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Review Paper

Pesticidal prospectives of chitinolytic bacteria in agricultural pest management

A.R.N.S. Subbanna*, H. Rajasekhara, J. Stanley, K.K. Mishra, A. Pattanayak

ICAR-Vivekananda Institute of Hill Agriculture (VPKAS), Almora, Uttarakhand 263601, India

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ABSTRACT

Chitin metabolism is an essential life sustaining activity of phytophagous insects, phytopathogenic fungi and parasitic nematode which are the major limiting factors of agricultural production systems. Diverse bacteria, although non-chitinous life forms, are reported to degrade native chitin associated with the pestiferous organisms thereby exerting pathogenicity. So, the deployment of chitinolytic bacteria, associated genes and enzymes for plant protection against invading parasites and insect pests is well studied. Currently, worldwide research mainly focuses on finding novel strains and enzymes with potential implications in pest management. Owing to the effectiveness and synergistic potential, the putative chitinases and chitinolytic bacteria are formulated as biocontrol agents for direct application, utilized in the development of transgenics and supplemented with other pesticidal toxins. This fast progressing twig of pest management has the ability to replace hazardous chemical pesticides, if not so, can reduce their dosage. The present review critically discusses the available diversity of chitinolytic bacteria and the present status of pest management achieved through this approach. The possible levels of control and achievable synergism against major pest species are also presented in the context of latest research findings to understand subsistence pest management using bacterial chitinases.

1. Introduction

Chitin is the second most abundant, ubiquitous and renewable polysaccharide in nature, after cellulose (Shahidi and Abuzaytoun, 2005; Frederiksen et al., 2013). Chemically, it is an insoluble linear homopolymer of N-Acetyl-D-glucosamine residues linked by β 1–4 linkage. The biopolymer is a major constituent of the vital structures in majority of agriculturally important lower invertebrate taxa *viz.,* integument of arthropods, nematodes, and molluscs, the gut linings of insects, the cell walls of fungi and some algae and also the cysts of various protozoans (Flach et al., 1992). The distinctive role of this structural carbohydrate is to providing rigidity to the exoskeleton and act as a physical barrier against invading microorganisms. This prime importance of chitin in different biological functions of pest species makes it an ideal target in their strategic management.

The biological degradation of chitin is largely manifested by the catalytic action of hydrolytic enzymes; the chitinases (EC 3.2.1.14) which belongs to family 18, 19 and 20 glycosyl hydrolases. The chitinogenic organisms largely depend on chitinases for chitin catabolism. Interestingly, some non-chitinogenic organisms, despite lack of chitin, possess chitinolytic activity. However, recent studies reported the existence of chitinases in nearly all the life forms spanning from lower prokaryotes to higher plants, vertebrates (White et al., 1997) and even humans (Guan et al., 2009) and viruses (Gooday, 1995). The company of chitinolytic enzymes in these organisms confirms some additional functions spanning from nutrition to defense (Hamid et al., 2013).

Among the different reported microflora of non-chitinogenic chitinase producers, bacteria constitute a major biosystem for soil chitin degradation in the context of environmental recycling. Majority of soil dwelling bacteria have high levels of chitinolytic activity (Cody et al., 1990) which is mainly used by them in the utilization of chitin degradation products as nutritional resources (Frederiksen et al., 2013). On the other hand, the importance of chitinases in biological control of agriculturally important plant pathogenic fungi, nematodes and insect pests has become an emerging field of research (Ajit et al., 2006), in the present context of environmental degradation by injudicious use of pesticide for management of pests to sustain crop yields. Most of the pesticidal chitinases are derived from soil inhabiting bacteria like *Bacillus*

* Corresponding author.
E-mail address: subbanna.ento@gmail.com (A.R.N.S. Subbanna).

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(Thamthiankul et al., 2001; Jung et al., 2002; Wen et al., 2002; Liu et al., 2010; Prasanna et al., 2013), *Pseudomonas* (Lee et al., 2000; Lee and Kim, 2015; Zhong et al., 2015) and *Streptomyces* (Tanabe et al., 2000; Gongora et al., 2001; Singh and Chhatpar, 2011). The potential application of these enzymes come from their established direct toxicity to a variety of plant pathogenic fungi (Prasanna et al., 2013; Brzezinska et al., 2014), nematodes (Hallmann et al., 1999; Jung et al., 2002; Kalaiarasan et al., 2006) and synergism with Cry toxins in the control of phytophagous insect pests (Ding et al., 2008).

The omnipresence of the substrate, its degrading enzymes and practical utility of both in agriculture and industrial sectors leads to the worldwide exploration studies on chitinolytic organisms. Among them, bacteria and associated chitinases represent a diversified and practically applicable group of bio-entities for the management of agriculturally important and chitin containing pestiferous organisms. The present review mainly discusses existing diversity and recent advances in their use in pest management of insect pests, pathogenic fungi and parasitic nematodes.

2. Existing variability of chitinolytic bacteria

Microorganisms are the most abundant (Whitman et al., 1998) and diverse (Torsvik et al., 2002; Venter et al., 2004) life forms available on the earth. Chitinolytic bacteria comprise only 4% of the total heterotrophic bacteria (Swiontek et al., 2008). Although, the phylotypic richness and diversity of soil bacterial community is highly dependent on existing ecosystem processes (Schimel et al., 2007; Terahara et al., 2009) and anthropogenic interventions (Yasir et al., 2009; Someya et al., 2011; Nampally et al., 2015), the members of Gammaproteobacteria and Bacilli represent the culturable diversity of chitinolytic bacteria especially in chitin-enriched soils (Das et al., 2010). This type of ecosystem and environment dependent diversity is true for chitinolytic bacteria as well (Brzezinska and Donderski, 2006; Kamil et al., 2007; Terahara et al., 2009). The two major factors, soil type and pH play an important role in shaping the bacterial chitinases composition of given habitat (Terahara et al., 2009). However, the best predictor for abundance of chitinolytic bacteria was soil pH (Fierer and Jackson, 2006). Gooday (1997) reported an average of 2–10% bacteria from soil and natural waters are chitinolytic by production of chitinases. Further, similar studies reported only 1% of cultural bacteria can utilize chitin as their nutrient source (Kamil et al., 2007). Chitinolytic bacteria are highly varied group of chitin degrading candidates in diverse environments. They include the majority of known bacterial classes *viz.,* Gammaproteobacteria (primarily designated by *Serratia* and *Stenotrophomonas*) and Firmicutes (primarily designated by *Paenibacillus* and *Bacillus*) (Someya et al., 2011).

Even though, chitin is one of the most abundant polymers in nature, only diminutive studies represent the number, diversity and function of chitinases produced by microorganisms (Gohel et al., 2006). To date, studies have reported bacterial chitinase diversity within terrestrial ecosystems such as upland grassland (Metcalfe et al., 2002), rhizosphere (Kamil et al., 2007), alkaline soils (Tsujibo et al., 2003), sandy soils (Williamson et al., 2000), pastures (Krsek and Wellington, 2001; Metcalfe et al., 2002), arable soils (Terahara et al., 2009), Antarctica (Xiao et al., 2005), maize fields (Ikeda et al., 2007), vermicompost (Yasir et al., 2009) and subglacial lakes of north western Indian Himalayas (Yadav et al., 2015; Avupati et al., 2017). Bacterial chitinases have also been widely retrieved from aquatic ecosystems (Kirchman and White, 1999; Cottrell et al., 2000; Ramaiah et al., 2000; Teplyuk et al., 2017). All these studies adopted either culture dependent or independent methods for chitinase gene and chitinolytic bacteria diversity. In majority of the culture dependent estimates (Nampally et al., 2015; Yadav et al., 2015), members belonging to *Bacillus*, *Paenibacillus*, *Serratia*, *Sporosarcina*, *Exiguobacterium* were found to be most predominant in soils of diverse environments (Reyes-Ramírez et al., 2004; Huang et al., 2005; Aktuganov et al., 2008; Singh et al., 2009; Terahara et al., 2009; Someya et al., 2011). Contrasting to this regular phenomenon, interesting report of absence of chitinases activity in *Bacillus* sp. is also reported (Metcalfe et al., 2002). Studies on lake waters and rhizosphere showed predominance of *Aeromonas*, *Flavobacterium* (Donderski and Brzezińska, 2001; Brzezinska and Donderski, 2006) and *Pseudomonas* members (Nielsen and Sorensen, 1999; Haas and Defago, 2005), respectively, as culturable bacteria. Different species of *Stenotrophomonas* (*S. maltophilia*, *S. geniculata*, *S. rhizophila* and *S. hibiscicola*) are also found to be ubiquitous and dominant in chitin rich soils over other pseudomonads (Palleroni, 2005; Someya et al., 2011). Members of actinobacteria *viz., Arthrobacter, Micromonospora* and *Streptomyces* were also reported to play a significant role in soil chitin degradation as evidenced from predominant chitin bait colonization (Spiegel et al., 1987; Vionis et al., 1996; Williamson et al., 2000; Gomes et al., 2000, 2001; Metcalfe et al., 2002; Yasir et al., 2009; Terahara et al., 2009). However, it is reversed for coastal environments (Cottrell et al., 2000). Interestingly, *Streptomyces* sp. are reported to contain family 19 chitinases (Itoh et al., 2003; Yasir et al., 2009). Proteobacteria are also reported to be major contributors of chitinases (LeCleir et al., 2004; Xiao et al., 2005). It should not be ignored that the genera *Erwinia, Aeromonas*, *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Microbacterium* were also reported as chitin degraders but in less abundance (Someya et al., 2011).

Major *Bacillus* with potential chitinase production includes *B. amyloliquefaciens* (Sabry, 1992; Wang et al., 2002), *B. cereus* (Trachuk et al., 1996; Pleban et al., 1997; Chang et al., 2007), *B. circulans* (Watanabe et al., 1992; Wiwat et al., 1999; Chen et al., 2004), *B. licheniformis* (Waldeck et al., 2006), *B. megaterium* (Sabry, 1992), *B. pabuli* (Frändberg and Schnürer, 1994), *B. stearothermophilus* (Sakai et al., 1994), *B. subtilis* (Wang et al., 2006; Chang et al., 2010) and different subspecies and strains of *B. thuringiensis* (Liu et al., 2002; Reyes-Ramírez et al., 2004; Driss et al., 2005; de la Vega et al., 2006; Liu et al., 2010; Usharani and Gowda, 2011).

The isolates belonging to *S. marcescens* had a special mention in chitinolytic activity (Someya et al., 2011) by being the most effective bacterium for chitin degradation (Ohno et al., 1996). It is noteworthy that this bacterium produces a variety of chitinolytic enzymes and chitin binding proteins when cultured in the presence of chitin (Ohno et al., 1996; Watanabe et al., 1997). Studies clearly showed that *S. marcescens* produces at least three chitinases (ChiA, ChiB, ChiC), a chitobiase and a putative chitin-binding protein (Ohno et al., 1996; Tews et al., 1996; Brurberg et al., 1996, 2001; Watanabe et al., 1997). This chitinolytic machinery of *S. marcescens* is of great interest because it is one of the best characterized chitinolytic machineries known till date (Brurberg et al., 2001).

It is known that only 1% of total bacterial species in nature are culturable (Amann et al., 1995). Cottrell et al. (2000) hypothesized a great variation between cultured and uncultured bacterial chitin degradation. However, their results supported chief occurrence of Alphaproteobacteria in both libraries. Some other clones were also reported to be similar but not identical to culturable Gammaproteobacteria, which might be the result of acquisition *via* horizontal gene transfer (Xiao et al., 2005). Studies also proposed the existence of novel uncultured strains and associated chitinases (Terahara et al., 2009).