Available online at www.sciencedirect.com

ScienceDirect

Procedia Technology 19 (2015) 965 – 971

Procedia Technology

8th International ConferenceInterdisciplinarity in Engineering, INTER-ENG 2014, 9-10 October 2014, Tirgu-Mures, Romania

Influence of chitinous waste on soil bacterial community: biofertilizer effect and antifungal activity

Djamel Eddine Aïzi[a,*], Ben Amar Cheba[a]

[a]*Department of Biothechnology, Faculty of Nature and life Scineces, University of Scince and Technology of Oran Mohamed Boudiaf, BP1505 Al Mnaour, Oran 31000, Algeria.*

Abstract

Today, the chitinous wastes are used by several companies to produce chitin and chitosan, which are used in various biotechnological applications, particularly in the agricultural sector which is a key sector for sustainable development. Improving the quality of the plant production requires an improvement in its rhizospheric microbial composition , which called Plant Growth Promoting Rhizobacteria (PGPR), the role of these microorganisms not only the improving the quality of nutrients present in the rhizosphere, but also assure the plant protection against phytopathogenic agents.

The objective of this work is to study the effect of chitinous waste on Triticum durum (wheat) rhizospheric microflora, by mixing (W/W) the wheat soil with chitinous waste at different concentration. Twentieth days of Monitoring, showed a significant effects on the soil microflora and pH. The results of the first ten days of treatment revealed in elevation in bacterial number with Bacillus genus dominance and absolute absence of fungi even saprophytic or phytopathogenic, whereas the results of the last ten days were characterized by the diversity of wheat rhizospheric microflora with appearance of Plant Growth Promoting Rhizobacteria such as N2 fixing bacteria and those involved in plant protection.

Peer-review under responsibility of “Petru Maior” University of Tirgu Mures, Faculty of Engineering

Keywords: marine chitinous waste; microflora; Triticum durum; microbial dynamics; rhizosphere.

* Corresponding author. Tel.: +213-775-670-709.
E-mail address: aizi_djameleddine@hotmail.fr

Peer-review under responsibility of “Petru Maior” University of Tirgu Mures, Faculty of Engineering
doi:10.1016/j.protcy.2015.02.138

1. Introduction

Chitin is the second most abundant polysaccharide exists in nature after cellulose [1]. About 1011 tons are produced every year in only the aquatic biosphere [2, 3]. These large quantities of chitin are linked to its major role in the terrestrial and marine ecosystems. It is the major component of fungi, insects exoskeleton and krill shellfish

[4]. Chitin's structure reminds cellulose's. Formally, in literature chitin indicates "animal cellulose" because of their structural similarity; the only difference is that chitin is an amino polysaccharide having acetamide groupes in C2 instead of hydroxyle group which is composed of N-acetylglucosamine long chains linked with β (1 →4) bonds arranged in antiparallel (α) in parallel or (β), which make its degradation difficult [5]. Enzymes responsible of the degradation and modification of chitin are chitinases that are produced generally by bacteria of which a small part only is identified until today like Actinobacteria (e.g. Streptomyces sp.) [6], β-proteobacteria (e.g. Burkholderia sp.) [7] and γ-proteobacteria (e.g. Xanthomonas sp.) [8]. These bacteria are involved in the chitin degradation process in a direct way (active agent) or indirectly (passive agent) by secreting of biofilm [4, 9].

Beside the fundamental role of chitin in ecosystems, it represents an important interest in biotechnology. Indeed, many applications are possible; In-situ microorganisms that have a chitinolytic activity are used as antifungal against plant pathogenic fungi. [6], while chitinous wastes can be used in enzyme and saccharide compounds production [10, 11].

2. Material and methods

2.1. Biological material

Chitinous wastes: shrimp shells were ground using a coffee grinder.
Chitosan: was prepared according next protocol [12, 13, 14].

2.2. Chitin and chitosan extraction

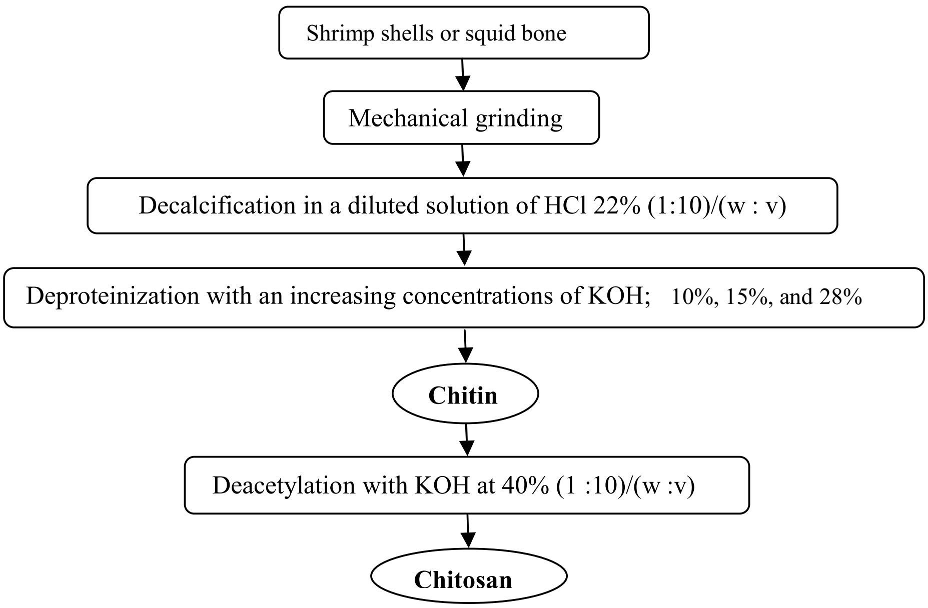

Fig. 1. Chitinous compounds extraction process

2.3. Chitinous wastes biofertilizer effect

Biofertlilizer effect is defined by the influence of chitinous wastes on the pH and microphlora of wheat rhizosphere (microflora and pH). Seven chitinous wastes mixtures were tested in pots containing 100g of soil (Table 1). All tests were repeated three times and in every pot 10 wheat caryopses were deposited. Microflora and pH changes of rhizosphere were assessed every 5 days during 20 days.

Table 1. Chitinous waste/soil mixtures concentrations in each pot.

	Test1	Test2	Test3	Test4	Test5	Test6	Test7
Quantity of soil [g]	100	100	100	100	100	100	100
Quantity of Chitinous waste [g]	0.6	1.2	1.8	2.4	3.0	3.6	4.2

2.4. Chitinous wastes antifungal activity

Chitinous wastes antifungal activity was performed on wheat phytopathogenic fungus Pyrenophora graminea using two tests:

Antifungal test: infected wheat leaves by Pyrenophora graminea were put on demeniralised chintinous waste agar (DCA medium) and others on potato dextrose agar (PDA medium) was incubated for one week at room temperature.

Antagonism test: isolated bacteria from chitinous wastes treated soil were cultivated on PDA medium with the fungus Pyrenophora graminea and then incubated at room temperature for one week.

3. Results and discussion

3.1. Chitinous waste effect on soil microbial dynamics

During the first phase that spreads on a period of 2 weeks; three phenomena were observed: first, a total elimination of the fungi were observed (fig 2).this result was correlated with the obtained by Abd el krim et al 2006 who reported that chitinous wastes exert an inhibitor effect on fungi [15]. The second phenomenon is bacteria predominance. And finally, an important pH decrease were noted, in 10 days pH decreased from 8,8 to 7,6 (Fig. 3).

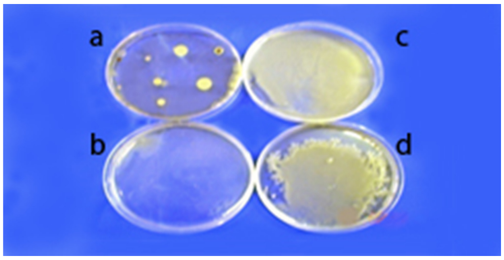

Fig. 2. Effect of chitinous wastes on wheat microflora number and diversity; a) Non-treated soil, b,c,and d) were soils treated with 0,6; 1,2 and 2,4g of chitinous wastes respectively.

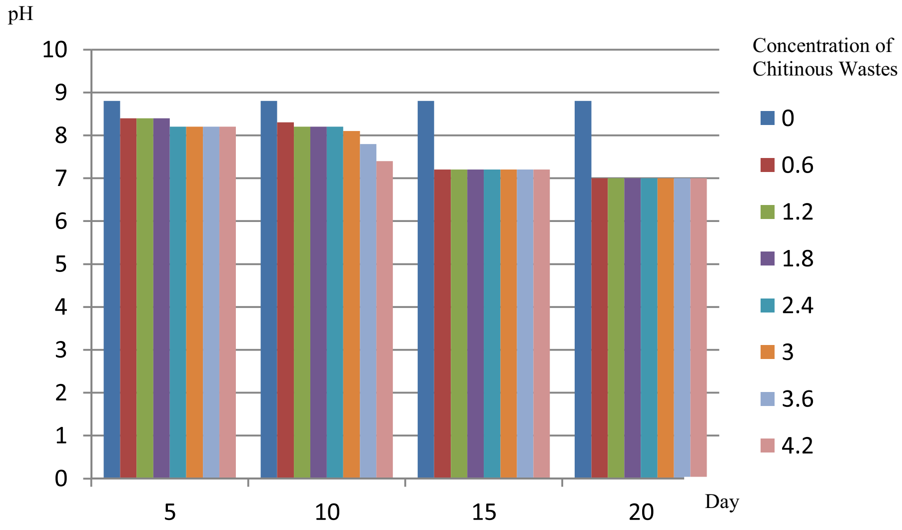

Fig. 3. Soil pH variation according to the mexture (soil/chitinous wastes) concentrations.

According to Mustin et al. (1987), the first composting phase of chitinous wastes is a degradation steps, it is charactirized by a high bacterial activity [16]. From a concentration [2,4] there is a positive correlation between bacterial presence levels dominated by the genus Bacillus, and the concentration of chitinous wastes. Several authors noticed that Organic fertilizer induced an important increase of bacterial existence [17, 18, 19], while the thrust presence of Bacillus is due to its chitinoulytic power. Cody et al. (1989) reported that 78% of Bacillus bacteria isolated from chitin enriched soil had a chitinoulytic activity [20].

Dupuy et al. noticed that the uniquity of chitinoulytic microflora in different ecosystems facilitate the composting of chitinous residues, for that several species of Bacillus are used as biofertilizer [21]. In this context, the most known Bacillus species to have this potential are: B.subtilis, B.liquefaciens, B.fluorescens, B.licheniformis. In 2005, Kokalis-burelle et al. reported that Bacillus sp is involved in chitinous wastes degradation [22].

The second phase begins from the 15th day of the experience. It is characterized by two phenomena: the first is the stabilization of pH at 7.1 (fig.3). The second is the change of the kind of the dominant microflora and the apparition of a diversity of mucosal bacteria (fig n 4). The microscopic observation after Gram staining showed a diversity of bacterial shaped (Fig.5).

Fig.5a shows a rod shaped bacteria named Dexia s. it has the capacity to fix atmospheric nitrogen. Fig 5b shows another bacteria of another genus i.e. Beijerikia sp, it is bacterial cells that have a pear shape, it produces globular lipid reserves de poly-β-hydroxybuturate. (Fig 5 d and e) shows two different forms of Azotobacter sp. (e) being the vegetative form and (d) the cystic form. Fig 5c shows a cell of Azospirillum sp. a stringy cell characterized by it capacity to fixe atmospheric nitrogen in a free manner.

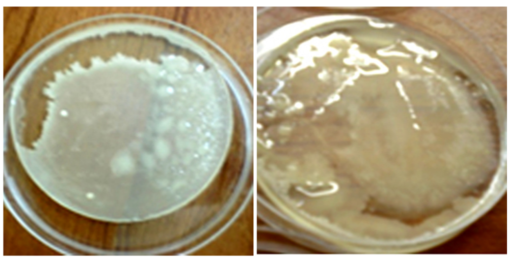

Fig. 4. Mucoid bacteria grown on nutrient agar medium.

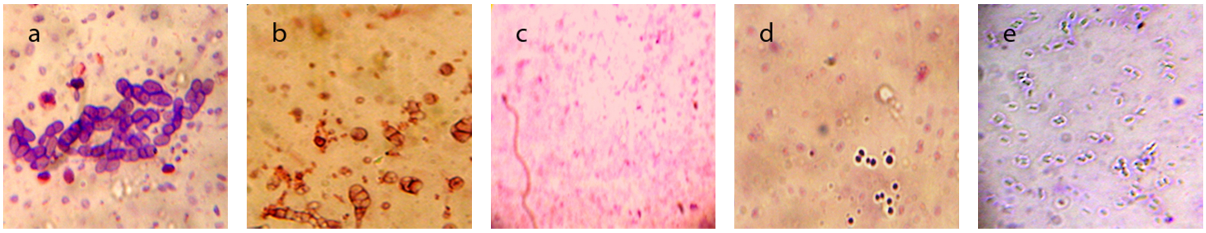

Fig. 5. Microscopic aspects of some wheat rhizobacteria from chitinous wastes treated soil: Mucoid bacteria susceptible genera, a: Derxia sp.; b: Beijerinkia sp.; c: Azospirillium sp.; d and e: were Azotobacter sp. cystic and vegetative forms, respectively.

According to Doroty et al. (2002) Azospirillum sp., Azotobacter sp., Beijerinkia sp. and Bacillus sp., are classified as plant growth promoting rhizobacteria (PGPR) [23], these genera are able to fix nitrogen in a free manner in the presence of a carbon source, like cellulomonas sp. that can ensure the nitrogen fixation by degrading wheat straw cellulose. Keeling et al. 1998 reported that nitrogen fixation potential in diazotroph bacteria increase in the presence of carbohydrate source [24]. In this study , by the mean of Bacillus , chitinous wastes represents an assimilable carbon source by nitrogen fixative microorganisms such as Azospirillum sp., Azotobacter sp., Beijerikia sp., and Derxia sp.

Steenhoudt et al. (2000) reported that among PGPR group, Azospirillum is the rhizosphere bacteria that have the most considerable effect on plant growth [25]. It occurs frequently associated to wheat plant roots. The same author noticed that the interaction between plant roots and Azospirillum is characterized by four aspects: natural habitat, roots interaction, nitrogen fixation and plant growth hormones biosynthesis. Keeling et al. 1998 reported the positive effect of nitrogen fixation on harvest [24].

Fig. 6. Chitinouse wastes biofertilizing effects: a) wheat root development; a: non-retreated soil, b: chitonous waste treated soil; b) wheat stems development: a non-treated; b, c, treated soil with 0,6 and 3.0 g of chitnous wastes concentration.

A massive germination with an invasive and important hairy root development was noticed in Pot b, treated with 3.0 g of chitinous wastes, compared to Pot a (control) (Fig. 6a). Pot c1 (Fig. 6b) showed a better growth of the wheat plantlets characterized with deep green leaves compared with the untreated pot (a1) and pot b1 at 0.6 g chitinous wastes, where the growth was low and the leaves were yellowish green.

Fraser et al. (1978) noticed the nitrogenase activity and its correlation with the biosynthesis of glutamin to obtain a better harvest quality [26]. Margaret et al in 2002, reported that pH affects nitrogenase activity of witch optimal is obtained at pH 7-7,5 [27]. This could have a relation with pH change noted in the study. Indeed, rhizosphere pH varied from basic ph 8,8 to neutral pH 7,1 (Fig 3) during the second phase of composting. Other studies demonstrated that it is not only pH that affect nitrogenase activity. According to Shekar et al. (2007) the roots exudates of green plants affect also on the microbial composition of the rhizosphere [28].

3.2. Chitinous wastes antifungal activity

Concerning Infected leaves by the fungus Pyrenophora Graminea, on PDA medium a propagation of the mycelium was observed. While the propagation was totally inhibited on DCA medium Fig. 8. This confirms that chitinous wastes have an inhibitory effect on phytopathogenic fungi.

3.3. Antifungal activity of micoid bacteria isolated from CW treated soil

Fig. 7a the total fungal growth inhibition on DCW medium chowen, it improve the effectiveness of DCW as fungal biocontrol agent, this may be due to the presence of antifungal chitooligosaccharides.

Fig. 7b. mucoid bacteria isolated from wheat treated soil exhibit strong antagonistic effect against phytopathogenic fungi Pyrenophora Graminea that has infected the wheat plantlet in the untreated soil. This, it may be due to the secretion of antibiotic antifungal compounds or enzymes that attack fungal cell wall components. Thus, chitinous wastes soil treatment stimulates the bacteria having a role in soil fertilization and inhibits plant pathogenic fungi and this by the induction of systemic resistance (ISR) and / or systemic acquired resistance (SAR) [29].

Fig. 7. a) Antifungal effect of chitinous wastes; a1: fungal invasive growth in GN medium (control); a2: total fungal growth inhibition on DCW medium (test); b) Antagonistic effect of micoid bacteria against phytopathogenic fungi.total fungal inhibition surrounding the mucoid colony.

4. Conclusion

Chitinous wastes present a very important biofertilizer source. In addition to their antifungal power they activity the presence of PGPR that have a role in plant growth regulation and the plant self-defense induction. The realized work permetted to improve that the chitinous waste addition in soil leads to a microbial dynamism by the elimination of phytopathogenic fungi and enhance of the Bacillus bacteria presence in the first phase, followed by a dominance of nitrogen fixating bacteria in the next phase. This change in the microbial composition of the rhizosphere of Triticum durum wheat has meant that the quality of the plant will be better. Improving the quality of the plant is linked to the increased accumulation of the reserves, elimination of phytopathogenic fungi, stimulation of the secretion of growth hormone, secretion of antimicrobial metabolites and reduced phytotoxic microbial communities in the rhizosphere.

References

[1] Theuruvathil KS, Sethumadhavan S, Paruthapara TM. Study ln the production of chitin and chitozan from shrimp shell by using Bacillus subtilis fermentation. Carbohydrate Research; 2007. 26:2423-2429.

[2] leCleir GR, Buchan A, Maurer J, Moran MA, Hollibaugh JT. Comparison of chitinolytic enzymes from an alkaline hypersaline lake and an estuary. Environ Microb; 2007. 9:197–205.

[3] Muzzarelli RA. Native, Industrial, and fossil chitin; 1999. 87:1–6.

[4] Gomes RC, Semedo LT, Soares RM, Linhares LF, Ulhoa CJ. Purification of a thermostable endochitinases from *Streptomyces RC1071* isolated from a cerrado soil and its antagonism against phytopathogenic fungi. J Appl Microbiol; 2001. 90:653–661.

[5] Jung W J, Jo G H, Kuk, J H, Kim Y J, Oh K T, Park W R D. Production of chitin from red crab shell waste by successive fermentation with lactobacillus paracaseibKCTC-3074 and Serratia marcescens FS-3. Carbohydrate Polymers; 2006. 68:746-750.

[6] Hjort K, Bergström M, Adesina M, Jansson J, Smalla K. Chitinases genes revealed and compared in bacterial isolates, DNA extracts and a metagenomic library from a phytopathogen-suppressive soil. FEMS Microbiol Ecol; 2009. 71:197–207.
[7] Ogawa K, Yoshida N, Kariya K, Ohnishi C, Ikeda R. Purification and characterization of a novel chitinase from *Burkholderia cepacia strain KH2* isolated from the bed log of Lentinus edodes, Shiitake mushroom. J Gen Appl Microbiol; 2002. 48: 25–33.
[8] Yamaoka H, Hayashi H, Karita S, Kimura T, Sakka K. Purification and some properties of a chitinase from *Xanthomonas sp*. *strain AK*. J Biosci Bioeng; 1999. 88:328–330.
[9] Gooday GW. The ecology of chitin degradation. Adv Microb Ecol; 1990. 11:387–430.
[10] Horn SJ, Sikorski P, Cederkvist JB, Vaaje-Kolstad G, Sørlie M. Costs and benefits of processivity in enzymatic degradation of recalcitrant polysaccharides. Proc Natl Acad Sci USA; 2006. 103:89–94.
[11] Han SJ, Park H, Lee SG, Lee HK, Yim JH. Optimization of cold-active chitinase production from the Antarctic bacterium, *KOPRI 21702*. Appl Microbiol Biotechnol; 2011. 89:613–621.
[12] Synowiecki J, Sikorski Z, Nacz KM. Immobilisation of amylases on krill chitin. Food chem; 1982. 8:239-246.
[13] Monreal J, Reese E T. The chitinase of Serratia marcescens. Can. J. Microbiol; 1969. 15:689-696.
[14] Kurita K. Controlled functionalization of the polysaccharide chitin, Prog. Polym. Sci; 2001. 26:1921-1971.
[15] Abd-El-kreem F, Nehal S, El-Mougy N, El-Gamal G, Fotouh Y O. Use of Chitin and Chitosan against Tomato root rot Disrase under Greebhouse Conditions. Research Journal of Agriculture and Biological Scince; 2006. 4:147-152.
[16] Mustin M. le compost : gestion de la matiere organique. 2eme Ed., François Dubusc. Paris France, 1989. 954p.
[17] Nur O, Seluck G, Yuksel T. Effect of organing manure application and solarization on soil microbial biomass and enzyme activities under green jouse conditions. Biology of Agriculture and Horticulture; 2006. 23:305-320.
[18] Dinesh R, Dubey R P, Ganeshamurthy A N, Prasard G S. organing manure in rice-based cropping system: Effect on soil microbial biomass and selected enzyme activities. Current Scince; 2000. 79:1716-1720.
[19] Appireddy G K, Saha S, Mina B L, Kundu S, Selvakumar G, Gupta H S. Effect of organic manures and integrated nutrient management on varieties and soil properties. Agronomic Soil Scince; 2008. 24:127-137.
[20] Cody R M. Distribution of chitinase and chitobiase in Bacillus. Current microbiology; 1989. 19:201-205.
[21] Dupuy A. Hydrolyse enzymatique de la chitine et de son produit déacétylé le chitosane effectuée par des actinomycètes isolés à partir de différent sols canadiens. mémoire de M.Sc. université de Sherbrooke, 1991. 173p.
[22] Kokalis-burelle N, Kloepper, J W, Reddy M S. Plant growth-promoting ehizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Applied Soil Ecology; 2005. 31: 91-100.
[23] Dorothy M, Alan H. Notrogenase of range of diasotrophic bacteria on straw breakdown products and related compounds. SoilnBiology and Biochemistry; 2002. 23:987-998.
[24] Keeling A, Cook J A, Wilcox A. Effects of carbohydrate application on diazotroph population and nitrogen availability in grass swards established in garden waste field conditions. Applied Soil Ecology; 1998. 45:71-77.
[25] Steenhoudt O, Vanderleyden J. Azospirillum. A free-living nitrogen-fixing bactrrium closely associated with grasses. Microbiology Reviews; 2000. 24:487-506.
[26] Fraser J B, Graham L T. Activity of nitrogenase and glutamine synthetase in relation to availability of oxygen in continuous cultures of a strain of cowpea Rhizobium sp. Supplied with excess ammonium. Biochimica et Biophysica Acta; 1978. 538:406-416.
[27] Margaret M R, Neil A S. Straw decomposition and nitrogenase activity (C2H2 reduction) by free-living microorganism. Soil Biology and Biochemistry; 2002. 23(3): 275-283.
[28] Shekhar C N, Sangeeta M D. Screening of plant Growth-Prometing Rhizobacteria. Advanced Techniques in Soil Microbiology; 2007. 11:363-375.
[29] Liu W, Zeng H, Liu Z, Yang X, Guo L, Qui D. Mutational analysis of the Verticilium dahlia protein elicitor PevD1 identifies distinctive regions responsible for hypersensitive response and systemic acquired resistance in tobacco. Microbiological Research; 2014. 169:5-6.