ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

MULTIPLE MECHANISMS OF ACTIONS AND ROLE IN ECTOMYCORRHIZAE SYMBIOSIS BY MYCORRHIZA HELPER BACTERIA: A MINI REVIEW

Papan Chowhan, Arka Pratim Chakraborty*

Department of Botany, Raiganj University, Raiganj, Uttar Dinajpur, West Bengal, India.
**Corresponding author: arkapratimchakraborty83@gmail.com*

ABSTRACT

Mycorrhiza helper bacteria (MHB) are organisms that specifically promote mycorrhiza formation especially ectomycorrhizal fungi by producing growth metabolites that encourages easy proliferation of the fungal hyphae thereby increasing the chances of the fungal hyphae to colonise plant roots with a large surface area. Mycorrhizal fungi are the best-known examples of fungal and bacterial interactions as the hyphae offer good ecological niches for other microbes. AM fungal spores harbour Bacteria Like Organisms (BLOs) also referred to as endosymbionts in their cytoplasm and these organisms complete their life cycle within the eukaryotic cells giving rise to a further level of symbiosis. MHB may play a vital role in root-fungus recognition, in fungal growth, in the modification of the rhizospheric soil. It may act as stimulus to the formation of lateral roots in mycorrhizal plants as well as help in in germination of fungal propagules. The specificity of the interaction between MHB and the fungi and between MHB and the symbiont plant have also been explored.

Keywords: Mycorrhiza Helper Bacteria (MHB), Diversity, Multiple mechanisms of actions, Interaction between MHB and ectomychorrhizal symbiosis

1. INTRODUCTION

Mycorrhizae is a symbiotic association or a delicate and complex partnership between a fungus and a plant in which both the symbionts benefit from each other by the exchange of nutrients, though this is true that the mycorrhizosphere does not contain only the roots and mycorrhizal fungi but several other microorganisms such as bacteria. Therefore, mycorrhiza are not made of root and fungal tissues only, but also shelter complex bacterial communities, with which they interact physically and metabolically. The microbes in the mycorrhizosphere affect mycorrhizal functioning and thus some bacteria may interact with the mycorrhizal fungi on more than one metabolic level. It has been reported that some organisms especially those belonging to the genera *Bacillus* can be multifunctional. This means that they are able to perform functional roles such as being N_2 fixers, P solubilizers or grouped as Plant Growth Promoting Rhizobacteria (PGPR) or Mycorrhiza Helper Bacteria (MHB). Scientist [1] proposed the term Mycorrhiza Helper Bacteria (MHB), referring only to bacteria that promoted the establishment of the root-fungus symbiosis with positive effects on mycorrhizal associations, but negative effects on the root pathogens. However, there are data concerning MHB stimulating phytopathogenic fungi and this should be considered in the bio-technological applications of MHB, for instance, as inoculums for plants.

Whether MHB could promote the colonization of the roots by pathogenic fungi- future more research is needed to establish.

2. EVIDENCE OF OCCURRENCE OF MYCORRHIZA HELPER BACTERIA

Mycorrhiza helper bacteria (MHB) is a generic name given to those bacteria which stimulate the formation of mycorrhizal symbiosis [2]. MHB are a group of organisms that form symbiotic association with both ectomycorrhiza and arbuscular mycorrhiza. Researcher [1] proposed for the first time the term mycorrhization helper bacteria (MHB), referring only to bacteria that promoted the establishment of the root fungus symbiosis. Two functional categories of MHB- the first-bacterial strains that positively impact on the functioning of mycorrhizal symbiosis called mycorrhization helper bacteria strictly referring to those that stimulate the process of mycorrhiza formation and the second- mycorrhiza helper bacteria for those that interact positively with the functioning of the already established symbiosis [3]. Both the categories can be represented by different groups or by overlapping groups of micro-organisms and the term MHB is used to represent both the groups. Some MHB can also have additional beneficial effects such as plant growth stimulations, protections against pathogens and adverse conditions. There was first report about some MHB associated with the endomycorrhizal fungus such as *Glomus* sp [4]. There after many bacteria stimulating endomycorrhizal symbiosis have been reported. Researcher [5] mentioned the genera and role of mycorrhiza helper bacteria, found in ectomycorhiza in their review article. Mycorrhiza helper bacteria found in arbuscular mycorrhiza have been represented in Table 1.

Table 1: Mycorrhiza helper bacteria found in arbuscular mycorrhiza

Mycorrhizal fungi	*Identity of the MHB isolates*	*Host plant*	*Ecological origin of MHB isolates*	*Effect of MHB*	*References*
Gigaspora margarita	*Azospirillum brasilense*	*Pennisetum americanum*	Not available	1.1-fold increase in the percentage of root infections	[6]
Endogone sp.	*Pseudomonas* sp.	Different *Trifolium* species, *Cucumis sativum* , *Allium cepa*	Contaminated cultures of mycorrhizal plants	Significant increase in the number of plants with arbuscular mycorrhizal infections	[4]
Glomus clarum	*Azotobacter diazotrophicus Klebsiella* sp.	*Ipomoea batatas*	Sugar cane roots	1.4-1.6-fold increase in the arbuscular mycorrhizal colonization of the roots	[7]
Glomus fasciculatum	*Azotobacter chroococcum*	*Lycopersicum esculentum*	Not available	1.2-fold inc of arbuscular mycorrhizal infection of the roots rease in the percentage	[8]
Glomus intraradices	*Bacillus subtilis*, *Enterobacter* sp.	*Allium cepa*	Not available	1.1-1.3-fold increase in the arbuscular mycorrhizal colonization of the roots	[9]
Glomus mosseae	*Paenibacillus* sp.	*Sorghum bicolor*	Growth substrate of *G. mosseae*-inoculated plants	1.3-fold increase in the arbuscular mycorrhizal colonization of the roots	[10]
Glomus mosseae	*Bradyrrhizobium japonicum*	*Glycine max*	Nodules	4.5-fold increase in the arbuscular mycorrhizal colonization of the roots	[11]
Glomus mosseae	*Pseudomonas* sp.	*Lycopersicum esculentum*	Rhizosphere	1.6-fold increase in the arbuscular mycorrhizal colonization of the roots	[12]
Glomus mosseae	*Brevibacillus* sp.	*Trifolium pratense*	soil	1.4-17.5-fold increase in the arbuscular mycorrhizal colonization of the roots	[13]

3. DIVERSITY OF MYCORRHIZA HELPER BACTERIA

Mycorrhiza Helper Bacteria belong to many groups and bacterial genera, such as Gram-negative Proteo-bacteria *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Bradyrhizobium*, *Enterobacter*, *Pseudomonas*, *Klebsiella* and *Rhizobium*), Gram-positive Firmicutes (*Bacillus*, *Brevibacillus*, *Paenibacillus*) and Gram-positive Actinomycetes (*Rhodococcus*, *Streptomyces* and *Arthrobacter*). Thus MHB are diverse and belong to different bacterial phyla including both gram-negative and gram-positive bacteria. Some of the most common types are *Pseudomonas* and *Streptomyces*. Most of the bacteria are associated with both ectomycorrhiza and arbuscular mycorrhiza, but some show specificity to particular type of fungus. The common phyla that MHB belong are as follows:

3.1. *Proteobacteria*

The proteobacteria are a large and diverse group of gram-negative bacteria. *Pseudomonas* is the most common type. These MHB bacteria belong to this genus are strongly associated with both ectomycorrhiza and arbuscular mycorrhiza in rhizosphere. *Pseudomonas fluorescens* has been examined in several studies to understand how they work in benefitting the mycorrhiza and plant [3]. It was noticed that the bacteria helped the ectomycorrhizal fungi to promote a symbiotic relationship with the plant by increase in formation of mycorrhiza. Some others MHB improve the root colonization and plant growth in association with arbuscular mycorrhiza. The improvement of growth has been hypothesized that MHB help the plant to defend against pathogens by improving the nutrient uptake. However the mechanisms are remain still unknown.

3.2. *Actinobacteria*

These are gram-positive bacteria found in soil. *Streptomyces* is the most common bacteria associated with MHB [3]. One study reported that *Streptomyces* increased root colonization, growth, mycorrhizal colonization and fungal growth. It has also been found that *Streptomyces* interact with both ectomycorrhiza and arbuscular mycorrhiza.

3.3. *Firmicutes*

These are gram-positive bacteria. Some genera which act as MHB, but the one most common is *Bacillus* which are rod shaped organisms that can be free living or pathogenic [3]. However in the presence of mycorrhiza some species can be beneficial and are considered as MHB. They can form relationship both with ectomycorrhiza and arbuscular mycorrhiza. *Bacillus* help in the establishment and growth of the mycorrhiza and also help in nitrogen fixation in the rhizosphere in some cases.

4. SPECIFICITY OF INTERACTION BETWEEN MYCORRHIZA HELPER BACTERIA AND ECTOMYCHORRHIZAL SYMBIOSIS

Mycorrhiza Helper Bacteria are fungus specific but they are not plant specific [2]. Many studies were carried out to determine the specificity of interaction between MHB and the fungi and between MHB and the plant by which a diverse results were found [2,3,14,15]. Researcher [3] reported the role of *Streptomyces* sp ACH505 as Mycorrhiza Helper Bacteria which not only promoted growth of *Amanita muscaria* but also increased the formation of ectomycorrhizae. *Streptomyces* sp ACH505 showed inhibition of pathogenic fungi. In general MHB showed a degree of specificity with the mycobiont, in which some strains were specific to certain ectomycorrhizal fungi [16] and other capable of stimulation of mycorrhization by different ectomycorrhizal fungi [15]. There are evidences that fungi belonging to Basidiomycetes have a role in interacting with mycorrhiza helper bacteria. The fungal specificity of MHB has been considered a very important property, for these characteristics, MHB enhance mycorrhiza formation by some fungi but also inhibit the other fungi to make symbiosis at same time. By this way MHB inhibited the pathogenic fungi [1]. However some MHB had a broader impact on mycorrhiza formation in a large number of hosts, for example the bacterium *Paenibacillus* EJP73 enhanced the ectomycorrhizal formation in *Lactarius rufus*, *Pinus sylvestris* and the arbuscular mycorrhizal symbiosis of *Glomus mossae-Cucumis sativus* [17]. However, some of MHB were reported to help the both symbiotic and pathogenic fungi [3, 18]. Mycorrhiza helper bacteria not only help in mycorrhiza formation and functioning but some MHB also help in growth of the plant through direct or indirect effects by plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas* sp. Many MHB are considered now-a-days as Plant Growth Promoting Rhizobacteria (PGPR). Some strains of *Pseudomonas* GM41 showed positive effects in the formation of secondary roots of *Populus trichocarpa* and *P.deltoide* [19]. *P. fluorescens* CECT 5281 modified the response of *Pinus halpensis* roots in drought [20]. MHB were reported as potent microorganisms that showed positive role in nitrogen fixation [21]. These bacteria are found in the surface of the ectomycorrhiza but it is still unknown that whether this nitrogen help the ectomycorrhizal fungus or plant. MHB protect the host plant from different type of pathogens by inhibiting the development of the pathogens through the biocontrol mechanism [18]. Mycorrhiza helper bacteria had a role in plant immune response against different pathogens through induction of jasmonic acid and salicyclic acid pathway [22]. Both ectomycorrhizal and endomychorrhizal fungi had effects on the host immune system by secreting small effectors [23-24].

5. MULTIPLE MECHANISMS OF ACTIONS

Researcher [2] proposed five different mechanisms of action of MHB, these are-

i) Increased the survival and germination rate of the fungal spore in the rhizosphere.
ii) Stimulation of the pre-symbiotic growth of the fungal mycelium.
iii) Increased the receptivity to fungal signals of the roots.
iv) MHB stimulated the root mycelium recognition.
v) Modification of the physio-chemical properties which help in mycorrhiza formation (**Fig. 1**).

The detailed mechanisms of actions by Mycorrhiza Helper Bacteria are as follows

5.1. Effects on the plant root system

MHB help in formation of short roots [19, 25, 26]. By increasing the lateral root formation MHB stimulate the ectomycorrhiza. Auxin and ethylene producing bacteria often stimulate the root development [27-28]. Auxin regulates the ectomycorrhizal formation [29]. Thus MHB induce growth hormone in a plant which help the mycorrhiza to interact with the plants lateral roots. An increase in root formation noticed when *Pseudomonus putida* inoculated with *Gigaspora rosea* on a cucumber plant. Two non auxin producing MHB were found to stimulate the dichotomus root branching in *Pinus* seedlings [14].

5.2. Effects on the fungal growth

The growth of the fungal mycelium and its survival at the presymbiotic stage were influenced by mycorrhiza helper bacteria. It increased the probability of the fungal mycelium to reach and interacted with the lateral roots of the plant and by this it helped to form the mycorrhizae [2]. However many bacteria which stimulated the fungal growth did not have any MHB characteristics and effects on mycorrhiza formation [19, 30]. Some MHB strains had no effects on fungal growth e.g. *Streptomyces* sp AcH505, *Paenibacillus* sp, *Pseudomonas monteilii* HR13 [31-34]. However how MHB stimulate the presymbiotic fungal growth is not clearly understood. It is observed that MHB produces thiamine at certain level which promote the growth of the fungus *in vitro* [35]. *Paenibacillus validus* produced raffinose which stimulated the growth of mycelia of *Rhizophagus* sp [36]. There were reports that some MHB inhibited the fungal growth by detoxifying some fungal metabolites [1]. MHB also influenced hyphal branching and hyphal density *in vitro* [19, 33, 35]. Increased hyphal branching make higher infection of short roots by the fungi in the symbiosis.

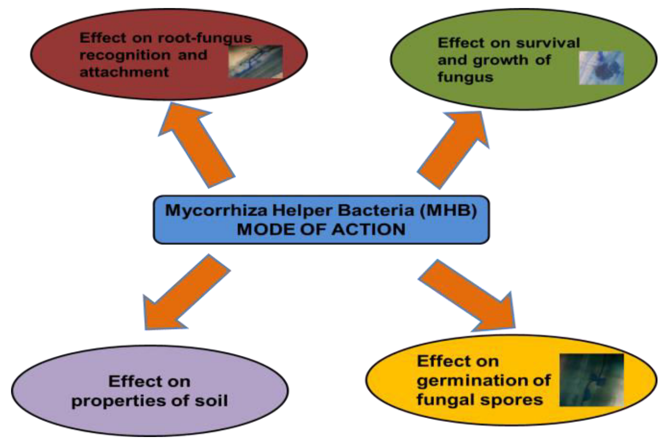

Fig.1: Schematic diagram with multiple modes of actions by Mycorrhiza Helper Bacteria.

5.3. Growth promotion by nutrients

MHB also help in breakdown of some molecules to a more usable form. The bacteria make available different inorganic and organic nutrients in the soil through a process known as mineral weathering. This process release proton and iron in the soil. Many MHB such as *Pseudomonas*, *Burkholderia* and *Collimonas* reported to participate in the mineral weathering process [37]. MHB also help in phosphate uptake [38]. These bacteria release phosphate degrading compounds in soil which break down the organic and inorganic phosphate. As a result, MHB make phosphate available for the mycorrhiza to use and help the mycorrhiza to grow in a phosphate limited conditions. MHB also able to fix nitrogen in the rhizosphere making it available for the plants, however MHB do not cause plant modification like legumes [39].

5.4. Detoxification of soil

MHB help the fungus to establish a symbiotic association with plants in stressed environment like rich in toxic metals [40]. In harsh environment the MHB help in acquiring nutrients like nitrogen, phosphorus. MHB prevent the uptake of toxic metals like cadmium, zinc, lead through blockade method, thus allow the fungus to form a better symbiotic association with the plant [41-42]. MHB create a balance between macro and micro nutrients.

Mycorrhiza helper bacteria promote the formation of mycorrhiza specially ectomycorrhiza by producing some growth metabolites which encourage the proliferation of the fungal hyphae and increase the chance of better colonisation of fungal hyphae with the plant roots. The stimulus of fungal growth appears to be the primary effect of MHB. The germination of fungal spores and the mycelia growth stimulated through the production of growth factors, detoxification of antagonistic substance or by inhibition by competitors [3]. The stimulus of fungal growth by MHB represents an adaptive advantage, thus the fungus become heavily associated with the host plant and become more competitive against other mycobiont in that area [34]. MHB also stimulated the formation of the lateral roots in mycorrhizal plants, it led to an increase in the number of possible interaction sites between the plant and fungus [43]. This way MHB promote a greater plant mycorrhization by the mycobiont. Different MHB may show different helper mechanisms, even for the same pair of mycorrhizal symbiont. As for example the MHB-*Burkholderia* sp EJP67 isolated form *Pinus sylvestris-Lactarius rufus* ectomycorrhizae stimulated both first and second order mycorrhizal roots formation while *Paenibacillus* sp EJP73 isolated from the same ectomycorrhizae only promoted the formation of second order mycorrhizal roots formation [25]. The contact between MHB cells and the symbiosis is necessary for the helper effect [14]. MHB also improve the nutrition of the fungus through the provision of nitrogen or by contribute to the solubilisation of minerals. Some strains of MHB compete with bacteria that inhibit the mycorrhization [2] and reduce the concentration of antifungal metabolites in the mycorrhizosphere. MHB stimulate the production of phenolic compounds by the fungus, such as hypaphorine and enhance the aggressiveness of the fungus. The fungus release some exudates that serve as nutrients for the bacteria. It was reported that the fungus *Amanita muscaria* secreted substances (organic acids and protons) that modulated the spectrum of antibiotics production by MHB [3]. The metabolite auxofuran produced by *Streptomyces* sp AcH505 stimulated the pre-symbiotic growth of *Amanita muscaria* but inhibited the growth of pathogenic fungi [44]. MHB may have selective mechanism of interaction with surrounding micro-organisms, with neutral or positive effect on the root pathogens [3]. However MHB may stimulate the phytopathogenic fungi and this should be a matter of concern in the application of MHB. Further researches are needed to determine whether MHB could promote the colonization of the roots by pathogenic fungi and lead to development of disease or not.

6. CONCLUSION

The studies of MHB on the establishment and development of mycorrhizae may help us to generate a comprehension about the interaction between these organisms and the other components of the environment. The study of MHB may pave the way towards gaining knowledge of how mixed microbial communities stimulate the formation of mycorrhizae. MHB could be very useful in controlled mycorrhization in forest management, through its application to soil in nurseries. Co-inoculation with the mycobiont enables the growth of fungal inoculum and help to improve the quality of the mycorrhizal association in seedlings. Although bacteria with the potential to act as MHB occur everywhere, the activities of most of the MHB have been demonstrated in laboratories or greenhouses and the extension of these results to natural conditions remain to be elucidated. Little is known about the molecular mechanisms involved in promoting the growth of mycobiont induced by MHB. A deeper study on MHB could generate a model for genomic analysis of bacteria-fungus interactions, that may benefit other research areas such as protection of plant species.

7. REFERENCES

1. Duponnois R, Garbaye J. *Annalesdes. Sci. Forestitres.*, 1991; **48:**239-251.
2. Garbaye J. *New. Phytol.*, 1994; **128:**197-210.
3. Frey-Klett P, Garbaye J, Tarkka M. *New. Phytol.*, 2007; **176:**22-36.
4. Mosse B. *J. Gen. Microbiol.*, 1962; **27:**509-520.
5. Gupta S, Chakraborty AP. *Int. J. Res. Rev.*, 2020; **7:**387-391.
6. Rao NSS, Tilak KVBR, Singh CS. *Pl. Soil.*, 1984; **84:**283-286.
7. Paula MA, Urquiaga S, Sijqueira JO. *Biol. Fertl. Soils.*, 1992; **14:**61-66.
8. Bagyaraj DJ, Menge JA. *New. Phytol.*, 1978; **80:**567-573.
9. Toro M, Azcon R, Barea JM. *Appl. Environ. Microbiol.*, 1997; **63:**4408-4412.
10. Budi SW, van Tuinen D, Martinotti G, Gianinazzi S. *Appl. Environ. Microbiol.*, 1999; **65:**5148-5150.
11. Xie ZP, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ, Vogeli-Lange R, Boller T. *Pl. Physiol.*, 1995; **108:**1519-1525.
12. Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, Bonfante P, O'Gara F, Azcón-Aguilar C. *Appl. Environ. Microbiol.*, 1998; **64:**2304-2307.

13. Vivas A, Marulanda A, Ruiz-Lozano JM, Barea JM, Azcon R. *Mycorrhiza.*, 2003; **13:**249-256.
14. Aspray TJ, Jones EE, Whipps JM, Bending GD. *FEMS. Microbiol. Ecol.*, 2006a; **56:**25-33.
15. Bending G. *New. Phytol.*, 2007; **174:**707-710.
16. Duponnois R, Garbaye J, Bouchard D, Churin JL. *Pl. Soil.*, 2003; **157:**257-262.
17. Aspray TJ. Frey-Klett P, Jones JE, Whipps JM, Garbaye J, Bending GD. *Mycorrhiza.*, 2006b; **16:**533-541.
18. Lehr NA, Schrey SD, Bauer R, Hampp R, Tarkka MT. *New. Phytol.*, 2006; **174:** 892-903.
19. Labbe JL, Weston DJ, Dunkirk N, Pelletier DA, Tuskan GA. *Front. Pl. Sci.*, 2014; **5:** doi: 10.3389/fpls.2014.00579.
20. Dominguez-Nuez J, Munz D, de la Cruz A, Saiz de Omenaca J. *Agronomy.*, 2013; **3:**571-582.
21. Khaitov B, Patino-Ruiz JD, Pina T, Schausberger P. *Ecol. Evolu.*, 2015; **5:**3756-3768.
22. Cameron DD, Neal AL, van Wees SCM, Ton J. *Trend. Pl. Sci.*, 2013; **18:**539-545.
23. Kloppholz S, Kuhn H, Requena N. *Curr. Biol.*, 2011; **21:**1204-1209.
24. Plett JM, Daguerre Y, Wittulsky S, Vayssières A, Deveau A, Melton SJ. *Proceed. Natio, Acad. Sci., U.S.A.* 2014; **111:**8299-8304.
25. Poole EJ, Bending GD, Whipps JM, Read DJ. *New. Phytol.*, 2001; **151:**743-751.
26. Schrey SD, Schellhammer M, Ecke M, Hampp R, Tarkka MT. *New. Phytol.*, 2005; **168:**205-216.
27. Spaepen S, Vanderleyden J, Remans R. *FEMS. Microbiol. Rev.*, 2007; **31:**425-448.
28. Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Loccoz YM, Muller D, Legendre L, Wisniewski-Dye F, Prigent-Combaret C. *Front. Pl. Sci.*, 2013; **4:**1-19.
29. Felten J, Legue V, Ditengou FA. *Pl. signal. Behav.*, 2010; **5:**864-867.
30. Zhao L, Wu XQ, Ye JR, Li H, Li GE. *Biol. Fertl. Soils*, 2013; **50:**593-601.
31. Bending GD, Poole EJ, Whipps JM, Read DJ. *FEMS. Microbiol. Ecol.*, 2002; **39:**219-227.
32. Riedlinger J, Schrey SD, Tarkka MT, Hampp R, Kapur M, Fiedler HP. *Appl. Environ. Microbiol.*, 2006, **72:**3550-3557.
33. Aspray TJ, Jones EE, Davies MW, Shipman M, Bending GD. *Mycorrhiza.*, 2013; **23:**403-410.
34. Duponnois R, Plenchette C. *Mycorrhiza.*, 2003; **13:**85-91.
35. Deveau A, Palin B, Delaruelle C, Peter M, Kohler A, Pierrat JC, Sarniguet A, Garbaye J, Martin F, Frey-Klett P. *New. Phytol.*, 2007; **175:**743-755.
36. Hildebrandt U, Ouziad F, Marner FJ, Bothe H. *FEMS. Microbiol. Lett.*, 2006; **254:**258-267.
37. Uroz S, Calvaruso C, Turpault MP, Pierrat JC, Mustin C, Frey-Klett P. *Appl. Environ. Microbiol.*, 2007; **73:**3019-3027.
38. Barea JM, Azcon R, Azcon-Aguilar C. *Antonie. van. Leeuwenhoek.*, 2002; **81:**343-51.
39. Antoun H, Prevost D. PGPR: *Biocont. Biofertl.*, Springer, Dordrecht, 2005; pp. 1-38.
40. Bonfante P. Anca IA. *Ann. Rev. Microbiol.*, 2009; **63:**363-383.
41. Vivas A, Barea JM, Azcon R. *Environ.Pollu.,* 2005; **134:**257-266.
42. Vivas A, Barea JM, Biro B, Azcon R. *J. Appl. Microbiol.*, 2006; **100:**587-598.
43. Shilev S, Lopez AF, Prieto MS, Puebla EDS. *J. Environ. Engineer. Landscape. Manag.*, 2007; **15:**221-226.
44. Keller S, Schneider K, Sussmuth RD. The J. Antibiotics., 2007; **59:**801-803.