Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 126-130

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 126-130**
Journal homepage: http://www.ijcmas.com

Review Article

http://dx.doi.org/10.20546/ijcmas.2016.507.011

A Review on Identification of *Bacillus* spp. using Molecular Characterization

Tamalika Sarangi* **and S. Ramakrishnan**

Department of Nematology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

**Corresponding author*

Keywords

Bacillus spp, biochemical test, molecular technique.

Article Info

Accepted:
12 June 2016
Available Online:
10 July 2016

A B S T R A C T

Nowadays the use of molecular tool is gaining importance for the proper and precise identification of any biological organisms. The technique is successfully adopted for the precise identification of *Bacillus* spp. The available literature on identification of *Bacillus* spp. at molecular level were collected and presented below. The review will be useful to the researchers working on this line as the identification of *Bacillus* spp. using morphological and cultural characters, biochemical tests etc. are time consuming, cumbersone and lacks precision.

Introduction

The endophytic bacteria, *Bacillus* spp. are generally classified based on their morphology and aggregation. The shape of the endophytic *Bacillus* spp. are spheres (coccus), rod shaped and round ended cylinders according to Wang *et al.*,(2008). Further the *Bacillus* spp. showing the cultural characters of colonization of bacteria on nutrient agar medium and serrated margin also used to identify the bacteria. (Hung and Annapurna, 2004 and Parvathi *et al.*, 2004). Besides biochemical tests of employing the methods of simple staining, gram staining, utilization of citrate, catalase test, vogos proskaecur test, growth in Nacl, starch hydrolysis, methyl red test, growth at 4^0C and KOH test are also in practise to identify the bacteria as *Bacillus* spp. (Rangasamy and Bagyaraj, 1993, Vetrivelkalai *et al.*, 2010 and Manikandan and Muthuselvan, 2014).

Molecular Characterization of Genus *Bacillus*

For the precise identification of the endophytic genus various morphological, cultural and biochemical tests were performed as above. Since it is difficult to confirm the identity of the endophytes based on only morphological and biochemical characters as opined by Frietas *et al.*, (2008),

currently molecular techniques like t DNA PCR (tDNA-intergenic spacer length polymorphisms) and ITS-PCR analyses (16S–23S intergenic transcribed spacer region) are being used for the precise identification of the *Bacillus* endophytes.

Hence the information furnished on the identification of *Bacillus* spp. based on molecular characters in the Table will be useful as ready reference to researchers working on isolation and identification of the bacterium.

Table.1 Identification of *Bacillus* spp. using PCR technique

***Bacillus* spp.**	**References**
Bacillus spp.	Raton *et al.,* (2012), Sihem *et al.,* (2011), Absalon *et al.,* (2012), Ettoumi *et al.*, (2013)
B.amyloliquefaciens	Wulff *et al.,* (2002), Matarante *et al.,* (2004), Lisboa *et al.,* (2006), Sarangi *et al*.(2009), Athukorala *et al*. (2009)
B. anthracis	Hill *et al*.(2004)
B.cereus	Matarante *et al.(*2004), Hill *et al*. (2004), Nagesh *et al*. (2005), Azokpota *et al*. (2007), Kang Cheng *et al*. (2009), Reddy *et al*. (2009), Wahyudi *et al*. (2010), Oliveira *et al*. (2014)
B. firmus	Ettoumi *et al*. (2013)
B. fusiformis	Wahyudi *et al*.(2010)
B. licheniformis	Tendulkar *et al*.(2006), Gomaa and Momtaz.(2006), Azokpota *et al*. (2007), Xio *et al*. (2009), Ettoumi *et al.,*(2013)
B. mojavensis	Sihem *et al.,*(2011), Youcef-ali *et al.,*(2014)
B. mycoides	Athukorala *et al.,*(2009), Sarangi *et al.*, (2009)
B. pumilus	Wulff *et al.,* (2002), Matarante *et al.,*(2004), Parvathi *et al.,* (2009), Ettoumi *et al.,* (2013), Moghaddam *et al.,*(2014)
B. simplex	Gomaa and Momtaz. (2006)
B. subtilis	Wulff *et al.,* (2002), Matarante *et al., (* 2004), Azokpota *et al.,*(2007), Kang Cheng *et al.,* (2009), Athukorala *et al.,*(2009), Sarangi *et al.,* (2009), Wahyudi *et al.,* (2010), Xia *et al.,* (2011), Sihem *et al.,* (2011), Ettoumi *et al.,*(2013), Yap Chin Ann.(2013), Youcef-ali *et al.,*(2014), Oliveira *et al.,*(2014), Wei *et al.*, (2014)
B. thuringiensis	Hill *et al.,* (2004), Reddy *et al.,*(2009), Yap Chin Ann. (2013), Zahner *et al.*, (2013)
B.weihenstephanensis	Biswas and Das.(2011)
B.subtilis	Tamalika Sarangi (2014)

References

Absalon, S.C., M. M.del, C.O. Mosqueda, M.M.M. Pacheco, R.F. Rodriguez, M. Govindappa, G. Santoyo. 2012. Isolation and molecular characterization of a novel strain of *Bacillus* with antifungal activity from the sorghum rhizosphere. *Genetics and Molecular Res.*, 67: 210-221.

Athukorala, Y., W.K. Jung, T. Vasanthan, Y.J. Jeon. 2006. An anti-coagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*.

Carbohydr. Polym., 66: 184-191.

Azokpota, P., P.L. Moller, J.D. Hounhouigan, M. Jakobsen. 2007. Biodiversity of predominant *Bacillus* isolated from *afitin*, *iru* and *sonru* at different fermentation time. *Int. J. Biol. Chem. Sci.*, 1(3): 211-222.

Biswas, S., S. Das. 2011. Peerless Hospital and B. K. Roy Research Centre, Dept. of Microbiology, Kolkata, WB/IN.

Ettoumi, B., A. Guesmi, L. brusetti, S. Borin, A. Najjari, A. Boudabous and A. Cherif. 2013. Microdiversity of deep-sea Bacillales isolated from tyrrhenian sea sediments as revealed by ARISA, 16S rRNA gene sequencing and BOX-PCR fingerprinting. *Microbes Environ.*, 28(3): 361–369.

Gomaa, O.M., O.A. Momtaz. 2006. 16S rRNA characterization of a *Bacillus* isolate and its tolerance profile after subsequent subculturing. *Arab J. Biotechnol.*, 10(1): 107-116.

Hill, K. K., O. Lawrence, L. Ticknor, J.J. Paul. 2004. Fluorescent amplified fragment length polymorphism analysis of *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* isolates. *Appl. Environ. Microbiol.*, 70(2): 1068-1080.

Hung, P.Q., K. Annapurna. 2004. Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.). Omonrice.12: 92-101.

Kang, S.M., J.Y. Min, Y.D. Kim, C.S. Karigar, S.W. Kim, G.H. Goo and M.S. Choi. 2009. Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. *J. Biotechnol.*, 139: 84-88.

Lisboa, M. P., D. Bonatto, D. Bizani, J.A.P. Henriques, A. Brandelli. 2006. Characterization of a bacteriocin like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic Forest. *Intl. Microbiol.*, 9: 111-118.

Manikandan, S., K. Muthuselvam. 2014. Isolation and characterization of phosphobacterial isolates from bhendi rhizosphere soils of Cuddalore district. *Indian Streams Res. J.*, 4(5): 87-93.

Matarante, A., F. Baruzzi, P. S.Cocconcelli and M. Morea. 2004. Genotyping and toxigenic potential of *Bacillus subtilis* and *Bacillus pumilus* strains occurring in industrial and artisanal cured sausages. *Appl. Environ. Microbiol.*, 70(9): 5168.

Moghaddam, M.R., S.B. Ravari and H. Rouhani. 2014. The nematicidal potential of local *Bacillus* species against the root-knot nematode infecting greenhouse tomatoes. *Biocontrol Sci. Technol.*, 24(3): 279-29.

Nagesh, M., R. Asokan and K.S. Mohan. 2005. Partial characterization of novel nematicidal toxins from *Bacillus cereus* and their effect on root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. *J. Biol. Control*, 19(1): 65-70.

Oliveira, A., J. Dobereiner J. Baldani. 2014. The effect of inoculating endophytic N2-fixing bacteria on micropropagated sugarcane plants. *Plant Soil*, 242: 205–215.

Parvathi, A., K. Krishna, J. Jose, N. Joseph and S. Nair. 2009. Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in Cochin, India. *Brazilian J. Microbiol.*, 40: 269-275.

Rangaswami, G., D.J. Bagyaraj. 1993. Agricultural Microbiology II Edition. Prentice Hall, India, 214p

Raton, T.M.O., R. Yano, O.R. Gamez, E.I.S. Floh, M.J.S. Diaz and H.R. Barbosa.

2012. Isolation and characterisation of aerobic endospore forming Bacilli from sugarcane rhizosphere for the selection of strains with agriculture potentialities. *World J. Microbiol. Biotechnol.,* 28: 1593–1603.

Reddy, D.M., D. Paul, H. K. Reddy and G. Reddy. 2009. Characterization and identification of *Bacillus cereus* GMHS: An efficient 2-picoline degrading bacterium. *Int. J. Integrative Biol.,* 5(3): 187.

Sarangi, N.P., W.G. Dilantha Fernando and Y. Khalid Rashid. Identification of antifungal antibiotics of *Bacillus* species isolated from different microhabitats using polymerase chain reaction and MALDI-TOF mass spectrometry. *Canadian J. Microbiol.,* 55(9): p 1029.

Sihem, Ben Maachia, R., Errakhi, R. Mathieu, M. Chérif, A. Lebrihi. 2011. Identification and partial characterisation of antifungal and antibacterial activities of two *Bacillus* sp. strains isolated from salt soil in Tunisia. *African J. Microbiol. Res.,* 13: 1599-1608.

Tamalika Sarangi. 2014. Utilization of antinemic or antimicrobial peptide genes associated with *Bacillus* spp. in the management of root knot nematode *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 on Tomato (*Solanum lycopersicum* Mill). Ph.D dissertation,Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. P.371.

Tendulkar, S.R., Y.K. Saikumari, V. Patel, S. Raghotama, T.K. Munshi, P. Balaram and B.B. Chattoo. 2007. Isolation, purification and characterization of an antifungal molecule produced by *Bacillus licheniformis* BC98 and its effect on phytopathogen *Magnaporthe grisea. J. Appl. Microbiol.,* 103: 2331–2339.

Vetrivelkalai, P., M. Sivakumar and E. I. Jonathan. 2010. Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in bhendi. *J. Biopesticides.,* 3(2): 452–457.

Wahyudi, A.T., J. P. Bramantyo and N. R. Mubarik. 2010. Diversity of antifungal compoundsproducing *Bacillus* spp. isolated from rhizosphere of soybean plant based on ARDRA and 16S rRNA. *J. BioSci.,* 17(3): 145-150.

Wang, W., M. Sun. 2008. Phylogenetic relationship between *Bacillus* species and related genera inferred from 16s rDNA sequences. *Brazilian J. Microbiol.,* 40: 505-521.

Wei, G., J.W. Kloepper, S. Tuzun. 2014. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathol.,* 86: 221–224.

Wulff, E.G., C.M. Mguni, K. Mansfeld-Giese, J. Fels, M. Lübeck and J. Hockenhull. 2002. Biochemical and molecular characterization of *Bacillus Amyloliquefaciens, B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris. Plant Pathol.,* 51: 574–584.

Xiao, Z., P. Xu. 2009. Acetoin Catabolism and Acetylbutanediol formation by *Bacillus pumilus* in a Chemically Defined Medium. *PLOS ONE,* 4(5): 5627-5635.

Xia, Y., S. Xie, X. Ma, H. Wu, X. Wang and X. Gao. 2011. The purL gene of *Bacillus subtilis* is associated with nematicidal activity. *FEMS Microbiol*

Lett., 322: 99–107.

Yap Chin Ann. 2012 . Screening for nematicidal activities of *Bacillus* species against root knot nematode (*Meloidogyne Incognita*), Research and Development Division, Malaysian Pepper Board. p 86.

Youcefali, M., N. K. Chaouche, L. Dehimat, I. Bataiche, M. Karaali, H. Cawoy and P.Thonart. 2014. Antifungal activity and bioactive compounds produced by *Bacillus mojavensis* and *Bacillus subtilis. African J. Microbiol. Res.*, 8(6): 476-484.

Zahner, V., A.C. Telles de Carvalho e Silva and I. de Filippis. 2013.Extended genetic analysis of Brazilian isolates of *Bacillus cereus* and *Bacillus thuringiensis. Mem Inst Oswaldo Cruz.*, 108(1): 65–72.

How to cite this article:

Tamalika Sarangi and S. Ramakrishnan. 2016. A Review on Identification of *Bacillus* spp. using Molecular Characterization. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 126-130.
doi: http://dx.doi.org/10.20546/ijcmas.2016.507.011