# جداسازی و شناسایی جدایه‌های *باسیلوس* تهران و ارزیابی فعالیت ضدقارچی آن‌ها علیه جدایه‌های قارچ *فوزاریوم* عامل بیماری بلایت فوزاریومی سنبله گندم اردبیل

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## چکیده

**بیماری بلایت فوزاریومی سنبله گندم از لحاظ اقتصادی یکی از مهمترین بیماری‌های قارچی گندم در سرتاسر جهان می‌باشد. هدف از انجام این تحقیق بررسی و شناسایی متابولیت‌های ضدقارچی جدایه‌های بومی *باسیلوس* بود. جدایه‌های *باسیلوس* از خاک مزارع کشاورزی گندم و جو (واقع شده در جنوب تهران در پاییز ۹۱) جداسازی شد و علیه چندین جدایه *فوزاریوم* بومی جداسازی شده از دانه‌های گندم آلوده فعالیت آنتاگونیستی نشان داد. تخلیص و شناسایی متابولیت‌های ضدقارچی تولید شده توسط جدایه منتخب *باسیلوس* در محیط نوترینت براث انجام شد. روش کروماتوگرافی مایع با کارایی بالا بر روی عصاره تخلیص شده حضور آنتی‌بیوتیک‌های لیپوپپتیدی ایتورین را به عنوان پیک اصلی نشان داد به طوری که با ایتورین آ خالص شرکت سیگما قابل مقایسه بود. ساختار متابولیت‌های ضدقارچی تخلیص شده به وسیله روش‌های طیف‌سنجی مادون قرمز و طیف‌سنجی جرمی بر پایه کروماتوگرافی مایع مورد شناسایی قرار گرفت و ساختار ایتورینی آن تأیید گردید. آنالیز طیف‌سنجی جرمی همچنین توانایی *باسیلوس* منتخب را در تولید فنجی‌سین علاوه بر ایتورین آ اثبات نمود. توالی ژنومی جدایه *باسیلوس* منتخب ۹۹/۹ درصد با توالی ژنومی *باسیلوس آریاب‌هاتایی* و ژنوم جدایه منتخب *فوزاریوم* ۹۹ درصد با *فوزاریوم گرامینئاروم* قرابت ژنومی نشان داد. بر اساس نتایج حاصل از این تحقیق، اثرات ضدقارچی جدایه‌های بومی *باسیلوس* و نیز اثرات آن‌ها در کنترل بیولوژیک بیماری بلایت فوزاریومی سنبله گندم مورد تأیید قرار گرفت.**

**کلیدواژه: *باسیلوس*، گندم، بلایت فوزاریومی سنبله، ایتورین، فنجی‌سین**

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# Isolation and characterization of *Bacillus* isolates from Tehran and investigation of their antifungal activity against some species of Fusarium head blight fungi from Ardebil

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## Abstract

Fusarium head blight (FHB) is economically one of the most important fungal diseases of wheat in the world. The aim of this research was to determine the antifungal metabolites of native *Bacillus* isolates. *Bacillus* strains isolated from soil of wheat and barley fields (Rural areas of southern Tehran, autumn 2012), and exhibited in vitro antagonistic activity against some *Fusarium* species isolated from infected wheat seeds (Wheat fields of Parsabad at Moghan, Ardebil Summer 2012). An attempt was made to partially purify and characterize the diffusible antifungal metabolite/s produced by the selected *Bacillus* strain in Nutrient broth medium. High Performance Liquid Chromatography (HPLC) of partially purified extract of the strain showed the presence of lipopeptide antibiotic iturin as a major peak that was comparable to that of standard iturin A (11.80 min) from Sigma–Aldrich. The structure was further confirmed by Fourier Transform-Infrared Spectrum (FTIR) and Liquid Chromatographic Mass Spectrometric (LCMS) analysis as iturin A. LCMS analysis also showed the presence of fengycin with iturin A. The genome of the selected isolate of *Bacillus* had shown 99/9 percent similarity by *B. aryabhattai* and the genome of the selected isolates of *Fusarium* had shown 99 percent similarity by *F. graminearum*. According to the results of this experiment, the antifungal effects of native *Bacillus* strains and also their effects in the biological control of wheat FHB disease were confirmed.

**Keywords:** *Bacillus,* Wheat, Fusarium head blight, Iturin, Fengycin

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## Introduction

Bread wheat (*Triticum aestivum* L.) is a major agricultural crop and the main cereal consumed by humans in Iran. Northern parts of Iran are one of the main wheat cultivation areas. This region with hot-temperate and wet climates has conducive conditions for *Fusarium* growth at the time of kernel formation. Fusarium head blight (FHB) or scab is one of the most economically important and destructive fungal diseases of wheat (Abedi-Tizaki and Sabbagh 2012). Apart from reducing the yield, FHB damages grain quality by contamination from toxic secondary metabolites (Mycotoxins), which cause a health risk to both humans and animals. The *F. graminearum* species complex, which consists of at least 11 phylogenetically distinct species, is the predominant species causing FHB worldwide (O'donnell *et al.* 2000; Ban *et al.* 2008). In northern parts of Iran, *F. graminearum* and *F.culmorum* have shown pathogenicity to wheat (Zamani-zadeh and Khoursandi 1995). Plant diseases such as FHB are the major cause of yield loss. Bio-pesticide has become a tendency for global pesticide development because of its relatively low side-effects and friendliness to environment (Yu 2000). The control of FHB has relied on using fungicides and resistant varieties. Biological control using bacterial antagonism has been explored as an additional or alternative means for managing the disease. A range of bacterial isolates obtained from rhizosphere and kernel of wheat was reported in a previous study (Stockwell *et al.* 2002). *Bacillus* species, as a group offer several advantages over other gram-positive bacteria, including longer shelf life because of their ability to form endospores and the broad-spectrum activity of their antibiotics (Bais *et al.* 2004, Kim *et al.* 1997). These species produce a variety of secondary metabolites with antifungal and pharmacological activities. Most of these metabolites are small peptides that have unusual components and chemical bonds with a high potentiality leading to a variety of biotechnological and pharmaceutical application. Prominent classes of such antifungal compounds are the lipopeptides fengycin and the other members of the iturin family (iturin, mycosubtilin, bacillomycin) (Kim *et al.* 2010). The iturin compounds are cyclic lipoheptapeptides that contain a β-amino fatty acid as lipophilic component. Fengycin has a β-hydroxy fatty acid in its side chain. The lipopeptides belonging to the iturin family are potent antifungal agents which can be used as biopesticides for plant protection (Arrebola *et al.* 2010). In the present investigation an attempt was made for isolation of native *Bacillus* strains from soil samples and native *Fusarium* strains from infected wheat seeds, detection of antifungal activity of *Bacillus* isolates against *Fusarium* species and partial purification and characterization of antifungal metabolite/s produced by the selected *Bacillus* isolate. Optimization of culture conditions for the selected *Bacillus* antifungal activity was the other objective of this research.

## Materials and methods

### *Isolation and identification of Bacillus species*

A total of 15 soil samples were collected from fields of wheat, barley and corn, in the depth of 10-15 cm, in the rural areas located in south of Tehran, Iran. The procedure adopted was as follows: 10 gram of each soil sample was diluted in 90 ml of sterile distilled water in 250 ml conical flask and kept in a orbital shaker at 150 rpm to get a homogenized soil suspension. Serial dilutions from $10^{-1}$ to $10^{-8}$ were made and 1 ml of each solution was added into sterile plate and then melted Plate Count Agar (PCA) (contains the following per liter: peptone, 5g; yeast extract, 2.5g; dextrose, 1g; Agar 15g. pH 7±0.2) added and mixed by sample and incubated at 37°C for 24 h (Amara and Salem 2009; www.hach.com 2012). *Bacillus*-like colonies were sub-cultured on new Nutrient Agar (NA) plates (contains the following per liter: peptic digest of animal tissue, 5g; sodium chloride, 5g; beef extract, 1.5g; yeast extract, 1.5g; Agar 15g. pH 7.4±0.2) until pure cultures were obtained and they were kept at 4°C in slant NA medium for further identification. Biochemical properties of isolates such as gelatinase, amylase, oxidase, urease, indol production, voges-proskauer test and nitrate reduction test were determined. All tests were repeated two times. Gram and malachite green staining methods were also used to determine morphological properties and slides were examined by light microscopy (Amara and Salem 2009, Horikoshi 1991).

**Table 1: Nucleotide sequences of primers**

| Primer name | Primer sequence | Tm* |
|---|---|---|
| 1492R | 5'-GGTTACCTTGTTACGACTT-3' | 54 |
| 27F | 5'-AGAGTTTGATCMTGGGTCAG-3' | 56.3 |

*Temperature of Melting

*16S rRNA gene sequencing*

For sequencing analysis, the genomic DNA was extracted from the isolate, using Roche kit. The amplification of the16S rRNA was performed through PCR (BIORAD version 1/065, USA), using Taq DNA polymerase, genomic DNA as a template, and 3' forward and 5' reverse universal primers (27F and 1492R). PCR steps were as follows:

1-Primary denaturation: in 95°C for 4 min; 2-Proliferation cycles: 30 cycles in denaturation temperature of 95°C for 1 min, annealing temperature of 54-60°C for 1 min and extention temperature of 72°C for 1 min; 3-Final hold: in 72°C for 10 min.

Table 1 shows nucleotide sequences of primers (Altschul *et al.* 1997, Kim *et al.* 2012, Myers and Miller 1988).

PCR products were sent to SQ lab Co. (Germany). By receiving the results, the 16S rRNA nucleotide sequence of isolate has been deposited in GenBank and aligned with the 16S rRNA sequences available in nucleotide database in NCBI, (National Center for Biotechnology Information, Available at: http://www.ncbi.nlm.nih.gov/), using BLAST software, (Basic Local Alignment Search Tool) (Lyon *et al.* 2000).

*Isolation and identification of Fusarium species*

A total of 12 samples were collected from infected wheat fields of Parsabad at Moghan located in Ardebil province in north west of Iran. Some of the samples had white heads with pink spots on spikes, and small and shrinked seeds. The samples were submerged in 0.5% sodium hypochlorite for 3 to 5 min (Nourozian *et al.* 2006). Then the seeds were washed by sterile distilled water, placed on Petri dishes containing potato dextrose agar (PDA) (contains the following per liter: potato infusion from 200g, 4g; dextrose, 20g; agar, 15g; Beef extract, 3g; $K_2PO_4$, 2. pH 5.6±0.2) and incubated at 24°C for one week. All *Fusarium* isolates were sub-cultured and then purified on PDA using a single-spore technique. Culture characteristics were assessed by eye and microscopic examination. The morphology of macroconidia, microconidia, conidiogenous cells and chlamydospores was assessed from cultures grown on PDA. Morphological identifications of isolates were carried out using the criteria of Leslie *et al.* 2006.

Molecular identification of the species was carried out using a species-specific PCR assay. Species are also determined based on versatile differences in a single characteristic. Therefore, for complete identification of the selected *Fusarium* spp., PCR assays with specific primers was performed. The following set of primers was used: F: 5' CTCCGGATATGTTGCGTCAA 3' and R: 5' GGTAGGTATCCGACATGGCAA 3' (Altschul *et al.* 1997, Kim *et al.* 2012, Myers and Miller 1988). For DNA extraction, *Fusarium* isolate was grown on PDA plates for 7 days and mycelia were harvested and ground in liquid nitrogen. Total DNA was extracted from ground mycelium of isolate (~100 mg wet weight) using a DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The reaction mixtures were prepared in a total volume of 25 µl with a final concentration of 50 mMKCl, 10 mMTris-HCl (pH 8.3), 0.2 mM of each dNTP and 1.5 mM $MgCl_2$. For each reaction, 1.5U of Taq polymerase (Fermentase, Sinagen, Iran), 15 pmol of each primer and approximately 25 ng of fungal template DNA were used. Reactions were performed in a thermal cycler (Eppendrof, Germany) using the following PCR conditions: denaturation at 95ºC for 5 min, 35 cycles of denaturation at 94ºC for 50 sec, annealing at 56ºC for 50 sec, extension at 72°C for 1 min, final extension at 72°C for 7 min, followed by cooling at 4°C until recovery of the samples. Amplification products were visualized in 1.2% agarose gels stained with ethidium bromide (Mule *et al.* 2004) and photographed under UV light in the Bio-Imaging system.

*In vitro antifungal activity*

The purified *Bacillus* isolates were pre-evaluated against the isolates of *Fusarium* by using dual culture in petri dishes containing PDA. Agar-well diffusion assay was used for the detection of antifungal activity. PDA plates containing $10^4$ *Fusarium* species spores per mL were prepared. A well with a diameter of 6 mm was then cut in the

agar using a sterile cork-borer. A droplet of agar was added to the well in order to seal it to avoid leakage. Then, 100 lL of Bacterial suspension grown in NB with a concentration of $10^8$cfu/ml was added into the well and allowed to diffuse into the agar during a 5 h pre-incubation period at room temperature, followed by aerobic incubation at 30°C for 24 h. The antifungal zone was recorded in each case (Zhang *et al.* 2008).

*Optimizations of the selected Bacillus isolate antifungal activity*

This part of study was performed to increase the amount of the selected *Bacillus* species antifungal activity. Role of different factors {temperature (25°C to 40°C) and time of incubation (24, 48, 72, 96 and 120 hours), Carbon and Nitrogen source (glucose, lactose, starch, yeast extract, beef extract peptone), pH (6 to 10 using 1% $Na_2CO_3$) and agitation rate (50,100, 150 and 200 rpm)} were investigated separately using agar well diffusion method as described before.

*Extraction and partial purification of antifungal metabolite/s*

For production of antifungal metabolites the organism was grown aerobically on optimized Nutrient Broth (NB) at pH 7.0 in 750 ml Erlenmeyer flasks containing 250 ml of medium, maintained at 30°C±1°C for 96 h with 150 rpm agitating rate in a shaker incubator. Then vials were centrifuged at (5000*g* for 20 min). Each supernatant was acidified to pH~2.0, adding concentrated HCl (12 N) and the precipitate formed was separated by centrifugation (20,000*g* for 15 min) using a refrigerated centrifuge (Sigma 3-CK, Germany). The supernatant was then discarded, the precipitate containing the antibiotics was solubilized in methanol and the alcoholic solution was centrifuged again (20,000*g* for 10 min). The supernatant was subsequently collected as methanol extract. The active fraction was dissolved in methanol and used for HPLC studies (Feng-Chia *et al.* 2008, and Mizumoto *et al.* 2007).

*High Performance Liquid Chromatographic (HPLC) Analysis*

A reverse phase HPLC technique was used for quantitative analysis. Partially purified extract was analyzed by HPLC. HPLC instrument (KNAUER, Germany) equipped with degasser, quaternary pump, photo diode-array detector connected with rheodyne injection system and a computer was used for analysis. The stationary phase consisted of C-18 packed stainless steel column (250 mm×4 mm i.d). Acetonitrile:water (70:30) at 1 ml/min flow rate was used as mobile phase. HPLC analysis was performed at wavelength of 240 nm, which was detected for absorption maxima using photodiode array. Iturin A standard was procured from Sigma–Aldrich. All the chemicals and reagents were analytical grade. Twenty microliters of sample and standard iturin were injected into HPLC under standardized conditions. Each run was repeated twice and the detector response was measured in terms of peak areas.

*Analytical methods*

*Fourier Transform-Infrared spectrum (FTIR) and Liquid Chromatographic Mass Spectrometric (LCMS)*

An infrared spectrum of the purified antibiotic was obtained with a Thermonicolet FTIR-870 nexus (Shimadzu, Japan) with a DLATGS detector. The antifungal metabolites were detected by ultraviolet (UV) light (254 nm). The Rf value of antibiotic under these conditions was 0.29. LCMS (Finnigan LCQ DECA, the Xcalibur 2.0 SR2 software) of the partially purified fraction was done on Water Alliance HPLC system with auto-sampler coupled with a mass detector with positive and negative mode. The mass spectrometer was operated in positive ionization mode with selected ion recorder (SIR) acquisition. Mobile phase was acetonitrile and 10 mM ammonium acetate (60:40) at a flow rate of 0.3 ml/min. Major peaks were produced by SIR of 10 channels in the TIC.

*Statistical analysis*

Obtained data were subjected to analysis of variance (ANOVA) using SPSS 0.8 software for windows. The means were compared using the least significant difference test at P=0.01. Tests were repeated two times.Values in the step of antifungal activity investigation and optimization are the mean of triplicate.

**Table 2: In vitro antagonism of *Fusarium* no. 2 by 10 of the selected *Bacillus* isolates**

| Number of Bacillus isolate | Inhibition zone (mm)[a] ± SD |
|---|---|
| 1 | 9 ± 0.82 |
| 6 | 7.50 ± 0.72 |
| 9 | 8.25 ± 0.90 |
| 11 | 8.80 ± 0.78 |
| 16 | 7.85 ± 0.83 |
| 17 | 8.25 ± 0.75 |
| 23 | 7.50 ± 0.71 |
| 24 | 8.50 ± 0.83 |
| 30 | 7.25 ± 0.71 |
| 31 | 8.30 ± 0.84 |

[a] Values are the mean of triplicate

## Results

11 of the 34 *Bacillus* isolates which were isolated from the soil rhizospheres inhibited the in vitro hyphal growth of 5 *Fusarium* isolates due to the production of diffusible antifungal metabolites. Based on the size of inhibition zones, significant differences were observed at P=0.01 among *Bacillus* no. 11 and the other *Bacillus* strains against *Fusarium* no. 2 and these two isolates were selected for further bioassay studies (Table 2). According to the size of inhibition zones in the optimization step, the *Bacillus* no. 11 could inhibit the fungal growth of *Fusarium* no. 2 in different conditions of **carbon** and **nitrogen** sources but the best sources were **glucose** and **yeast extract**, respectively. The results indicated that the neutral pH and 150 rpm of shaker incubator were the best choices for the antifungal activity of the selected *Bacillus* isolate. The bacterium could inhibit the fungal growth in the different conditions of incubator temperature but 30°C had the highest number in this manner. So the best culture conditions for the antifungal activity of *Bacillus* no. 11 were assigned as: carbon source: Glucose, Nitrogen source: Yeast extract, pH: 7, Round per minute of shaker incubator: 150 rpm and temperature: 30°C. After incubation for 48 h, the size of inhibition zone was 4.5 mm which increased by more than four folds by 96 h (19 mm). Further incubation up to 144 h did not show any significant increase in the inhibition zone size, indicating that 96h incubation is sufficient for maximum production of the antifungal metabolite/s. Production of extracellular antifungal metabolite/s by the selected *Bacillus* strain was studied under shaking conditions in optimized NB at 30°C (data not shown). The methanol extract of the culture broth of the selected *Bacillus* was analyzed by HPLC. Methanolic extract of the selected *Bacillus* showed two extra peaks at retention time 8.92 and 11.80 min. When compared with iturin A standard, the peak at 11.80 min having the same elution profile as commercial iturin A, and was regarded as a positive result for iturin A production. HPLC analysis confirmed the production of iturin A by the selected *Bacillus* isolate (Fig. 1). Although HPLC comparison by standard iturin A indicated the presence of iturin A in the extract but the authenticity of the produced iturin A was further established by FTIR and LCMS analysis. For all fractions, the FT-IR analysis showed bands in the range of 1,630 to 1,680 $cm^{-1}$, resulting from the stretching mode of the CO-N bond (amide I band) indicating the presence of a peptide component; and also bands at 2,855 to 2,960 $cm^{-1}$, resulting from typical CH stretching vibration in the alkyl chain. FTIR analysis confirmed the ability of the selected *Bacillus* isolate (*Bacillus* no. 2) for the production of Iturin. The partially purified extract of the culture broth of the selected *Bacillus* isolate was also analyzed by LCMS. Mass spectrum profile of peak at retention time of 8.92 showed one well-resolved group of peaks at m/z (mass to charge ratio) values between 1.483 and 1.549. The group of peaks could be attributed to the isoform ensembles of fengycin which represent an important biosurfactant familly of *Bacillus* strains. Mass spectrum profile of peak at retention time of 11.8 showed one weak-resolved peak at m/z values between 1.082 and 1.110 which could be attributed to the isoform ensembles of iturin A which represent the well-known biosurfactant familly by *Bacillus* strains (Fig. 2). Mass numbers of the iturin A and fengycin peaks obtained by LCMS of partially purified extracts and tentatively identified on the basis of literature information are given in Table 3. The genome of the most selected isolates of *Bacillus* had shown 99/9 percent similarity by *B. aryabhattai* (data not shown). The isolate gave positive results for the Gram Stain, Oxidase, Urease, Gelatinase, Nitrate Reduction, Voges-Proskauer, Starch Hydrolysis assays and negative results for the Indole Production Assay. It showed cold tolerance to as low as 4°C but was intolerant to temperatures higher than 30°C (Table 4). The

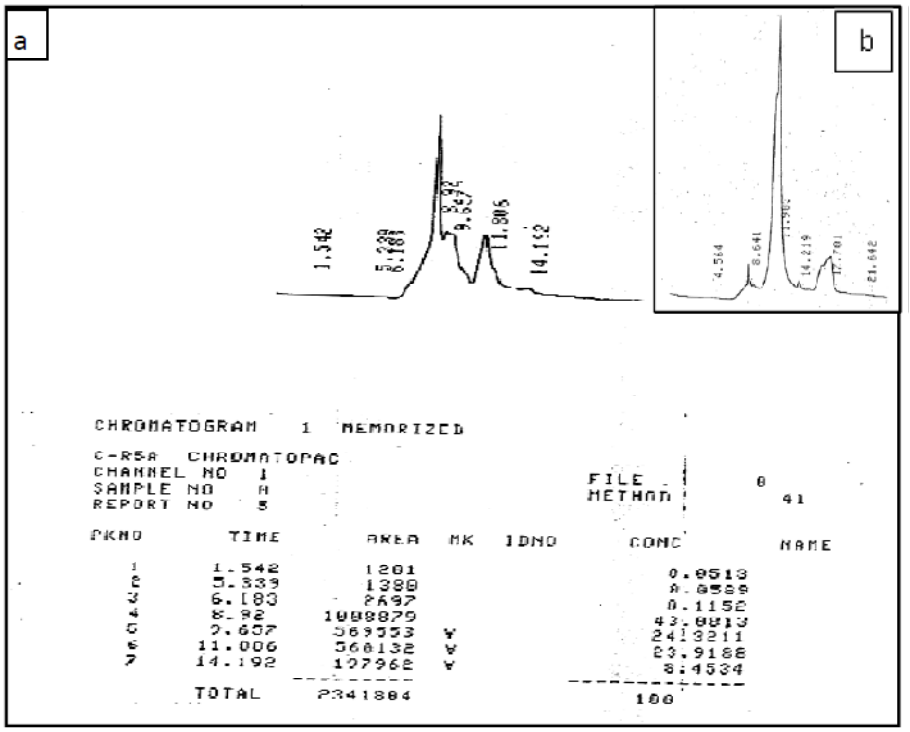


**Fig1: HPLC analysis of partially purified extract of the selected *Bacillus* isolate (a) and standard iturin A (b).**

**Table 3: Assignment of all mass peaks produced by *Bacillus subtilis* by LCMS**

| Mass peaks (m/z) | Probable assignment |
|---|---|
| 1073.25 | $C_{16}$iturin $(M+H)^+$ |
| 1082.35 | $C_{17}$iturin $(M+H)^+$ |
| 1095.52 | $C_{18}$iturin $(M+H)^+$ |
| 1110.79 | $C_{19}$iturin $(M+H)^+$ |
| 1467.03 | $C_{15}$fengycin $(M+Na)^+$ |
| 1483.35 | $C_{16}$fengycin $(M+Na)^+$ |
| 1497.8 | $C_{17}$fengycin $(M+Na)^+$ |
| 1513.9 | $C_{16}$fengycin $(M+Na)^+$ |
| 1529.42 | $C_{16}$fengycin $(M+K)^+$ |

The mass data represent the monoisotopic mass numbers

**Table 4: Biochemical Test of the selected *Bacillus* isolate**

| Biochemical test | Result |
|---|---|
| Gram Stain | Positive |
| Oxidase Test | Positive |
| Urease Test | Positive |
| Gelatinase Test | Positive |
| Indole Production | Positive |
| Voges-Proskauer Test | Positive |
| Nitrate Reduction Test | Positive |
| Starch Hydrolysis | Positive |

genome of the most selected isolates of *Fusarium* had shown 99 percent similarity by *F. graminearum* (data not shown). According to the results of this experiment, the antifungal effects of *Bacillus* isolates and also their effects in the biological control of FHB disease were confirmed.

## Discussion

*Bacillus* strains exhibit broad spectrum of action against different plant pathogens due to their ability to produce a great abundance of antibiotics with an amazing varieties of structures (Han *et al.* 2005). These compounds include predominantly peptides that are resistant to hydrolysis by proteinases and proteases. Their activity is also resistant to high temperature and a wide range of pH (Gong *et al.* 2006). In our study, the selected *Bacillus* strain, which were isolated from soil samples, exhibited in vitro antagonism against the native *Fusarium* strains isolated from the infected wheat seeds due to the production of diffusible antifungal metabolites. Glucose as carbon source, yeast extract as nitrogen source, neutral pH, 150 rpm of shaker incubator, 30°C temperature and 96 h incubation time were found to be optimum

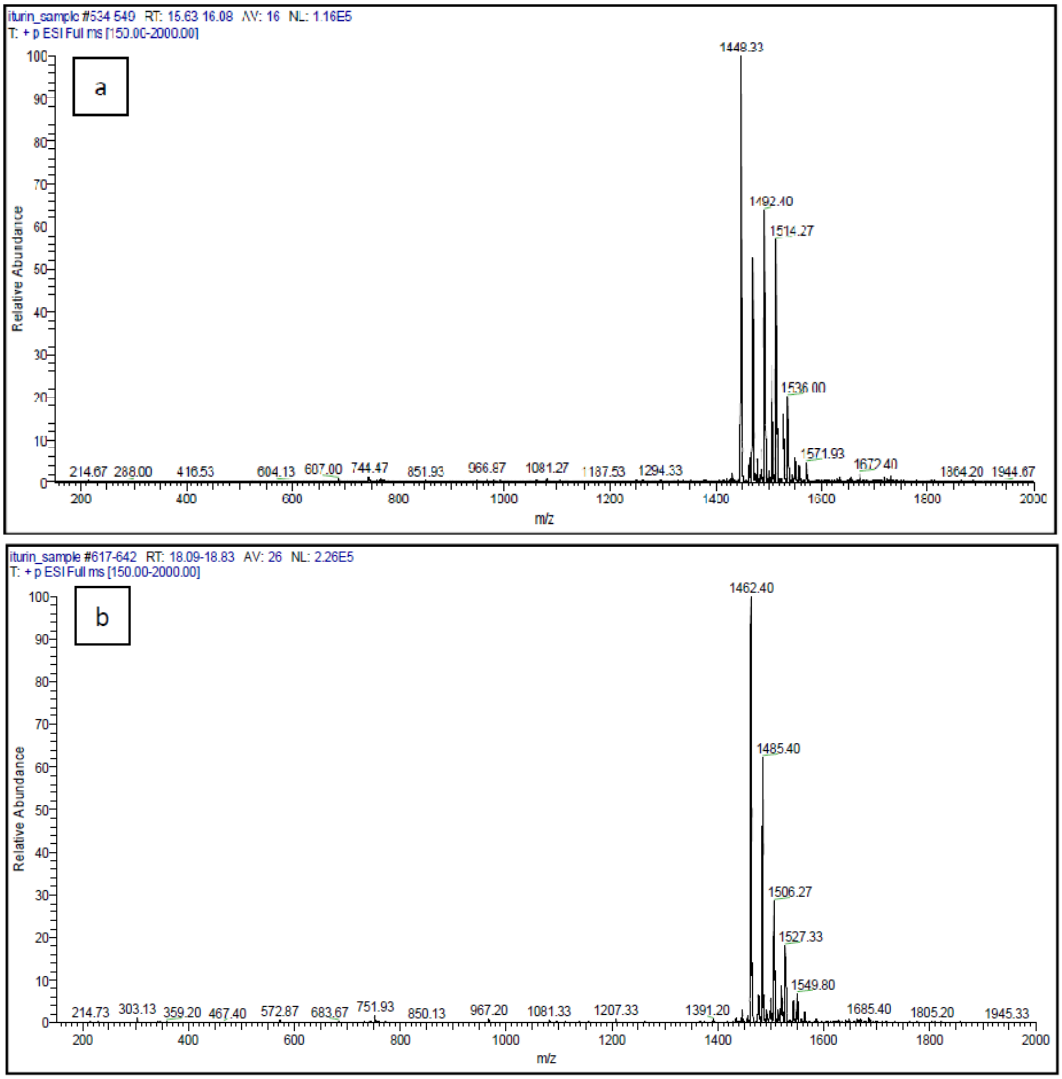


**Fig 2. MS profile of peaks at retention time of 11.80 (shows one weak-resolved peak at m/z values between 1.082 and 1.110) (a) and 8.92 (shows one well-resolved group of peaks at m/z values between 1.483 and 1.549) (b) which are attributed to iturin and fengycin, respectively.**

conditions for the maximum production of antifungal metabolites by the selected *Bacillus* strain in NB. The antifungal metabolite/s was thermostable, pH stable, soluble in methanol, ethanol, and acetic acid but insoluble in water indicating toward lipopeptide nature of the metabolite/s. The chromatographic analysis using HPLC, FTIR and LCMS showed the occurrence of two different lipopeptide antibiotics, iturin A (as major fraction) and fengycin (as minor fraction) in the partially purified extract of the selected *Bacillus* strain. Thus the production of two different lipopeptide antibiotics could be related with the biocontrol efficiency of the selected strain. The simultaneous excretion of different lipopeptides in *Bacillus* spp. is often observed. *B. subtilis* GA1 is a producer of a wide variety of lipopeptides, iturin A, surfactin, and fengycin with various lengths of the fatty acid chains from C14 to C18 (Toure *et al.* 2004). Coproduction of iturin A, fengycin, and surfactin by *B.subtilis* strains UMAF6614 and UMAF6639 was found responsible for the biocontrol of cucurbit powdery mildew *Podosphaerafusca* (Romero *et al.* 2007). Mixture of surfactin and iturin produced by *B. subtilis* RB14 and *B. amyloliquefaciens* BNM 122 increased the antifungal activity since the former compound is able to form mixed micelles with iturin and thereby improves its activity (Thimon *et al.* 1992). Furthermore, lipopeptides of iturin group seem to help the organisms in biofilm formation thus contributing to the protective activity by

preventing the growth of other microorganisms as shown in Arabidopsis against *Pseudomonas syringae* (Bais *et al.* 2004). Increasing the diversity of antibiotics excreted by the organism to the soil might result in an increase of the range of action on different phytopathogens. The target site for lipopeptide antibiotics is the fungal cytoplasmic membrane. Iturin antibiotics increase the membrane permeability of the target microorganism due to the formation of ion channels on the cell membranes thereby increasing the permeability to $K^+$ that is associated with fungicidal activity. Modification of membrane permeability and lipid composition of *Saccharomyces cervisiae* cells by iturin A has been reported (Besson *et al.* 1984, Yu *et al.* 2002). In the present study, an attempt was also made to determine the identity of the selected isolate of *Bacillus* and *Fusarium*. Molecular identification of the species was carried out using a species-species PCR assay. These observations need some practice and are difficult for a non-specialist (Bluhm *et al.* 2002). The genome of the selected isolate of *Bacillus* had shown 99/9 % similarity with *B. aryabhattai*. This was followed by phylogenetic analysis based on partial 16S rRNA gene sequences, to establish the bacterial isolate as *Bacillus aryabhattai*. This species of *Bacillus* was found in the Indian Sub-continent by Ray *et al.* 2012. The isolate gave positive results for the Gram staining process, Oxidase, Urease, Gelatinase, Nitrate Reduction, Voges-Proskauer and Starch Hydrolysis assays and negative results for the Indole Production Assay. It showed cold tolerance to as low as 4°C but was intolerant to temperatures higher than 37°C. This is the first proof of this particular extra-terrestrial microorganism to have antifungal activity. The genome of the selected isolate of *Fusarium* had shown 99% similarity with *F. graminearum*. *F. graminearum* cause root rot, food rot, crown rot, stem rot and head blight in wheat. Head blight causes reduced kernel set and kernel weight, destruction of starch granules and storage proteins and seed infection (Nourozian *et al.* 2006). Iturin and fengycin are lipopeptide antibiotics with a broad antifungal spectrum. They have wide application in industries and medicine (Tendulkar *et al.* 2007, Souto *et al.* 2004). In our present observation, an efficient iturin A producing the selected *Bacillus* strain, which had shown 99/9% similarity by *B. aryabhattai*, along with fengycin provide a broad antifungal spectrum which can be further exploited as a biocontrol agent and for the commercial production of antifungal compounds.

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