



Microbial inhabitants of agricultural land have potential to promote plant growth but they are liable to traditional practice of wheat (*T. aestivum* L) straw burning



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ABSTRACT

Soil organic carbon (SOC) is closely associated with the soil fertility level. Crop straw return is an important conservation agricultural practice to restore SOC in the soil. Soil microbial communities are actively involved in decomposition of crop straws. Decomposition of straws releases bonded nutrients, water, organic carbon and energy in the soil, which enhance plant growth as well as increase microbial biomass. This study was conducted to analyze the effect of the conventional practice of wheat straw burning on bacterial populations present in the soil shortly after crop harvesting. Results indicate that wheat cultivated soil is inhabited by many plant growths promoting bacterial genera like *Acinetobacter*, *Azotobacter*, *Bacillus*, *Lysinibacillus*, and *Raoultella*, however, vegetation fire has dramatically decreased number of bacterial cells in the soil. Plant growth promoting assessment indicates that isolated strains have the greatest potential to boost plant growth. For example, some isolates have remarkable ability to synthesize auxin under *in-vitro* conditions like *B. safensis* AB-81, and *B. thuringiensis* AM-16 and some are able to solubilize inorganic phosphate, for instance, *A. pittii* AM-12 and *B. aryabhathi* AB-51. The rooting assay has shown that bacterial isolates had significantly improved plant growth. It is concluded that wheat cultivated land is inhabited by several beneficial bacterial communities while the straw burning practice has a strong negative impact on soil bacterial count.

1. Introduction

Soil organic C (SOC) plays an imperative part in land fertility and productivity and it bears a substantial influence on biological, chemical and physical properties of soil. Application of fertilizers, crop straw return and tillage are some key management practices that are responsible to shape the SOC level (Zhu et al., 2015). Due to the continuous mineralization process in cultivated land, it is important to restore the soil C level and for this purpose, crop straws (i.e. rice/wheat straw) are considered an important source that significantly improves total organic C content (TOC) of agricultural land, as well as they, have

strong influence on soil microbial populations (Zheng et al., 2015; Zhao et al., 2016). However, traditional agricultural practices like crop residue burning, crop rotation, and intensive tillage cause significant disturbance of soil structure, nutrient cycles, and composition of microbial communities.

The best and most biodiverse habitat of bacteria on earth is soil (Quince et al., 2008). Remarkable bacterial diversity, present in the soil habitats, is disclosed during the last few years because of rapid development and innovation in molecular biology and data analysis techniques (Raynaud and Nunan, 2014). It is estimated that more than 10^{10} bacterial cells may be present in 1 g of soil. Species diversity in the same

Abbreviations: SOC, Soil organic carbon; CONS, Conservation Agriculture Practices; TOC, Total organic carbon; CFU, Colony Forming Units; IAA, Indole-3-Acetic Acid; IGP, Indo-Gangetic Plains; PGPR, Plant Growth Promoting Rhizobacteria

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quantity of soil may range from 10^3 to 10^5 . It is also reported that more than 33000 bacterial and archaeal taxa can be detected from a soil sample using PhyloChip (Mendes et al., 2011). Soil microorganisms are responsible for up to 90% of dead organic matter decomposition and nutrient turnover. They assist in the preservation and regulation of various biochemical processes like purification and storage of water and organic carbon as well as N and P cycles (Cheng et al., 2018). Chen et al. Has reported that soil microbial players actively participate in straw decomposition, however, some factors like soil texture, climate pattern and straw quality significantly affect this process (Chen et al., 2014). Moreover, it is also reported that soil microbes are an important agent for structure maintenance of soil to sustain plant growth and their niche is to make the nutrients available for plant uptake (Vos et al., 2013). Some management practices like crop residue burning results in complete disturbance of microbial communities that can be linked with low crop yield.

Maintenance of the diverse and complex populations of soil microorganisms is essential for prolonged soil fertility. So many factors like nutrient content, pH, land use and type of vegetation along with SOC define microbial diversity in the soil. In other words, the effect of environmental factors and agricultural practices on the fertility of the soil can be assessed by analyzing the microbial content of the agricultural land (Fierer et al., 2012; Hartmann et al., 2015). Moreover, the drastic effect of a vegetation fire on soil microbial communities has also been reported by various studies as analyzed by Pressler et al. (Pressler et al., 2018). Generally, fungal cells were more intolerant to heat as compared to the bacteria and experiments have shown that sometime heat favors bacteria over fungi (Lupwayi et al., 2017). Withal, the final result, whatever it may be, holds a long-lasting effect on soil microflora and when it is disturbed, it alters many other processes happening in the near vicinity.

Potential ways to increase crop production include improvements in soil biological, chemical and physical attributes and all these qualities are mostly associated with soil nutrient level. According to the conservation agricultural practices (CONS), disturbance in soil organic matter results in reduced crop production (Malhi et al., 2006). Management of crop residue is, thus, an important factor in this regard which, if left unbridled, will throw a devastating effect on soil fertility and ultimately in the farming sector. In this view, the main theme of CONS is to increase the amount of crop residue over the soil surface to restore the SOC (Guo et al., 2016). Sometimes, crop residues are intentionally burned due to some short-term advantages like to decrease crop disease/pathogens and to advance weed control (Wang et al., 2010). A study had confirmed an increase in crop yield because last crop residues were burned in the field. But it is suggested that the increment was due to better preparation of seedbed in the burned field as compared with the unburned (Kutcher and Malhi, 2010). Additionally, combustion of crop leftovers results in a significant rise in atmospheric aerosols and emission of greenhouse gases (GHG) which not only have harmful effects on human health but also have a strong negative impact on regional and global climate.

The Indo-Gangetic Plain (IGP) located in four different countries (Pakistan, India, Nepal, and Bangladesh) is a highly intensive agricultural region of the world. It is reported that one-fifth of the total geographical area of these four countries is included in IGP (Sharma et al., 2010). In the IGP region, located in Punjab province, Pakistan, rice-wheat crop rotation is a dominant agricultural practice. Under this rotation, rice crop residues are burnt during the month of October/November and wheat straws are burnt during April/May as a regular management practice. Intensity and duration of fire vary per the weather, soil conditions and plant biomass. Farmers prefer burning practice because the management of crop left over is much difficult and labor intensive (Badarinath et al., 2009; Wang et al., 2015a,b). No previous study has been conducted to analyze effect of residues burning practice on soil microbial inhabitants, in this regard, present study is a first ever attempt to define negative consequences of fire practice on

soil biological properties. We hypothesized that fire has a significant negative effect on soil microbial communities.

2. Materials and methods

2.1. Study area

Wheat crop field located at 74°54 East longitude, 32°9 North latitude geographical location, Narowal city, Punjab province, Pakistan was selected for sampling. This area faces severe fog and haze as the climate of this region is freezing during the winter but it changes and become moderate to scorching during the summer. The annual temperature ranges between 4 °C and 20 °C during winter and rise to 40 °C during summer. This region also faces a long rain fall (Monsoon Season) with annual rainfall range is around 1200 mm (PMD).

The wheat crop in this region is sown in November/December. During the whole season, nitrogen fertilizer is applied for three times and irrigation is usually applied twice. The crop is usually reaped in April by combined harvester machine and crop left over are burned as a regular activity of field management practice.

2.2. Sample collection

Soil samples were collected from the wheat field by the help of soil core sampler (depth up to 20 cm and diameter 7 cm). Sampling was performed from three different sites just before (Sample AM-1) and after the regular activity of plant residues burning (Sample AM-2). Samples of different sites were then pooled and transferred to the lab aseptically.

2.3. Bacterial isolation

For isolation of bacterial strains, 1 g of soil sample was mixed with 99 ml of the autoclaved distilled water and 10^{-2} , 10^{-4} and 10^{-6} dilutions were prepared. Luria Bertani (LB) agar was employed as a growth medium and 0.1 ml volume from each dilution was plated on media plates which were then incubated at 37 °C for 24 h. Viable bacterial cells had grown over night and following incubation, total colony forming units (CFU) were counted and colony morphology was documented. Well isolated colonies were purified by streaking on the same medium and preserved in glycerol (at -70 °C) for further analysis. Isolated strains were then characterized by biochemical profiling and representative isolates were selected for sequencing and subsequent analysis.

2.4. 16S rRNA gene sequencing

16S rRNA gene sequencing technology was used to identify selected bacterial strains. For this purpose, genomic DNA was extracted with the help of genomic DNA purification kit (Promega, USA). As 16S rRNA gene was targeted so the 1.5 kb long gene fragment was amplified by using the method as described by Akhtar and Ali (2011) (Akhtar and Ali, 2011). PCR amplicon was purified and sequenced by using the same primers by sending to First-Base Laboratories (Singapore).

2.5. Cellulose digestion test

Selected bacterial strains were also analyzed for their potential to digest cellulose by using the procedure described by Hendricks et al., (Hendricks et al., 1995). Briefly, a fresh culture of selected bacterial strains was streaked on cellulose-Congo red agar media plates and incubated at 30 °C for 24 h. Bacterial isolates that were able to digest cellulose formed a clear zone around them. Following incubation period, change in color of media was recorded.

2.6. Nitrate reduction test

Nitrate reduction medium and reagent solutions were prepared according to the protocols given in microbiology laboratory manual (Cappuccino and Sherman, 2008). Briefly, sterile liquid media was dispensed in glass culture tubes which were then inoculated with respective strains and placed at 37 °C for 5 days. After incubation period, ability of the isolated strain to reduce nitrate was tested by adding a few drops of reagents into the growth media. Development of red/pink color was a clear indication of positive nitrate reduction test.

2.7. Ammonia production test

The ability of selected strains to synthesize ammonia has been evaluated by using the protocol as described by Rana et al., (Rana et al., 2012). Glass test tubes containing peptone media was prepared, inoculated with respective strains and incubated at 25 °C for 3 days. A change in color of peptone to brown/yellow was an indication of a positive test reaction (Dye, 1962).

2.8. Phosphate solubilization test

For phosphate solubilization test, the fresh culture of selected bacterial strains was streaked on Pikovskaya's agar plates and incubated at 28 °C for 7 days. Plates were wrapped with parafilm-sheet to avoid the chance of contamination. Size of the clear zone around bacterial colonies was measured after incubation period (Gupta et al., 1994).

2.9. Indole acetic acid production test

Proficiency of selected strains to synthesize auxin was assessed both in the presence and absence of L-tryptophan (500 µg/ml). For this purpose, media was prepared in 50 ml flasks and inoculated with fresh bacterial culture. Flasks were incubated at 37 °C in a shaker incubator (120 rpm) for 72 h. Cells were harvested by centrifugation at 2300g for 15min. For colorimetric analysis, 1 ml of supernatant and 2 ml of Salkowski's reagent were mixed in a test tube and incubate in the dark for 30min. Change in color was then measured at a 535 nm wavelength by a spectrophotometer and absorbance values were compared with a standard curve of IAA to calculate total auxin production.

2.10. Rooting assay

Rooting assay was performed to examine the efficiency of bacterial IAA to enhance the growth parameters of *Zea mays* under in-vitro conditions. Sterile petri plates with two filter papers (soaked with 10 ml autoclaved water) were used for this assay. Seeds were co-inoculated with isolated strains separately and allowed to grow for 7 days. After the incubation period, different growth parameters were analyzed and results were documented.

2.11. Statistical analysis

Data of all experiments were recorded in numerical form and saved in.csv file format where applicable for further analysis. Data were then subjected to analysis of variance using SPSS 20 bioinformatics program and means were separated using Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. Bacterial isolation

Following the incubation period, the total number of CFUs of sample AM-1 and AM-2 were calculated (Table 1, Supplementary Fig. 1). Results had shown that, 168×10^6 bacterial cells were present

Table 1
Total CFU per gram of soil samples.

No.	Samples	Dilution	No. of colonies	Volume plated (ml)	CFU $\times 10^6$ /gram of soil
1	AM-1	10^{-5}	168	0.1	168
2	AM-2	10^{-5}	86	0.1	86

in 1 g of AM-1 soil sample whereas, only 86×10^6 bacterial cells were viable in same amount of soil in AM-2 sample. It means fire regime has markedly decreased CFU count in AM-2 and nearly half of the bacterial cells were died. Thus, wheat straw burning practice has devastating effect on soil bacterial flora.

To assess the plant growth promoting potential of bacterial cells normally present in the soil and to envisage how much loss farmer face due to burning practice, initially, 40 morphologically different bacterial strains had been isolated and purified from sample AM-1. Gram staining and microscopic examination had shown that most of the isolated strains were gram-positive (GP) rods and only one isolate AM-12 was gram-negative (GN) cocci (Supplementary Fig. 2). A possible explanation for GP dominance over GN is a special support provided by both soil and environmental conditions to GP. They also have remarkable ability to survive under unfavorable conditions i.e. spore formation (Sun et al., 2016).

3.2. 16s rDNA gene sequencing

Draft sequencing result was received in.ab1 file-format from First-Base laboratories. After trimming the noise from ends, sequences were assessed for homology with previously reported strains by using the NCBI BLAST tool. Homology result had shown that the isolated strains belonged to Genus *Acinetobacter*, *Azotobacter*, *Bacillus*, *Lysinibacillus*, and *Raoultella*. All sequences were submitted to NCBI gene bank and accession numbers were obtained (Table 2). Phylogenetic relationship among all isolated strains has been shown in Fig. 1.

3.3. Plant growth promoting traits analysis

Performance of isolated strains against the designed testes has shown that isolated strains are able to significantly stimulate plant growth under field conditions. Almost all strains were positive for nitrate reduction and ammonia production, however, only six and ten strains were able to solubilize inorganic phosphate and hydrolyze cellulose respectively (Table 3).

Table 2
Isolated bacterial strains identified by 16S rDNA gene sequencing.

Sr. No	Isolate	Identified as	Accessions
1	AM-11	<i>Bacillus methylotrophicus</i>	KT027743
2	AM-12	<i>Acinetobacter pittii</i>	KT027744
3	AM-15	<i>B. thuringiensis</i>	KT027745
4	AM-16	<i>B. thuringiensis</i>	KT027746
5	AM-21	<i>B. licheniformis</i>	KT027747
6	AM-22	<i>B. mojavensis</i>	KT027748
7	AM-25	<i>B. toyonensis</i>	KT027749
8	AM-26	<i>B. amyloliquefaciens</i>	KT027750
9	AB-13	<i>Lysinibacillus macrolides</i>	KT027751
10	AB-21	<i>B. subtilis</i>	KT027754
11	AB-33	<i>B. pumilus</i>	KT027757
12	AB-51	<i>B. aryabhathi</i>	KT027756
13	AB-61	<i>B. subtilis</i>	KT027758
14	AB-62	<i>Raoultella planticola</i>	KT027759
15	AB-71	<i>L. macrolides</i>	KT027760
16	AB-72	<i>B. subtilis</i>	KT027752
17	AB-74	<i>B. subtilis</i>	KT027753
18	AB-81	<i>B. safensis</i>	KT027755
19	AB-83	<i>B. subtilis</i>	KT027761

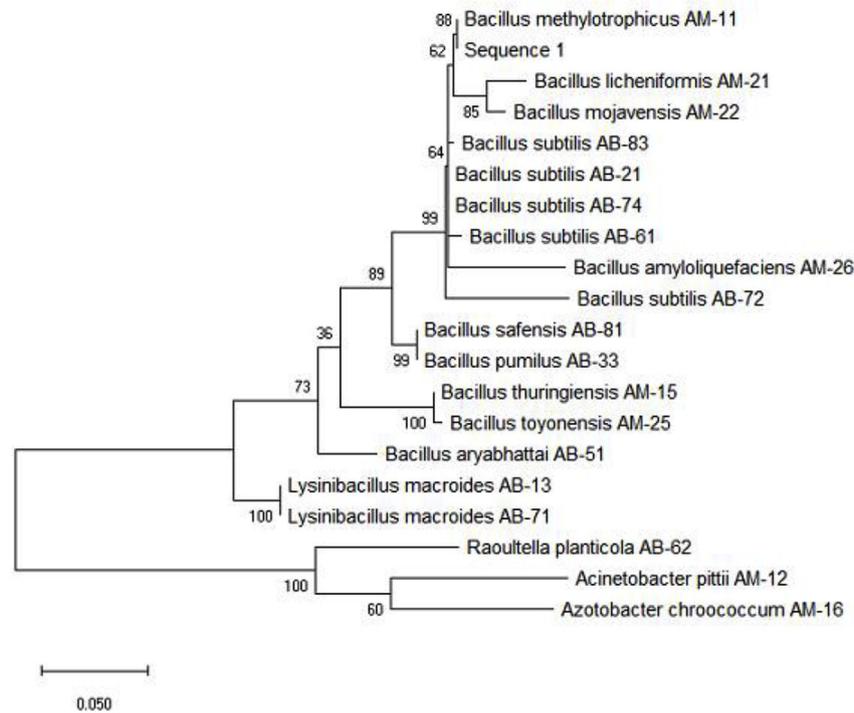


Fig. 1. Phylogenetic relationship among selected isolates. Phylogenies were inferred using the neighbor-joining algorithm and tree was generated using software MEGA 6. Numbers at branches points indicates the percentage of 500 bootstrap resamplings. The scale bar represents mutations per nucleotide position.

The capability of bacterial isolates to synthesize ammonia; the available form of nitrogen for plants, is of vital importance. By the virtue of this trait, bacteria can enhance plant growth directly by providing ready to use N as well as indirectly by producing plant growth hormones (Breedt et al., 2017). Among tested strains, *B. thuringiensis* AM-15, *B. thuringiensis* AM-16, *B. aryabhatai* AB-51, *B. subtilis* AB-74 and *B. subtilis* AB-83 were highly efficient for ammonia production and *B. thuringiensis* AM-15, *B. toyonensis* AM-25, *B. toyonensis* AM-25, *B. amyloliquefaciens* AM-26, *B. subtilis* AB-61 were competent for nitrate reduction. Although plants need only a small amount of phosphorus, yet it is a vital nutrient and phosphate-solubilizing bacteria are important agents that provide soluble P to the plants (Supraja et al., 2011). Among identified strains, *A. pittii* AM-12 and *B. aryabhatai* AB-51 were efficient for phosphate solubilization and *A. pittii* AM-12, *B. licheniformis* AM-21

and *B. aryabhatai* AB-51 were more promising for cellulose degradation (Table 3, Fig. 2). A possible explanation for least number of positive phosphates solubilizers may be the fact that bacterial isolates lose their characteristics with successive sub-culturing on artificial media as reported in the literature (Kucey, 1983; Tang et al., 2018). Similar results about the loss of bacterial ability to grow on the N-free medium have also been reported by da Saliva et al., (Silva et al., 2011). However, competency of bacterial isolated to hydrolyze cellulose is not changed over time or with subsequent sub-culturing (Tang et al., 2018).

3.4. Indole acetic acid production test

Isolated bacterial strains have shown similar behavior towards IAA biosynthesis as shown in Fig. 3. Almost all strains were able to produce

Table 3
Qualitative results of plant growth promoting traits of selected isolates.

Sr. No	Name of strain	Ammonia production	Nitrate reduction	Cellulose degradation	Phosphorus solubilization
1	<i>Bacillus methylotrophicus</i> AM-11	-	+	-	-
2	<i>Acinetobacter pittii</i> AM-12	+	+	++	++
3	<i>B. thuringiensis</i> AM-15	++	++	-	+
4	<i>B. thuringiensis</i> AM-16	++	++	-	-
5	<i>B. licheniformis</i> AM-21	+	+	-	++
6	<i>B. mojavensis</i> AM-22	+	++	-	-
7	<i>B. toyonensis</i> AM-25	+	++	+	-
8	<i>B. amyloliquefaciens</i> AM-26	+	++	-	+
9	<i>Lysinibacillus macrolides</i> AB-13	+	+	-	-
10	<i>B. subtilis</i> AB-21	+	-	+	+
11	<i>B. pumilus</i> AB-33	-	+	-	-
12	<i>B. aryabhatai</i> AB-51	++	+	++	++
13	<i>B. subtilis</i> AB-61	+	++	-	+
14	<i>Raoultella planticola</i> AB-62	+	+	-	-
15	<i>L. macrolides</i> AB-71	+	-	-	-
16	<i>B. subtilis</i> AB-72	-	+	+	+
17	<i>B. subtilis</i> AB-74	++	+	-	+
18	<i>B. safensis</i> AB-81	+	-	-	-
19	<i>B. subtilis</i> AB-83	++	+	+	+

+ + : strongly positive, + : positive, - : negative.

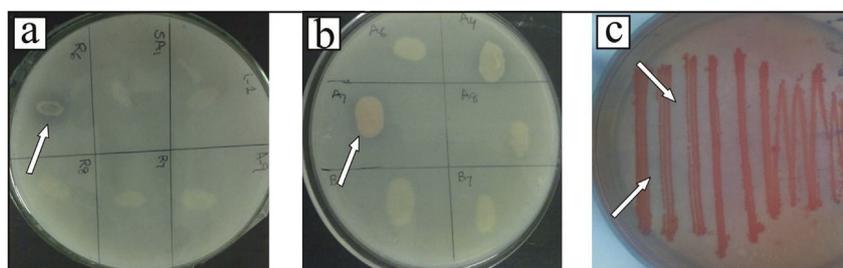


Fig. 2. Phosphate solubilization test (a, b). Cellulose degradation test (c). Arrows indicate clear zone around bacterial growth.

IAA in the absence of L-Tryptophan whereas the addition of originator greatly enhance auxin synthesis. Most promising strains for IAA synthesis in the absence of L-Tryptophan were *B. thuringiensis* AM-16, *A. pittii* AM-12 and *B. toyonensis* AM-25 which were able to synthesize 58, 56 and 55 μ g/ml of auxin respectively. However, *B. safensis* AB-81, *B. subtilis* AB-74, *B. subtilis* AB-61 and *L. macrolides* AB-13 with 136, 132, 132 and 130 μ g/ml of auxin production were highly biochemically active in the presence of L-Tryptophan respectively (Supplementary Fig. 3).

3.5. Rooting assay

Following the incubation period, growth parameters of seedlings were recorded (Table 4). Bacterial strains had efficiently enhanced almost all growth parameters of seedlings. Data analysis had shown that *B. subtilis* AB-21, *L. macrolides* AB-13, *B. pumilus* AB-33 and *B. subtilis* AB-74 were more promising for increasing root length. For example, *B. subtilis* AB-21 and *L. macrolides* AB-13 were able to stimulate root length up to 32 and 30% respectively. In addition to this, *R. planticola* AB-62, *L. macrolides* AB-71, *B. subtilis* AB-21 and *B. methylotrophicus* AM-11 were highly proficient to increase shoot length of seedlings. For instance, *R. planticola* AB-62 and *L. macrolides* AB-71 were able to increase shoot length up to 58 and 56% respectively. Similarly, *A. pittii* AM-12, *B. subtilis* AB-72, *B. toyonensis* AM-25, and *R. planticola* AB-62 were capable to significantly enhance the fresh weight of seedlings. As an example, *A. pittii* AM-12 and *B. subtilis* AB-72 had increased fresh weight of seedlings up to 12 and 11% respectively. In addition to this, *B. subtilis* AB-61, *B. toyonensis* AM-25, *L. macrolides* AB-71 and *B. licheniformis* AM-21 were highly competent to enhance the dry weight of seedlings. For example, *B. subtilis* AB-61 and *B. toyonensis* AM-25 were able to increase the dry weight of seedlings up to 24 and 22% respectively. Similar results have also been reported by Raheem and Ali. (2015) (Raheem and Ali, 2015).

4. Discussion

Results indicate that wheat straw burning management practice adversely affect soil microbial communities specifically PGPR population. Fire diminish the bacterial content of soil because almost all bacterial cells are intolerant to the high temperature (Bárcenas-Moreno and Bååth, 2009). Although, seasonal variations also affect bacterial diversity of land habitat, yet physical factors have a more obvious and drastic impacts. Many other studies designed to examine the effect of fire on microbial communities have also reported similar results for example (Pereg et al., 2018).

According to the CONS practices, it is strongly recommended that agricultural land must not be left uncovered and for this purpose, cultivation of cover crops is suggested (Bonanomi et al., 2016). So, it is of utmost importance that either the cover crops must be grown shortly after crop harvesting or straw must not be removed or burned (Wang et al., 2017). In addition to providing shelter to the soil, wheat straws are also responsible to increase SOC specifically in the upper 21-cm deep soil layer as reported by Zhu et al., (Zhu et al., 2015). Although SOC has large reservoirs and it is insensitive to occasional changes in management practices like straw-burning, yet, microbial populations are highly susceptible and the effects of only one fire regime may last for 10 years (Zhao et al., 2016). Therefore, the wheat straw burning practice has short as well as long-lasting adverse effects on soil properties and microbial populations.

Total organic carbon (TOC) is a representative of various C forms that are present in functional agricultural land. It is an important indicator of short-term change in SOC content and soil quality. In a recent study planned to analyze the effect of short-term straw return, Chen et al., have reported that if crop residues are retained in the field, they can increase microbial biomass C (MBC) up to 38% (Chen et al., 2017). Possible reason behind this boost is the release of plenty of nutrients, energy and soluble organic matter from decomposing crop-residues as

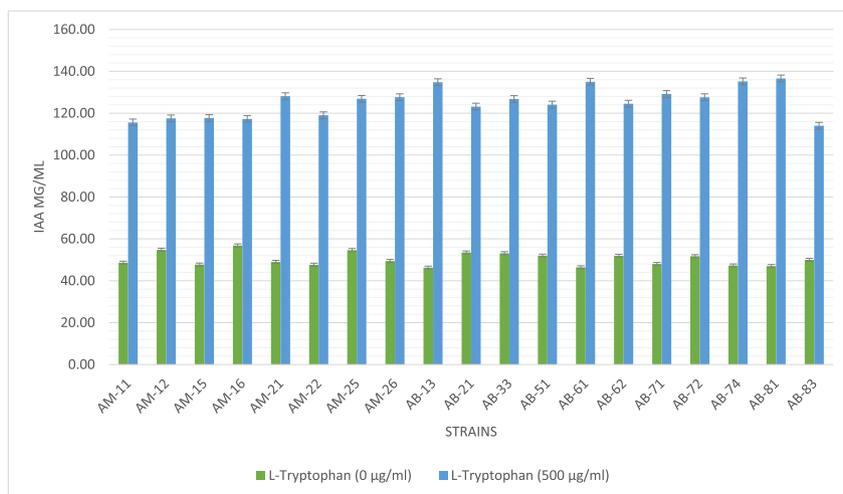


Fig. 3. In vitro auxin production test: Isolated bacterial strains are able to synthesize auxin in the presence of 0 and 500 μ g/ml concentration of L-tryptophan.

Table 4
Stimulation of root and shoot growth of *Zea mays* L. seedlings by bacterial isolates.

No.	Treatment	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
1	Control	17 ± 1.0 (a)	8.2 ± 0.3 (a)	2.34 ± .07 (a)	1.5 ± .03 (a)
2	<i>Bacillus methylotrophicus</i> AM-11	19.2 ± 0.8 (abc)	12.6 ± 0.5 (b)	2.5 ± .05 (abcd)	1.62 ± .04 (ab)
3	<i>Acinetobacter pittii</i> AM-12	18 ± 1.9 (ab)	11.3 ± 0.5 (ab)	2.64 ± .06 (d)	1.68 ± .04 (bc)
4	<i>B. thuringiensis</i> AM-15	20.3 ± 1.5 (abc)	10.4 ± 0.6 (ab)	2.42 ± .11 (abcd)	1.72 ± .04 (bcd)
5	<i>B. thuringiensis</i> AM-16	18.1 ± 0.7 (abc)	11.4 ± 0.5 (ab)	2.5 ± .07 (abcd)	1.76 ± .05 (bcd)
6	<i>B. licheniformis</i> AM-21	19.6 ± 1.2 (abc)	12.2 ± 0.7 (b)	2.58 ± .07 (bcd)	1.78 ± .04 (bcd)
7	<i>B. mojavensis</i> AM-22	18.9 ± 2.0 (abc)	10.3 ± 1.2 (ab)	2.46 ± .05 (abcd)	1.72 ± .04 (bcd)
8	<i>B. toyonensis</i> AM-25	21.4 ± 0.5 (abc)	10.6 ± 1.3 (ab)	2.6 ± .07 (bcd)	1.84 ± .02 (cd)
9	<i>B. amyloliquefaciens</i> AM-26	20.4 ± 1.8 (abc)	12.6 ± 1.0 (b)	2.46 ± .05 (abcd)	1.74 ± .07 (bcd)
10	<i>Lysinibacillus macrolides</i> AB-13	22.2 ± 0.7 (bc)	11 ± 0.8 (ab)	2.56 ± .10 (abcd)	1.76 ± .05 (bcd)
11	<i>B. subtilis</i> AB-21	22.6 ± 0.5 (c)	12.6 ± 0.6 (b)	2.42 ± .04 (abcd)	1.69 ± .06 (bcd)
12	<i>B. pumilus</i> AB-33	22.2 ± .5 (bc)	10.9 ± 0.9 (ab)	2.52 ± .04 (abcd)	1.72 ± .07 (bcd)
13	<i>B. aryabhathi</i> AB-51	19.7 ± 1.1 (abc)	11.4 ± 0.8 (ab)	2.38 ± .03 (ab)	1.72 ± .04 (bcd)
14	<i>B. subtilis</i> AB-61	18.4 ± 0.9 (abc)	11.4 ± 0.4 (ab)	2.58 ± .07 (bcd)	1.86 ± .02 (d)
15	<i>Raoultella planticola</i> AB-62	21.4 ± 1.1 (abc)	13 ± 1.3 (b)	2.6 ± .07 (bcd)	1.72 ± .06 (bcd)
16	<i>L. macrolides</i> AB-71	21.5 ± 2.6 (bc)	12.8 ± 0.4 (b)	2.4 ± .07 (abc)	1.78 ± .07 (bcd)
17	<i>B. subtilis</i> AB-72	20.2 ± 1.6 (abc)	12.2 ± 1.4 (b)	2.62 ± .03 (cd)	1.7 ± .07 (bcd)
18	<i>B. subtilis</i> AB-74	22 ± 0.7 (bc)	11.5 ± 1.3 (ab)	2.56 ± .06 (abcd)	1.68 ± .06 (bc)
19	<i>B. safensis</i> AB-81	19.6 ± 0.6 (abc)	12 ± 1.0 (b)	2.38 ± .05 (ab)	1.72 ± .07 (bcd)
20	<i>B. subtilis</i> AB-83	19.4 ± 1.2 (abc)	12 ± 1.1 (b)	2.56 ± .04 (abcd)	1.76 ± .05 (bcd)

Mean ± SE of 5 plants. Different letters within parentheses in same column indicate significant differences between treatments using Duncan's multiple range test (P = 0.05).

well as decomposition process also increase soil moisture and soil temperature and all these factors greatly intensify microbial growth (Wang et al., 2015a,b; Cheng et al., 2018). In addition to this, the presence of microbial populations, specifically the actinomycetes in the soil greatly enhance the rate of decay of crop straws and in return to this, their growth is also stimulated (Hai-Ming et al., 2014). Generally, when crop straws are returned to the soil, they significantly improve microbial content of the soil, specifically GP bacterial population which is a dominant group of microbes among functional microbial populations as compared with the soil in which crop straw are burnt. In this regard, our results are consistent with previous reports as wheat straw retention is much beneficial for soil C status and for bacterial populations (Guo et al., 2015; Zhao et al., 2016; Chen et al., 2017). In addition to crop residues, fertilization, climatic conditions and soil texture are important factors that define variety of microbial populations both in numbers and types. For example, it is reported that sand particles are less likely to support microbial growth as compared with silt and clay particles (Bach et al., 2010).

Rhizobacteria is a well-characterized bacterial group that actively enhance plant growth either directly or indirectly. The indirect way is to degrade crop residues and bring the bonded nutrients in such a form that plant can absorb easily. One such process is nitrogen fixation which is the most important phyto-stimulatory role that functional microbial species play in the soil (Fierer et al., 2012; Singh et al., 2015). Results of the present study indicates that isolated bacterial strains like *A. pittii* AM-12, *B. aryabhathi* AB-51, *B. toyonensis* AM-25 and *B. subtilis* AB-21 are able to produce and reduce ammonia and nitrates respectively and in this way, they play a substantial role in nitrogen cycle (Breedt et al., 2017) (Table 3). Crop residues increase the amount of available carbon in the soil that in-turn facilitate the microbial growth which subsequently increases nitrogen reserve of the soil. Higher nitrogen pool is helpful to promote the development of plantation in the next season (Pereg et al., 2018). Similarly, the capability of isolated strains to degrade and solubilize cellulose and inorganic phosphorus have also been analyzed respectively. For these two activities, auspicious potential of *A. pittii* AM-12, *B. licheniformis* AM-21, *B. aryabhathi* AB-51 and *B. subtilis* AB-74 has been documented (Table 3, Fig. 2). Similar results have also been reported by Breedet et al., and Tang et al., (Breedet et al., 2017; Tang et al., 2018).

Production and supply of growth regulators e.g. auxin, to the plants is a direct way by which rhizobacteria can stimulate plant growth under

normal and stress conditions (Sajid et al., 2017). Isolated bacterial strains were also tested for their ability to synthesize auxin and consequently enhance plant growth to evaluate their plant growth promoting potential. Almost all selected strains significantly enhance the growth parameters of *Zea mays* seedlings. *R. planticola* AB-62, *B. subtilis* AB-21 and *B. toyonensis* AM-25 strains were most promising in this regard. A similar effect of rhizobacteria on growth enhancement has also been reported by Raheem and Ali (2015) (Raheem and Ali, 2015).

In conclusion, the practice of wheat crop residues burning has an overall negative effect on soil microflora as well as fire also interfere with soil chemical and physical attributes. It is envisaged that the reduction in the bacterial count will likely disturb many biological and chemical processes happening in the soil. It is believed that burning of crop residues lowers the soil C and N content and results in soil degradation and deterioration. Therefore, it is much beneficial to leave crop leftover in the fields so that the SOC can be restored, which is responsible to maintain biological, physical and chemical properties of soil. In this regard, it is strongly recommended that wheat straws must not be burned and left untouched.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101060>.

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