both rice varieties were treated with CF and results revealed that there was a significant increase ( $P < 0.05$ ) in the total plant length of the "Dongjin" and "Waito-C" plants, treated with the CF of *P. temperata* M1021 in a comparison with control. Moreover, the results indicated that fresh and dry bio-masses of the plants of both varieties were also increased significantly ( $P < 0.05$ ) as compared to control. Similarly

Table 2 Effects of *P. temperata* M1021 culture filtrate on growth attributes of Dongjin and Waito-C rice plants

PM	Treatment	PL (cm)	CC (SPAD)	FB (g)	DB (mg)
Dongjin	LB (control)	23.48±2.11 <sup>b</sup>	24.35±4.19 <sup>b</sup>	1.08±0.04 <sup>b</sup>	0.119±0.00 <sup>b</sup>
	CF (treatment)	27.88±2.46 <sup>a</sup>	27.59±2.81 <sup>a</sup>	1.60±0.02 <sup>a</sup>	0.17±0.00 <sup>a</sup>
Waito-C	LB (control)	14.65±2.04 <sup>b</sup>	27.20±1.06 <sup>b</sup>	1.23±0.03 <sup>b</sup>	0.13±0.00 <sup>b</sup>
	CF (treatment)	16.71±2.09 <sup>a</sup>	30.62±1.84 <sup>a</sup>	1.78±0.01 <sup>a</sup>	0.18±0.00 <sup>a</sup>

(SPAD) stands for soil-plant analysis development unit for measuring leaf chlorophyll content; PM - plant material; PL - plant length; CC - chlorophyll content; FB - fresh biomass; DB - dry biomass

Note: Values are expressed as mean ± standard deviation with n = 3. Different alphabetical letters in the same column for each set of treatment indicate significant differences at  $P < 0.05$  levels as estimated by Duncan's multiple range test (DMRT).

the chlorophyll contents of plants were analysed in the response to treatments and observed a significant increase ( $P < 0.05$ ) in the chlorophyll content of the plants treated with the CF of the *P. temperata* M1021 as compare to control treatment.

#### 4 Discussion

Biological control agents prove for insect parasitoids and predators have two dimensional effects on field ecosystems. One effect is pathogenicity against the target pests and the other is on plant's morphology and physiology (San-Blas et al., 2013). Unfortunately, the second dimension has often been ignored during the development and commercialization of bio-control agents (Ullah et al., 2014). In order to explore the dual effects of the *Photorhabdus* spp., in the first round proteinous and non-proteinous toxins were assayed against *G. mellonella* larvae by injecting whole cells, soluble and CF of *P. temperata* M1021. Whereas, CF was exposed to heat and proteinase-K treatments to determine insecticidal activities of non-proteinous metabolites exclusively. Results are endorsed by previous studies suggesting that a part of the high molecular weight proteins complexes, e.g. toxin complexes (Tcs), *Photorhabdus* insect related (Pir) makes caterpillars floppy (Mcf) and *Photorhabdus* virulence cassettes (PVC), the insecticidal potential of *Photorhabdus* are also largely attributed to the secondary metabolites, which are equally effective as protein toxins (Hu & Webster, 2000; Daborn et al., 2002; Eleftherianos et al., 2007). Recently, San-Blas et al. (2013) have successfully applied the metabolites, extracted from the *Xenorhabdus* and *Photorhabdus* spp. against the *Dothiorella*, causing disease in guava plants and suggest that both genera of entomopathogenic bacteria have great potential to be exploited as biocontrol agent in agriculture.

In addition, GC-MS analysis of CF revealed a list of compounds including *trans*-Cinnamic acid, a well-known insecticidal compound (Chalabaev et al., 2008). A number of reports have confirmed a strong larvicidal activity of *trans*-Cinnamic acid against a diverse group of insects including, *Manduca sexta*, *G. mellonella*, *Culex quinquefasciatus* and *Armigeres subalbatus* larvae (Eleftherianos et al., 2007; Chalabaev et al., 2008). In addition, Seo et al. (2012); Salvadori et al. (2012) and Eleftherianos et al. (2007) investigated that the extracellular compounds i.e., 1,3-dihydroxy-2-(isopropyl)-5-(2-phenylethenyl) benzylideneacetone, proline-tyrosine, acetylated phenylalanine-glycine-valine indole, oxindole, cis-cyclo-PY, and *p*-hydroxyphenyl propionic produced by *Photorhabdus* bacteria are involved in the insecticidal activity against different larvae e.g. *Manduca sexta*, *Plutella xylostella*, *Salix exigua* and *G. mellonella*.

Moreover, the GC-MS analysis also detected indole in the CF of *P. temperata* M1021, which was further subjected to Salkowski's

assay to determine the auxin contents. Auxins are common indole compounds and well known plant growth regulators, therefore, some bacteria need L-tryptophan as precursor to produced auxins in the culture environment (Ullah et al., 2013). *P. temperata* M1021 produced equivalent concentration of auxin without and with L-tryptophan in LB broth. These results led to the proposal of a tryptophan-independent pathway for the biosynthesis of auxin (Dobbelaere et al., 2003; Bauer & Mathesius, 2004; Müller et al., 2009).

In addition, the presence of the auxins was confirmed through bioassay of the CF on rice plants and results showed the plant growth attributes i.e., plant length, chlorophyll content, and biomass were significantly increased in a comparison with control. Secretion of bioactive metabolites by microbes in their growing medium can extend greater benefits for plant improvement (Kang et al., 2012). Previous studies of Kang et al. (2009) suggest that application of bacterial CF containing bioactive secondary metabolites to various vegetable and crops are beneficial for the plant growth and development. Agar and water were used in current experiment as the growing media for the rice to devoid the seedling from any nutrients to accurately measure the sole effect of microbial CF (Ullah et al., 2013). The present results are in accord with the previous findings of Kang et al., (2012), suggesting that the application of bacterial culture, enhances the plant growth by direct augmentation of the growth hormones which induce the rapid cell division and cell elongation (Glick, 1995; Hallmann et al., 1999; Ikeda et al., 2002; Compant et al., 2005). Results of the application of CF on plant in the second round to experiment confirmed a dual role of *P. temperata* M1021 in agriculture. However further study is need to elaborate the role of *P. temperata* M1021 on plant health and growth.

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#### Conflict of Interest

There is no conflict of interest among the authors.

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