JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2015. Vol. **9**(1), p. 269-279

An Investigation of Antifungal Activity of Native *Bacillus* Strains against Fusarium Head Blight on Wheat

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(Received: 20 October 2014; accepted: 12 December 2014)

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 effects and metabolites of native *Bacillus* species. *Bacillus* strains isolated from soil of fields grown wheat and barley, exhibited *in vitro* and *in vivo* antagonism against some *Fusarium* species isolated from infected wheat seeds. An attempt was made to partially purify and characterize the diffusible antifungal metabolite/s produced by the selected *Bacillus* strain. High Performance Liquid Chromatography (HPLC) of partially purified extract 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 asiturin A. LCMS analysis also showed the presence of fengycin beside siturin 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*. The antifungal effects of *Bacillus* strains in the glasshouse conditions was also confirmed. Disease severity in plants inoculated with the pathogen and *Bacillus* strains was significantly less than the pathogen control. 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.

Key words: *Bacillus*, Fourier transform-infrared spectrum, *Fusarium*, Fusarium head blight, High performance liquid chromatography, Iturin, Liquid chromatographic mass spectrometric, wheat.

Bread wheat (*Triticum aestivum* L.) is a major agricultural crop and the main cereal consumed by humans in Iran. Northern parts of Iran are the main wheat cultivation areas. This region with hot-temperate and wet climates has favorable 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

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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). Many agricultural losses are due to plant diseases, such as FHB. Bio-pesticide has become a tendency for and goal of global pesticide development because of its relatively low side-effects and friendliness to environment. The control of FHB has relied on the resistant varieties and use of fungicides. Resistant cultivars are very rare and application of fungicides may be used for control of FHB. Biological control by using bacterial antagonism has been explored as an additional or alternative means of 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-negative bacteria, including longer shelf life because of their ability to form endospores and the broad-spectrum activity of their antibiotics (Kim *et al*. 1997; Bais *et al*. 2004). *Bacillus* species produce a variety of secondary metabolites with anti-metabolic 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 b-amino fatty acid as lipophilic component. Fengycin has a b-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 in vitro and in vivo 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, the depth of 10-15cm, in the rural areas of southern 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 it 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 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 sampleand incubated at 37°C for 24 h. 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 for further identification. Biochemical properties of isolates such as catalase, gelatinase, amylase, etc., were determined. 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 (Horikoshi1 991).

16S rRNA gene sequencing

For sequencing analysis, the genomic DNA was extracted from the isolate, using Roche kit. The amplification of the 16SrRNA was performed through PCR technique, using Taq DNA polymerase, genomic DNA as a template, and 3· forward and 5· reverse universal primers. Table 1 shows nucleotide sequences of primers.

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 12samples of wheat were collected from several infected fields of Parsabad

Moghan of Ardebil in north-western Iran. Some of these samples had symptoms of pink spots on spikes, small and shrinked seeds, and white heads. The samples were submerged in 0.5% sodium hypochlorite for 3 to 5 min. After this treatment, they were extensively 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, 2g. pH 5.6±0.2) and incubated at 24°C for one week. All *Fusarium* isolates were sub-cultured 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.

PCR assay

Currently, the differentiation of *Fusarium* spp. is based on physiological and morphological characteristics such as the shape and size of the macroconidia, the presence or absence of microconidia and chlamydospores, and colony morphology (Llorens *et al*. 2006). Species are also determined based on versatile differences in a single characteristic. However, these observations need some practice and are difficult for a non-specialist (Bluhm *et al*. 2002). Therefore, for complete identification of the selected *Fusarium* spp., additional molecular analysis such as species-specific PCR assays must be performed. Species- specific PCR assay with specific primers was used to identify the selected *Fusarium* species. The following set of primers was used: F: 5' CTCCGGATATGTTGCGTCAA 3' and R: 5' GGTAGGTATCCGACATGGCAA 3'. 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 ¼l 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 10^8cfu/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

In order to investigate the optimized conditions for antifungal effect of the selected *Bacillus* isolate, the role of different environmental factors, Carbon source, Nitrogen source, pH, agitation rate, temperature and time of incubation, were detected separately by using agar well diffusion method as was described in the previous step. Various carbon sources such as glucose, lactose and starch were used. Sources of nitrogen included yeast extract, beef extract, and peptone. The effect of pH on antifungal metabolites production was determined by growing the isolate in production media with an initial pH range of 6 to 10 using 1% Na_2CO_3.The effect of agitation rate was investigated by incubating culture flasks at different agitation speed of 50,100, 150 and 200 rpm. The effect of temperature was determined within a temperature range from 25°C to 40°C and the role of incubation time was identified after 24, 48, 72, 96 and 120 hours of incubation.

Extraction and partial purification of antifungal metabolite/s

For production of antifungal metabolites the organism was grown aerobically on optimized NB maintained at pH 7.0. The culture was grown at 30°C±1°Cfor 96h in 750 ml Erlenmeyer flasks containing 250 ml of medium with shaking at 150 rpm in a shaker incubator.After this period, for the extraction of the metabolites, after centrifugation (5000×g/20 min), each supernatant was acidified to pH~2.0, adding concentrated HCl and the precipitate formed was separated by centrifugation (20,000×g/15 min) using a refrigerated centrifuge. The supernatant was then discarded, the precipitate containing the antibiotics was solubilized in methanol and the alcoholic solution was centrifuged again (20,000×g/10 min). The supernatant was subsequently collected as methanol extract.The active fraction was dissolved in methanol and used for HPLC studies.

High Performance Liquid Chromatographic (HPLC)Analysis

A reverse phaseHPLC technique was used for quantitative analysis. Partially purified extract was analyzed by HPLC. HPLC instrument 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 mmÍ4 mm i.d). Acetonitrile:water (70:30) at 1 ml/minflow 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 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.

Biological control of *Fusarium* on wheat in glasshouse condition

Three *Bacillus* strains 6, 11 and 24 with great inhibition zone *in vitro* against *Fusarium* strain 2 were investigated for their ability to reduce the incidence of head blight in wheat. Ten seeds of susceptible wheat were sown in autoclaved potting mix consisting of two parts compost and one part of field soil in 20 cm diameter plastic pots. Before sowing, seeds were surface-disinfected by soaking in 1% sodium hypochorite for 1 min then rinsed three times in sterile distilled water (SDW). There were 4 replicate pots per treatment, arranged in complete randomized design. Treatments were: *Bacillus* strain 6 + *Fusarium* strain 2, *Bacillus* strain 11 + *Fusarium* strain 2, *Bacillus* strain 24 + *Fusarium* strain 2, *Bacillus* strain 6, *Bacillus* strain 11, *Bacillus* strain 24, Healthy control and *Fusarium* strain 2 only. Plants were maintained at constant temperature of 15ºC without supplementary lighting in August in Tehran, Iran, and then transferred to a glasshouse at constant temperature of 20ºC with a 14 h photoperiod of light. Bacterial antagonist was grown on PDA for 48 h then bacterial cells were washed with SDW. The concentration of bacterial suspension was adjusted to 10^9 using a spectrophotometer. Isolate of *Fusarium* was grown on PDA (90 mm diam.) for 14 d. Hyphae and conidia were harvested by pouring a few mL of sterile water (0.05% Tween 20) on the plates. The concentration of spores in the inoculum was approximately 2×10^5 spores/mL of *Fusarium* isolate, but hyphal fragment concentration was not determined. Inoculation with bacterial antagonist began when the main spikes emerged from the boot. Inoculation with *Fusarium* began at six h after the bacterial inoculation and continued every other evening for 10 d. Immediately after each inoculation, the plants were misted with overhead mister. The inoculum of

bacteria or *Fusarium* was applied with a sprayer at about 7 mL per spike. Head blight was evaluated by the severity, the number of necrotic spikelets in each spike divided into the number of spikelets in each spike 21 d after inoculation. The weight of 100 grain per replication was determined at harvesting time. The experiment was arranged as randomized complete design with 4 replications. Analysis of variance and Duncan's Multiple Range Test was used to determine differences among treatments (Little and Hills 1978).

Statistical analysis

Obtained data were subjected to analysis of variance. The means were checked by using Duncan's Multiple Range Test and ANOVA.

RESULTS

11 of the 34 *Bacillus* isolates which were isolated from the soil rhizospheres and were named as *Bacillus* strains number 1 to 11, inhibited the in vitro hyphal growth of 5 *Fusarium* isolates which were isolated from infected wheat seeds and were named as *Fusarium* strains number 1 to5, due to the production of diffusible antifungal metabolites. Based on the size of inhibition zones, *Bacillus* strains 6, 11 and 24 had shown the best antifungal activity among *Bacillus* strains against *Fusarium* strain 2. These three strains of *Bacillus* were selected for the *in vivo* assay but *Bacillus* strain 11 which had the greatest antifungal activity was selected for the optimization and antifungal metabolites purification and the other steps of *in vitro* assay (Table2). The other strains of *Bacillus* had a lesser ability to inhibit *Fusarium* species and had not been selected for further assays (data not shown). According to the size of inhibition zones in the optimization step, the *Bacillus* number 11 could inhibit the fungal growth of *Fusarium* number 2 in different conditions of carbon and nitrogen sources but the best items were glucose and yeast extract, respectively (Figure 1). The acidity of culture medium and the round per minute of shaker incubator were the other factors which were tested in this experiment. 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 efficiency. So the best culture conditions for the antifungal activity of *Bacillus* number11 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, 96, and 144 h, the antifungal activity of *Bacillus* was measured by agar well diffusion method. After incubation for 48 h, the size of inhibition zone was 12mm which increased by 96 h(19 mm) to 17 mm. Further incubation up to 144 h did not showany significant increase in the inhibition zone size, indicating that 96-h incubation is sufficient formaximum production of the antifungal metabolite/s (Figure 2). Production of extracellular antifungal metabolite/s bythe 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 as described in the "Materials and Methods" section. Methanolic extract of the selected*Bacillus* showed two extra peaks at retention time 8.92 and 11.80 min. When compared with iturinAstandard,

Table 1. Nucleotide sequences of primers for 16S rRNA gene sequencing of the selected *Bacillus* isolate.

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

*Temperature of Melting

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

Number of *Bacillus* isolate	Inhibition zone (mm)[a]
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

the peak at 11.80 min having the same elutionprofile as commercial iturin A, and was regarded as apositive result for iturin A production. HPLC analysisconfirmed the production of iturin A by the selected *Bacillus* isolate (Figure3).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 for the production of Iturin(Figure4). 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 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 family of *Bacillus* strains (Figure 5). Mass spectrum profile of methanolic extraction showed one weak-resolved peak at m/z values between 1.081 and 1.110 which could be attributed to the isoform ensembles of iturinA which represent the well-known biosurfactant family by *Bacillus* strains. 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 givenin Table3. The genome of the most selected isolates of *Bacillus* had shown 99.9

Fig. 1. Agar-well diffusion method: antifungal activity of the selected *Bacillus* isolate (which was determined as a sub-species of *B. aryabhattai*) against *Fusarium* number 2 (which was determined as a sub-species of *F. graminearum*)

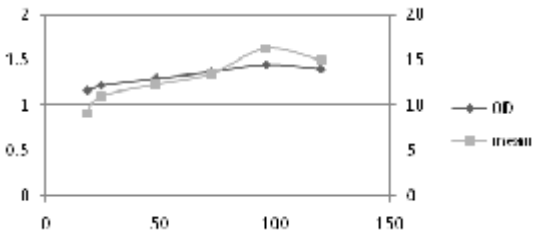


Fig. 2. Comparison of the selected *Bacillus* isolate suspention OD (Optical Density) and mean of *Fusarium* number 2 inhibition haloes in the different temperatures

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

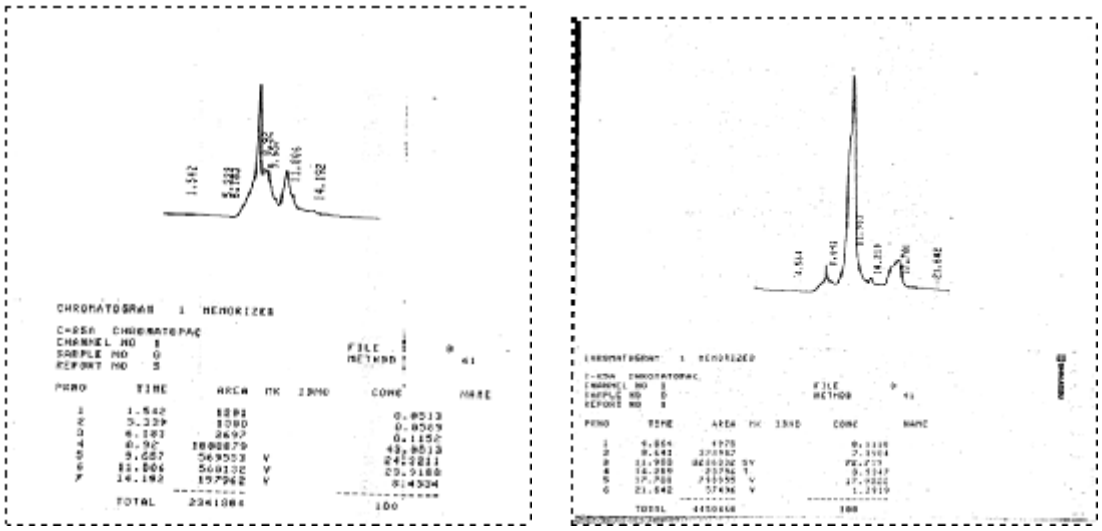
Table 4. Biochemical Tests of the selected *Bacillus* isolate

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

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 genome of the most selected isolates of *Fusarium* had shown 99 percent similarity by *F. graminearum* (data not shown). Disease severity in plants inoculated with the pathogen and *Bacillus* strains was significantly less than the pathogen control and *Bacillus* strain 11 had the greatest antifungal activity(Figure 6). The yield of wheat from plants treated with *Fusarium* and *Bacillus* strains specially strain 11, was significantly greater than of the control inoculated with pathogen alone. Treatments with *Bacillus* strain 11 alone increased the yield of wheat compared with the un-inoculated control (Figure 7). 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 AND CONCLUSIONS

Bacillus strains exhibit broad spectrum of action against different plant pathogens due to their ability to produce a great abundance of



Fig, 3. HPLC analysis of partially purified extract of the selected *Bacillus* isolate (a) and standard iturinA (b)

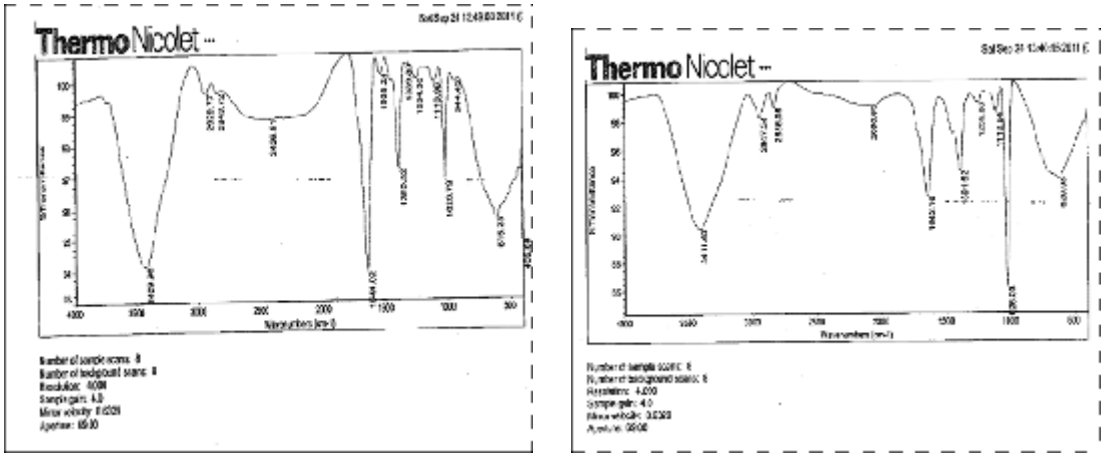


Fig. 4. FTIR profile of partially purified extract of the selected *Bacillus* isolate (a) and standard iturinA (b)

antibiotics belonging to the Iturin group with an amazing varieties of structures (Han *et al*. 2005). Iturin and fengycin are lipopeptide antibiotics with abroad antifungal spectrum. They have wide application in industries and medicine (Souto *et al*. 2004; Tendulkar *et al*. 2007). These compounds include predominantly popeptides 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). Head blight

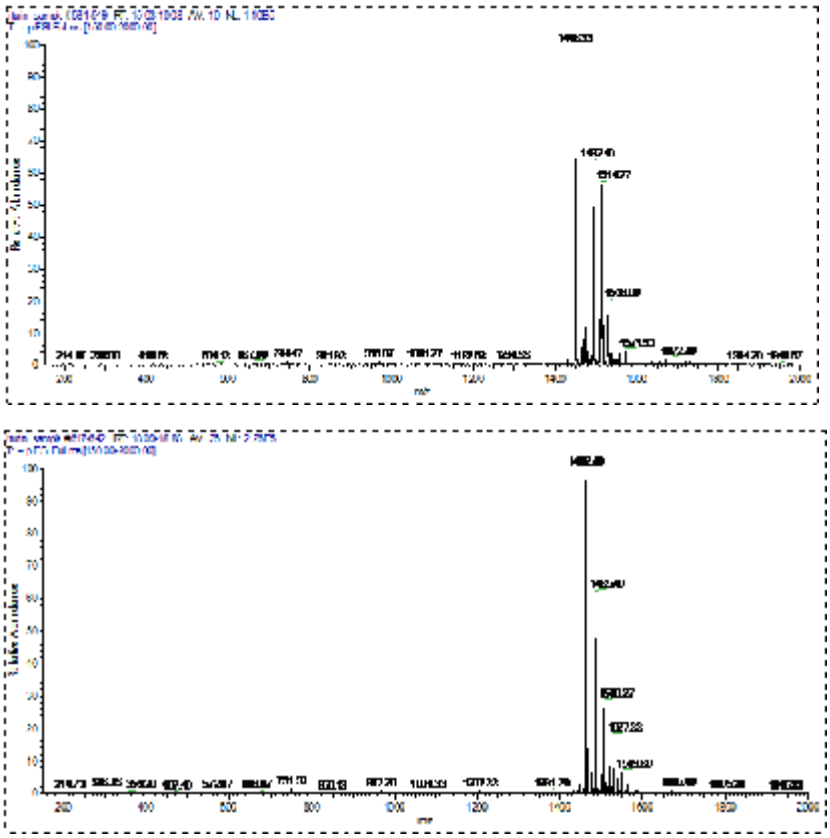


Fig. 5. ESI-MS spectra of the methanolic fraction (a), and commercially fengycin (b)

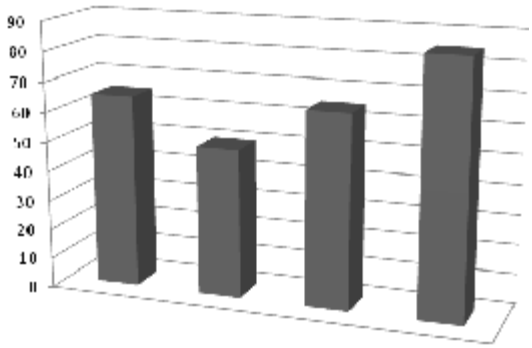


Fig. 6. Effect of bacterial strains on Fusarium head blight severity. Treatments with the same letters do not differ significantly ($p<0.05$) according to Duncan's Multiple Range Test. The vertical bars represent standard deviation with four replicates

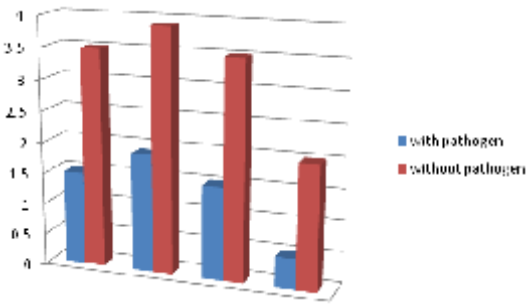


Fig. 7. Effect of bacterial strains plus *Fusarium* and bacterial strains only on weight of 100 wheat grains. Treatments with pathogen or without pathogen with the same letters do not differ significantly ($p<0.05$) according to Duncan's Multiple Range Test. The vertical bars represent standard deviation with four replicates

causes reduced kernel set and kernel weight, destruction of starch granules and storage proteins and seed infection (Nourozian *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 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, fengicin (as major fraction) and iturin A (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 is often observed in *Bacillus* spp. *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 foundresponsible 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*. 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. Inoculation of wheat in the green house showed that treatments with *Bacillus* strains reduce fusarium head blight severity. A work showed that the best isolates of *B. megatherium* and *B. subtilis* significantly diminished the *disease* incidence and severity up to 50% and 67%, respectively (Luz 2000). Nourozian *et al*., 2006, studied the antifungal activity of some strains of *Streptomyces* and *pseudomonas* against *F. graminearum* on wheat. Mycelial growth of the pathogen was reduced by cell free and volatile metabolites of bacterial antagonists by 37%-97%. Streptomyces strain 3 reduced disease severity of FHB 21 d after inoculation (Nourozian *et al*. 2006). In our present observation, an efficientiturin 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.

ACKNOWLEDGEMENTS

We are grateful to Cereal Research Center of Iran, complex of Sciences and Researches Campus of Islamic Azad University for the HPLC and FTIR results, Pharmaceutical Plant Research Center of Shahid Beheshti University for the LCMS results and Iranian National Biological Research Center for the 16S rRNA sequencing results.

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