B. Krishnaveni et al /J. Pharm. Sci. & Res. Vol. 7(4), 2015, 197-205

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Extraction and Characterization of Chitin and Chitosan from *F.solani CBNR BKRR*, Synthesis of their Bionanocomposites and Study of their Productive Application

B. Krishnaveni [1] and R.Ragunathan[2]
[1] *Ph.D Research Scholar,* [1] *Department of Biotechnology,*
Maharaja Co-education Arts and Science College, Perundurai, Erode, India-52,

[2] *Senior Scientist (R&D), Synkromax Biotech Private Limited, Chennai, India-16,*

Abstract
Chitin and chitosan have versatile biological activities and chemical applications and as such have been found to be immensely popular as biomedical addictives. *F.solani CBNR KKRR,* isolated from the marine soils of Pichavaram, Tamil Nadu was used for the economic production of Chitin and Chitosan using three different media-Sabouraud sucrose broth, Hesseltine and Anderson medium, Andrade et al medium. The polysaccharides were extracted by alkali-acid treatment, and characterized by infrared spectroscopy. The highest growth rate was with Andarde et al medium with a mycelial dry weight of 12.59 g/L. The best yields of the polysaccharides (mg per gram of dry mycelia biomass) are obtained with Sabouraud sucrose broth for chitosan (56.39 mg/g or 5.6%) and for chitin (769 mg/g or 76%) From the SEM image, Chitin AgNP's exhibited smaller powder particle size. Ag/Chitosan BNCs show strong needle shaped structures. The EDS spectrum of *F.solani CBNR BKRR* CH AgNP composite shows the peaks of C K, O K, Cl K and Ag L. The atomic ratio of was found to be 83:13:1.04:2.44 wt %. The EDS spectrum of *F.solani CBNR KRRR* CS AgNP composite shows the peaks of C K, O K, and Ag L. The atomic ratio of was found to be 24.67:16.46:58.87 wt %.The antibacterial activity of Chitin and Chitosan solution was found to be less than the Bionanocomposites indicating that the presence of the silver ion thereby increases the antibacterial strength of the polysaccharides. Chitin AgNP showed 98.5% dye inhibition in 72 hours and Chitosan AgNP showed 93.5% inhibition.

Keywords- *F.solani*, Chitin, Chitosan, Pichavaram, FTIR, SEM, EDS, antibacterial activity, Methylene blue

INTRODUCTION

Chitin, a homopolymer of N-acetyl-D-glucosamine (Glc-NAc) residues linked by ß-1-4 bonds, is the most widespread renewable natural resource following cellulose [1]. The main source of chitin is crustacean waste, which is also the main cell wall material in most fungi [2]. Chitin and its derivatives have high economic value owing to their versatile biological activities and agrochemical applications [3].The natural antibacterial and/or antifungal characteristics of chitosan and its derivatives have resulted in their use in commercial disinfectants. Both chitin and chitosan have been shown to activate the defence system of a host and prevent the invasion of pathogens [4]. From a technological point of view, it would be quite profitable to recover the by-products released from seafood processing because of its richness in compounds of high value added such as chitin products.

Infrared spectroscopy can be used to investigate the composition of a chitin sample [5]. Due to its simplicity, relative instrument availability, and independence of sample solubility, IR spectroscopy is one of the most studied methods for characterisation of chitin and chitosan [6].

The chemical properties of Ag nanoparticles (Ag NPs) are significantly different from those of silver ingot or Ag ion, and thus Ag NPs have been studied by many researchers due to their wide variety of potential applications [7]. The special and unique properties of Ag NPs can be attributed to their smaller size and the larger specific surface area relative to bulk materials, and many preparation processes have been proposed for controlling the physical and/or chemical characteristics of Ag NPs.

Most dyes used in the pigmentation of textiles, paper, leather, ceramics, cosmetics, inks and food-processing products are derived from azo dyes, which are characterised by the presence of one or more azo groups (-N=N-) in their structure [8]. Approximately 15% of the dyes produced worldwide are lost within waste water during synthesis and processing. This waste represents a great hazard to human and environmental health due to the toxicity of azo dyes [9]. Hence an efficient system for degradation of these dyes is required which is eco-friendly.

The present paper aims to investigate chitin and chitosan production using *F.solani CBNR KRRR* grown in three different traditional culture media, synthesis of their bionanocomposites , comparative of the antibacterial activity of the polysaccharides and AgNP's against MDR pathogens and their ability to degrade the dye-methylene blue.

MATERIAL AND METHODS

Isolation and characterization of Marine fungus

Collection of Samples

Pichavaram (Lat.11°428'E; Long.79°798`E), Cuddalore (dt) of Tamil nadu is home to the second largest Mangrove forest in the world, is rich in *Avicennia officinalis, Rhizophora mucaronata, Acanthus illicifoliu* and *Excoecaria agallocha* plants. Marine Mangrove sediments were collected from rhizosphere as well as non rhizosphere region of various parts of Pichavaram. The surface layer of the sediment was removed and the central portions of sediments were transferred into sterile plastic bags.

Isolation of fungi

The samples were taken separately for serial dilution. Ten grams of sample was suspended in 90 ml of sterile distilled water. The suspension was considered as 10^{-1} dilution. About 0.1 ml of the serially diluted sample was spread over the Potato Dextrose Agar (Potato Infusion 200, Dextrose 20, Agar 15 g/L) pH was adjusted to 5.6 ± 0.2.The medium was supplemented with 20 µg ml^{-1} Ciproflaxin to minimize the fungal and yeast contaminations respectively . After inoculation, the plates were incubated in an inverted position for 5-7 days at 25 ± 2^0C.

Microscopic Observation

The fungal isolates were observed using hand lens and the colony morphology was recorded with respect to color, shape, size and nature of colony. Fungal isolates were microscopically characterized by Lactophenol Cotton Blue mounting. The cell morphology was recorded with respect to spore chain morphology, hyphae and mycelium structure.

Isolation and Identification of Test Fungus.

Individual fungal colonies were picked and further purified by subculturing on potato dextrose agar medium. Further identity of fungus was confirmed by nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing using ABI-Big Dye Termintor v3.1 Cycle Sequencing Kit in the ABI 3100 automated sequencer by National Fungal Culture Collection of India (NFCCI), Pune, India. ITS region was amplified by using universal fungal primer set, (Forward Primer) 5'- GACTCAACACGGGGAAACT-3' and (Reverse primer) 5'-AGAAA GGAGG TGATC CAGCC-3'. Polymerase chain reaction amplified regions were sequenced. The analysis of nucleotide sequence was done in Blast-n site at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) .The alignment of the sequences was done by using CLUSTALW (www.ebi.ac.uk/clustalw).

Extraction and characterization of Chitin and Chitosan

Culture medium

F.solani CBNR KRRR was grown, for chitin and chitosan production, in three different culture media: a) Sabouraud sucrose (SS broth)-(bacteriological peptone (10 g) and sucrose (20 g) per litre of distilled water, pH 5.7); b) Hesseltine and Anderson (HA medium)- (glucose (40 g); asparagine (2 g); chloridrate of thiamine (0.05 mg); potassium phosphate (0.50 g) and magnesium sulphate (0.25 g) per litre of distilled water, pH 5.2); c) Andrade et al.(AD medium) (2000)- (glucose (60 g); asparagine (3 g); chloridrate of thiamine (0.08 mg); potassium phosphate (0.50 g) and magnesium sulphate (0.25 g) per litre of distilled water, pH 5.1)

Microbiological methods

Growth profile. The sporangioles of *F.solani CBNR KRRR* were harvested from cultures grown for seven days at 28ºC on Petri dishes containing PDA medium. A suspension was prepared and adjusted to 10^8 sporangioles/mL, using a hematocytometer for counting. For fungal submerse cultivation, 10 mL sporangioles suspension (10^8sporangioles/mL) were inoculated in Erlenmeyer flask of 1000 mL containing 290 mL of culture media, and the flasks were incubated at 28ºC in an orbital shaker at 150 rpm, during 96 hrs. The mycelia were harvested, washed twice in distilled and deionised water by filtration, utilizing a silkscreen nylon membrane (120 F), and were submitted to lyophilization process. After lyophilization the biomass was maintained in a vacuum dissecator until constant weight.

Chitin and chitosan extraction. The process of extraction involved deproteination with 2% w/v sodium hydroxide solution (30:1 v/w, 90ºC, 2 hrs), separation of alkali insoluble fraction (AIF) by centrifugation (4000 rpm,15 min), extraction of chitosan from AIF under reflux (10% v/v acetic acid 40:1 v/w, 60ºC, 6 hrs), separation of crude chitin by centrifugation (4000 xg, 15 min) and precipitation of chitosan from the extract at pH 9.0, adjusted with a 4 M NaOH solution. Crude chitin and chitosan were washed on a coarse sintered-glass funnel with distilled water, ethanol and acetone and air-dried at 20ºC (Franco et al. 2004).

Chitin and chitosan characterization

Infrared spectroscopy (Deacetylation degree – DD %).

The degree of deacetylation for microbial chitin and chitosan were determined using the infrared spectroscopy using the absorbance ratio A1655/A3450 and calculated according to equation : A (%) = (A1655/A3450) x 100 / 1.33

Two milligrams sample of fungal chitin and chitosan, which had been dried overnight at 60ºC under reduced pressure were thoroughly blended with 100 mg of KBr, to produce 0.5 mm thick disks. The disks were dried for 24 hrs at 110ºC under reduced pressure. Infrared spectrometer was recorded with a Bruker 66 Spectrometer, using a 100 mg KBr disks for reference. The intensity of maximum absorption bands were determined by the baseline method.

Preparation, characterization of Chitin Bionanocomposites

Preparation of AgNPs

Briefly, 0.50 g of silver-containing glass powder was dispersed in 50 mL of an aqueous solution of 0.25, 1, or 4.0 wt% glucose in a 100 mL glass vial. The mixture was at 121° C and 200 kPa for 20 min. The mixture was then gradually cooled to room temperature and centrifuged at 3000 rpm for 10 min. The supernatant containing the Ag NP suspension was removed and stored in the dark at 4° C.

Preparation of Ag NP/ Chitin Composites.

In this study, 10 mg of chitin (<5% DAc) was added to 1 mL of each Ag NPs suspension (about 60 μg/mL). The mixture was mixed well (at pH 7.0) on a shaker for 30 min. The insoluble Ag NP/chitin composites were centrifuged at 6000 rpm for 10 min. The centrifuged composites were washed twice with distilled water by centrifugation at 6000 rpm for 10 min. The washed composites were dried up at 70ºC on a blockheater for 2 h.

Preparation, characterization of Chitosan Bionanocomposites

Preparation of silver-chitosan nanocomposites

A solution of chitosan (1 - 3 mg/ml) in acetic acid solution (1 - 2 %) was first prepared. Due to the poor solubility of chitosan, the mixture was vortexed to achieve complete dissolution, and then kept overnight at room temperature. The solution was filtered through a 0.22 µm millipore syringe filter to remove any impurity before use. Silver-chitosan nanocomposites were obtained by chemical

reduction of the silver salt to yield the corresponding zero valent silver nanoparticles with $NaBH_4$. To ensure complete reduction, the concentration of $NaBH_4$ was 10 times that of the silver salt. The silver nanoparticles were separated by centrifugation at 15000 rpm and dried at 60 ºC for 24 h on a Petri dish, yielding a thin layer.

Characterization of nanoparticles

Scanning electron microscope (JEOL/EO, JSM-6390, Japan, magnification range 1500, acceleration voltage 20 kv) was used to evaluate the surface and shape characteristics of the particles after prior coating with gold. Elemental film composition was analyzed using Energy Dispersive Spectrometer (JEOL, JED-2300) at SAIF, Kochi, India. UV-VIS absorption spectra of the samples were recorded in the wavelength range of 300 to 500 nm using UV spectrophotometer (UV-Visible Perkin ElmerLambda) at the Center for Bioscience and Nanoscience Research, Coimbatore, India.

Comparative Study-Antimicrobial Activity of the polysaccharides and their bionanocomposites

Preparation of Chitin and Chitosan Solution

Chitin and Chitosan solution 1% (w/v) was prepared in 1% (v/v) acetic solution. The chitosan solutions and chitin suspensions were stirred overnight at room temperature, and the chitosan solutions were filtered using miracloth to remove potential impurities. Then solutions were then diluted by physiologic serum (0.9% NaCl solution) to get final concentration of 0.1% (w/v^{-1}). pH of the solution was adjusted to 5.5 by addition of 2M NaOH and the solutions were autoclaved at 121º c for 20 mins.

Antimicrobial Activity

The antibacterial activity of the polysaccharides and nanoparticles was evaluated against *E.coli, S.aureus, C.albicans and K.pneumoniea* by the agar diffusion method with Mueller Hilton agar as the medium. The four microbial cultures were procured from Department of Microbiology, Maharaja Co-education Arts and Science College, Erode. An aliquot of polysaccharide solution and nanoparticle dispersion (10 µl) was added into each of two wells in a plate, and then incubated for 24 h at 37°C. Amoxicillin was used as reference standard.

Photo catalytic Degradation of Dye

Typically 10mg of Methylene Blue dye was added to 1000 mL of double distilled water used as stock solution. About 10 mg of synthesized Chitin and Chitosan Nanoparticles were added to 100 mL of dye solutions. A control was also maintained without addition of silver nanoparticles. Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30 mins to clearly make the equilibrium of the working solution. Afterwards, the dispersion was put under the sunlight and monitored from morning to evening sunset. At specific time intervals, aliquots of 2-3 mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at the different wavelength. Concentration of dye during degradation was calculated by the absorbance value at 660 nm.

Percentage of dye degradation was estimated by the following formula:

$$\% \text{ Decolourization} = 100 \times [(C_0 - C)/ C_0]$$

Where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

RESULTS

Morphological identification of the fungal isolates obtained from the soil sample

The isolated fungi were purified by repeated sub-culturing on the Potato Dextrose Agar medium at regular intervals and incubating at 29°C. The isolates were identified based on the colony morphology, microscopic observation and molecular identification (Collier *et al.*, 1998; St-Germain, G and R. Summerbell, 1996) .The identification was done based on 18S rRNA gene sequencing. The 18S rRNA sequences of the isolates were compared with the data present in NCBI. The BLASTn of the isolates was showing 99% homology with *Fusarium* spp. The sequence was submitted to the Gene Bank under the name *Fusarium solani CBNR BKRR* and is awaiting accession number.

Microscopic Observation

The fungal isolates were observed and the colony morphology was recorded with respect to color, shape, size and nature of colony. Fungal isolates were microscopically characterized by Lactophenol Cotton Blue mounting. The cell morphology was recorded with respect to spore chain morphology, hyphae and mycelium structure.

Fusarium sps have cottony flat appearance on the surface and have reddish pigmentation. The reverse side of the fungi is cream in colour. Microscopic identification of the fungus showed hyaline septate hyphea and distinct macroconidia. These species showed the presence of Chlamydospores.

Extraction and characterization of Chitin and Chitosan

Biomass Production

The growth of the fungus *F.solani* in three different media was observed for 14 days at RT. The highest growth rate was with Henderson and Anderson medium with a mycelial dry weight of 14 g/L while the next highest growth rate was observed with Andarde *et al* medium 12.59g/L followed by Sabouraud sucrose broth with a mycelia dry weight of 7.89 g/L.

Chitin and Chitosan Extraction

The best yields of the polysaccharides (mg per gram of dry mycelia biomass) are obtained with Sabouraud sucrose broth for chitosan (56.39 mg/g or 5.6%) and for chitin (769 mg/g or 76%) In addition, the next best yield of chitin and chitosan per 1 g of biomass from *F.solani* are obtained using Henderson and Anderson medium and Andrade *et al.* medium for chitin 116.4 mg/g or 11% and 34.15 mg/g or 3% and chitosan 45 mg/g or 4% and 43.09 mg/g or 4% respectively.

Chitin and chitosan characterization

Infrared spectroscopy

In this study, the IR spectra of the three isolated samples of chitin and chitosan were analyzed and compared with the IR spectrum of commercial chitosan (Fig. 1-8).

The presence of bands at 3442 cm^{-1} in chitin samples from SS broth, 3308 cm^{-1} in HA Medium and 3426 cm^{-1} from

AD medium and in the extracted Chitosan samples from SS broth at 3442 cm^{-1}, HA medium in 3416 cm^{-1}, AD medium in 3386 cm^{-1} indicate strong dimeric OH stretch. Chitin from SS broth have spectra in the Amide I region 1657 cm^{-1}, those from HA medium showed spectra at 1657 cm^{-1} and those from AD medium showed spectra at 1642 cm^{-1} , while Chitosan from SS broth have bands at 1640 cm^{-1}, HA medium at 1631 cm^{-1},AD medium at 1641 cm^{-1} indicating presence of C=C stretch. The peaks around 1558 cm^{-1} in chitin samples from SS broth, 1561 cm^{-1} in HA medium and 1555 cm^{-1} in AD medium and bands around 1559 cm^{-1} in Chitosan samples from SS broth, 1557 cm^{-1} in HA medium and 1554 cm^{-1} in AD medium are due to stretching vibrations of C-O group (Amide II). Amide III region presence was indicated by bands at 1378 cm^{-1} in chitin samples from SS broth, 1375 cm^{-1} in HA medium and 1381 cm^{-1} in AD medium. Chitosan samples from SS broth showed bands at 1414 cm^{-1}, HA medium at 1412 cm^{-1} and Andrade et al medium at 1406 cm^{-1} indicating aromatic C-C stretch. Commercial Chitin samples showed similarity with the extracted samples by exhibiting bands at 3473 cm^{-1}, 1653 cm^{-1}, 1560 cm^{-1} and 1380 cm^{-1}. Similar results were obtained with commercial Chitosan samples which revealed bands at 3429 cm^{-1}, 1651 cm^{-1} and 1417cm^{-1}.

Deacetylation degree – DD %

In the present study, chitin and chitosan from *F.solani CBNR BKRR* grown in Sabouraud sucrose broth was found to have 22% DD and 60% DD, respectively. Chitin and Chitosan grown in Hesseltine and Anderson medium were found to have 80% DD and 40% DD. Andarde medium provided chitin and chitosan with 53% DD and 45% DD.

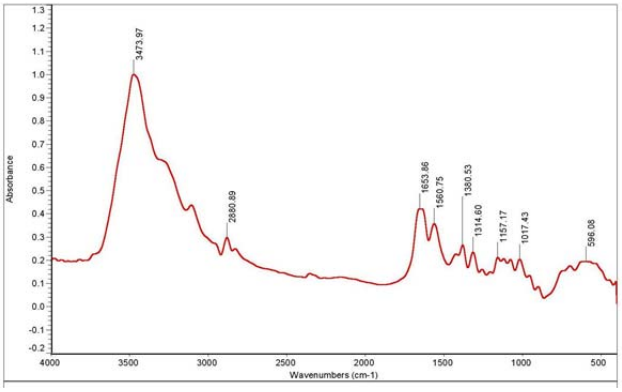

Fig.1 FTIR Spectrum of Commercial Chitin

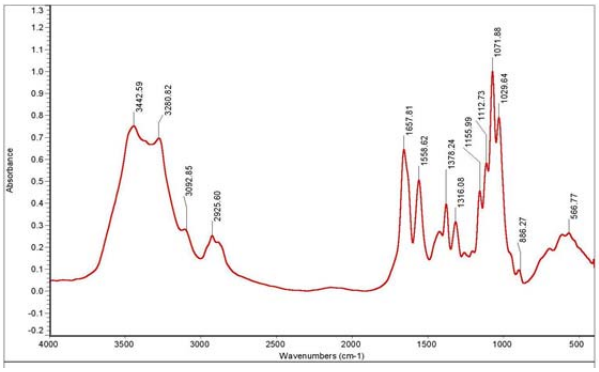

Fig. 2 FTIR Spectrum of Chitin extracted from using Sabouraud Sucrose broth

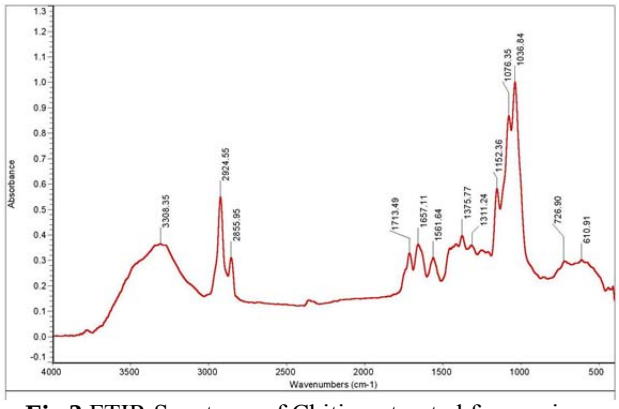

Fig.3 FTIR Spectrum of Chitin extracted from using Henderson and Anderson medium

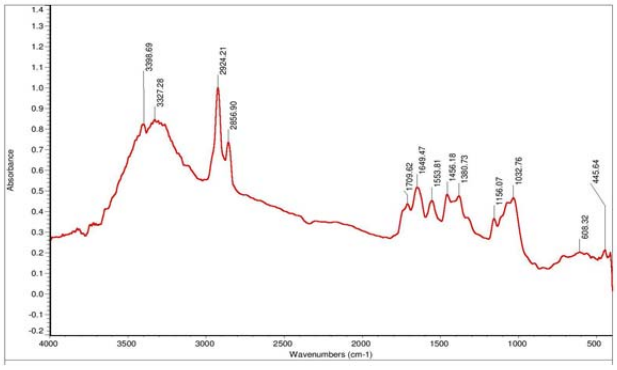

Fig.4 FTIR Spectrum of Chitin extracted from using Andarde et al medium

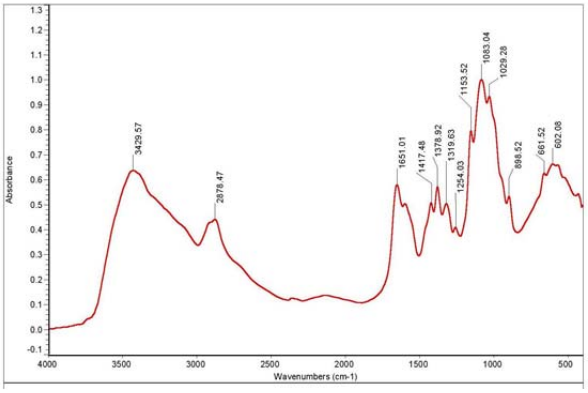

Fig.5 FTIR Spectrum of Commercial Chitosan

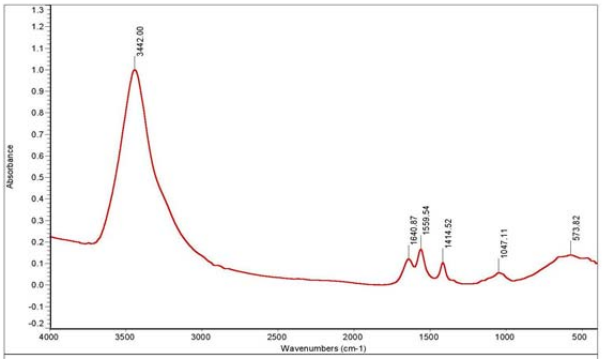

Fig.6 FTIR Spectrum of Chitosan extracted using Sabouraud Sucrose broth

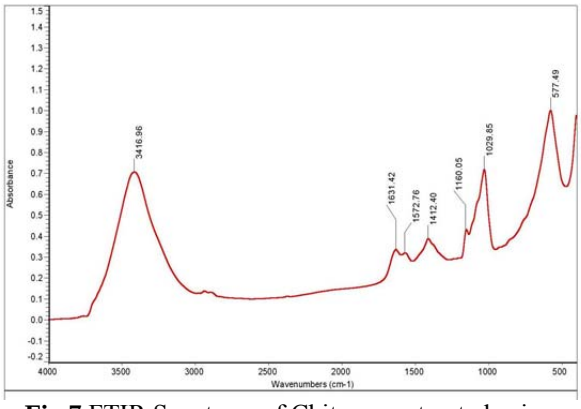

Fig.7 FTIR Spectrum of Chitosan extracted using Henderson and Anderson medium

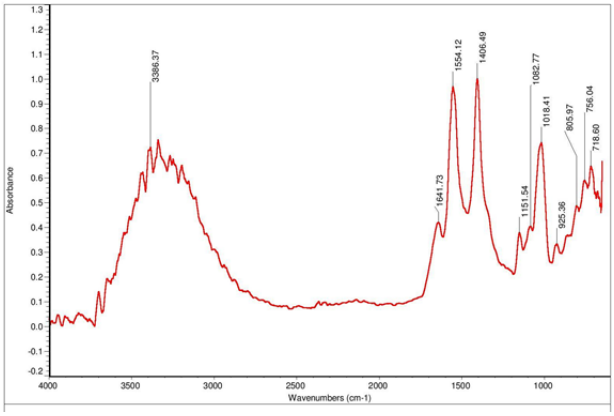

Fig.8 FTIR Spectrum of Chitosan extracted using Andarde et al medium

Fig.9 Bionanocomposites synthesized from Chitin and Chitosan extracted from *F.solani*

Synthesis of Bionanocomposites from Extracted Polysaccharides

Visual Appearance

Nanotechnology is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. Since the highest yields for both the polysaccharides were obtained from Sabouaraud Sucrose broth, the same were used for synthesis of bionanocomposites. Chitin (<5% DAc) was added as stabilizer to the AgNPs suspensions to remove the generated caramel and to prevent agglomeration and precipitation of the AgNPs. The composites so formed were twice with water to remove the caramel. The composites were brown coloured and indicated that surface plasmon vibrations, typical of silver nanoparticles. Similarly, addition of $NaBH_4$ leads to reduction of $AgNO_3$ whereby chitosan is added as stabilizer for synthesis of AgNP's. The AgNP's so produced are dark brown in colour (Fig.9)

UV-VIS Spectroscopy

Chitin Bionanocomposites

The UV-Vis spectra were recorded for the supernatants of the post-reaction mixtures in which chitin reacted with the AgNP. The peak was observed at 390 nm in case of the *F.solani CBNR KRRR.* (Fig.10)

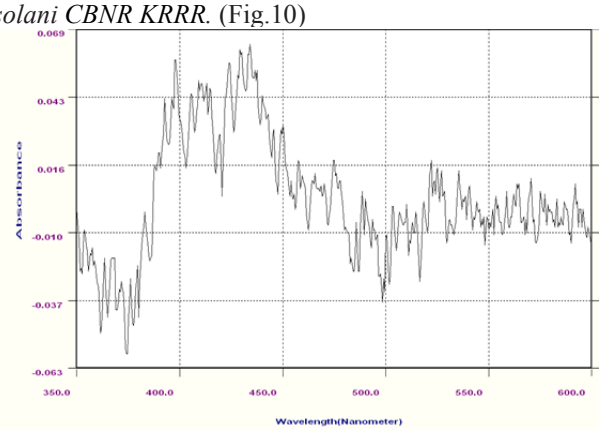

Fig.10 UV-Visible Spectroscopy of Chitin AgNP

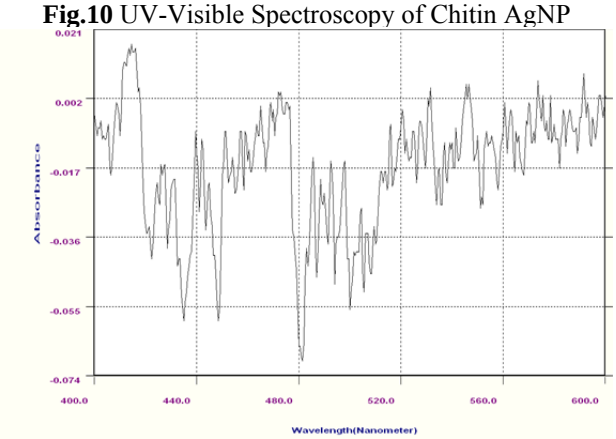

Fig.11 UV-Visible Spectroscopy of Chitosan AgNP

Chitosan Bionanocomposites

During the $NaBH_4$ reducing process, color of the $AgNO_3$ / Chitosan suspensions changed from colorless to brown. The color changes due to the formation of Ag NPs are proven by UV-visible spectra. After adding $NaBH_4$, the maximum absorbance bands for *F.solani CBNR BKRR* CS AgNP were detected at 420 nm respectively. (Fig.11)

Surface topography by Scanning Electron Microscopy

Chitin Bionanocomposites

The structural morphology of Chitin bionanocomposite was characterized by Scanning Electron Microscope. The samples were prepared by taking thin sections with a scalpel blade. The sections were platinum sputtered in vacuum. Chitins AgNP's exhibited smaller powder particle size indicating that they are well dispersed. The pore size was reported to be in the range of 5-10 µm. (Fig.12)

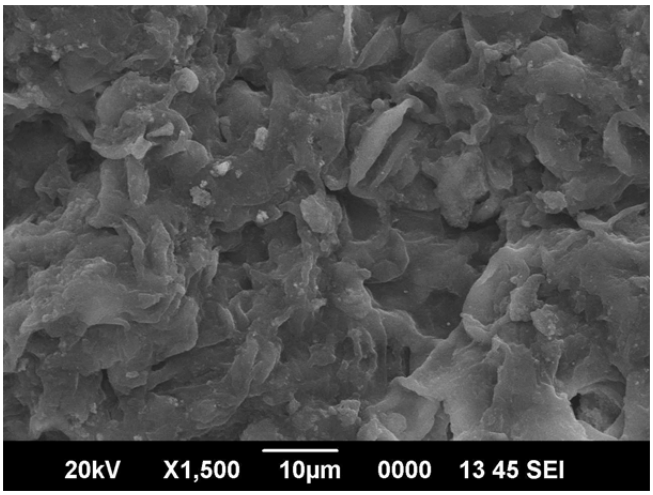

Fig.12 SEM Image showing the porous surface of Chitin AgNP

Chitosan Bionanocomposites

Surface morphology of polymer and Ag BNC's are illustrated in Figure 14. The samples for SEM analysis were prepared by solvent casting on petridish. From the SEM image, Ag/Chitosan BNCs showed strong needle shaped structures.

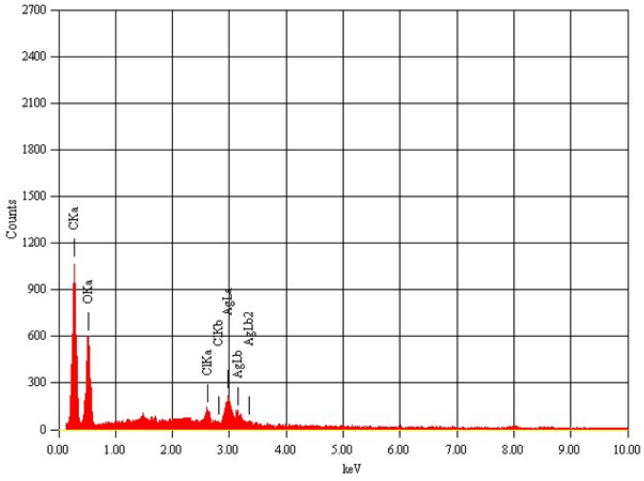

Fig.13 EDX analysis graph for Chitin AgNP

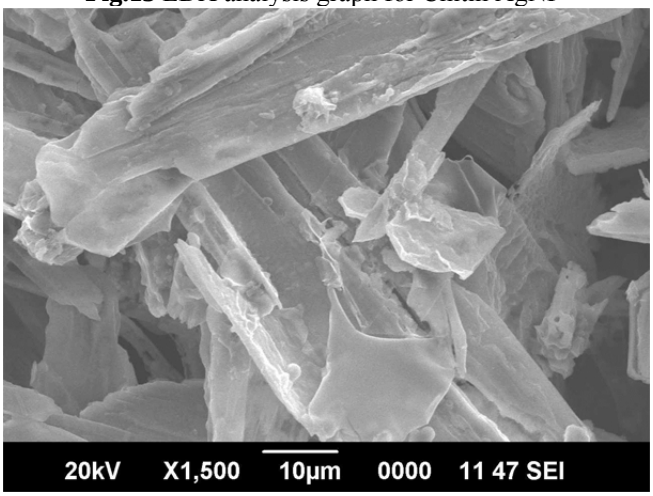

Fig.14 SEM Image showing the needle shaped surface of Chitosan AgNP

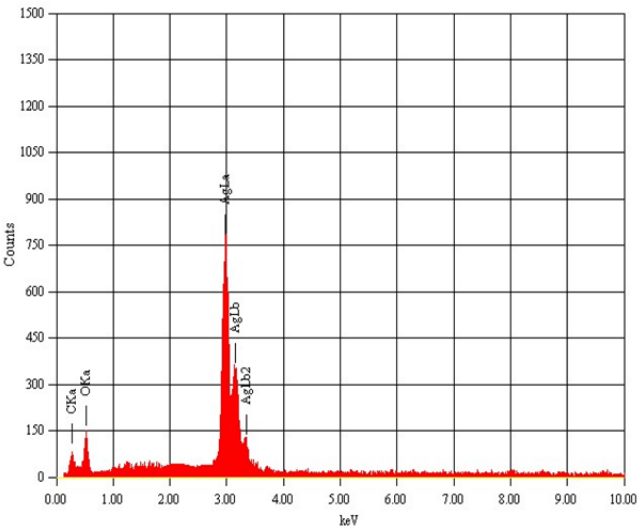

Fig.15 EDX analysis graph for Chitosan AgNP

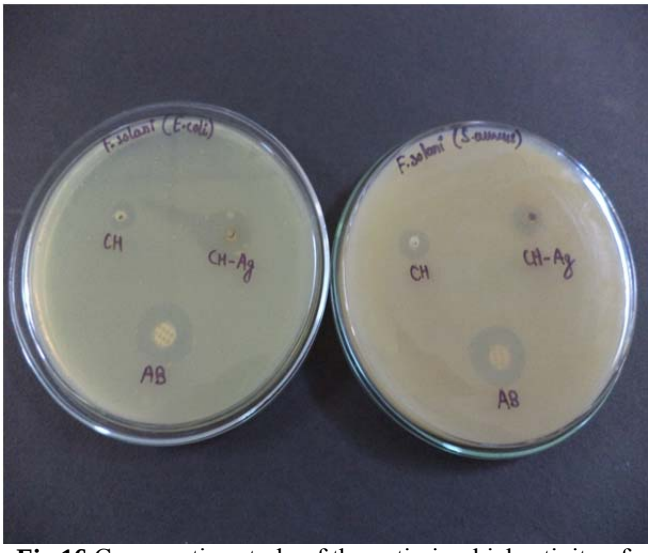

Fig.16 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites

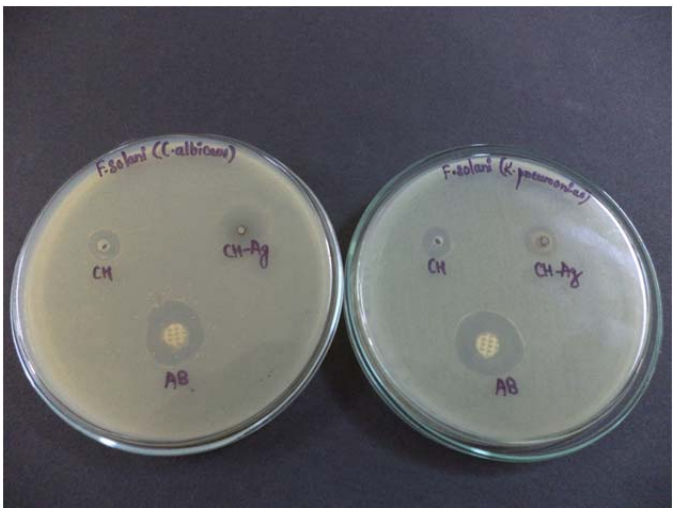

Fig.17 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites

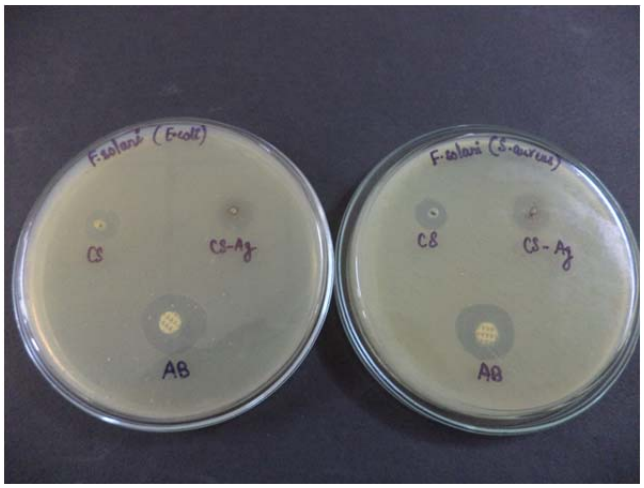

Fig.18 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites

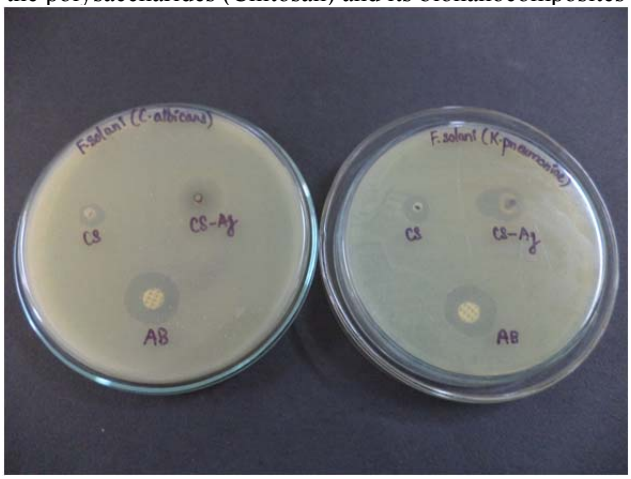

Fig.19 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites

Elemental Composition Analysis

Chitin Bionanocomposites

Energy-dispersive Spectroscopy (EDS) analysis was performed on JEOL, JED-2300. Thin section of scaffold was placed on carbon tape coated stub. The sample was then platinum coated. The EDS spectrum of *F.solani CBNR BKRR* CH AgNP composite shows the peaks of C K, O K, Cl K and Ag L. The atomic ratio of was found to be 83:13:1.04:2.44 wt %. (Fig.13)

Chitosan Bionanocomposites

The EDS spectrum of *F.solani CBNR KRRR* CS AgNP composite shows the peaks of C K, O K, and Ag L. The atomic ratio of was found to be 24.67:16.46:58.87 wt %. This represents a very good adsorption by the large surface area of paramagnetic Ag, with good stability and high storage of the chitosan layer. (Fig.15)

Disk Diffusion Assay

Inhibition zone values were obtained from the polysaccharides solution and the synthesized Ag NPs tested against Gram-negative bacteria (*E. coli and K.pneumoniea*) and Gram-positive (*S. aureus and C.albicans*). Figure (16-19) illustrate the images of each inhibition zones for the samples for antibacterial activity studies. Results of the inhibition zones are presented as average values in mm in the Table (1, 2).

The table shows that the Ag NPs had high and similar antibacterial activity against Gram-positive and Gram-negative bacteria. Due to their particle size, Ag NPs can easily reach the nuclear content of bacteria by disrupt the membranes of bacteria. The particle size smaller than 10 nm interact with bacteria and generate electronic effects that improve the reactivity of Ag NPs. The antibacterial activity of Chitin solution was found to be less than the Chitin AgNP's indicating that the presence of the silver ion thereby increases the antibacterial strength of the polysaccharides. Chitin AgNP's showed comparable antibacterial strengths as the antibiotic disks (Amoxicillin) employed. Similar results were obtained for Chitosan solution and Chitosan AgNP's with comparable antimicrobial activity to the antibiotic disk used. The antimicrobial activity of chitosan is described to be associated with molecular weight, degree of acetylation, concentration of chitosan and bacterial inoculum size was described.

Table.1.Antimicrobial Activity of the Polysaccharide solution (Chitin-*F.solani*) and the Bionanocomposites against MDR pathogens (in mm)

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitin Solution	**8**	**10**	**9**	**9**
Chitin AgNP	**15**	**9**	**10**	**16**
Antibiotic	**7**	**2**	**20**	**17**

Table.2.Antimicrobial Activity of the Polysaccharide solution (Chitosan- *F.solani*) and the Bionanocomposites against MDR pathogens (in mm)

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitosan Solution	**10**	**10**	**10**	**8**
Chitosan AgNP	**11**	**12**	**19**	**15**
Antibiotic	**20**	**18**	**16**	**16**

Photocatalytic Degradation of Dye

Visual Observation

Photocatalytic degradation of methylene blue was carried out by using AgNP's synthesized from the bionanocomposites synthesized from Chitin and Chitosan under solar light. Dye degradation was initially identified by color change. The color of dye shows blue color changed into light blue after the 4 h of incubation while exposed to solar light .Thereafter light blue was changed into light sheen of blue. Finally, the degradation process was completed at 72 h and was identified by the change of reaction mixture to colorless.

UV-Vis Spectrophotometer

Photocatalytic activity of AgNP's synthesized from the extracted Chitin and Chitosan on the degradation of dye was demonstrated by using the dye methylene blue, at different time in the visible region. The absorption spectrum showed the decreased peaks for methylene blue at different time intervals. The percentage of degradation

efficiency of Chitin and Chitosan AgNP was calculated to be 98.5% and 93.5% after 72 hrs. (Figure 20).

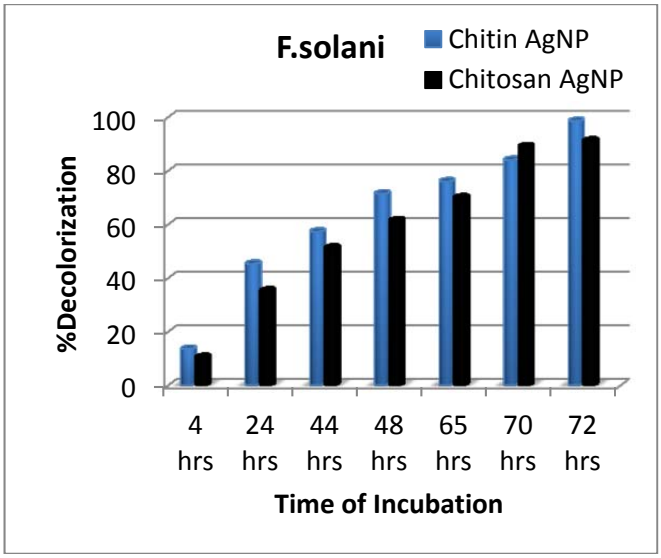

Fig.20 Dye degradation using Chitin and Chitosan AgNP's- *F.solani*

DISCUSSION

For fungal community analyses, PCR-based techniques are most powerful and generally used. The 18S rRNA gene (rDNA) and internal transcribed spacer (ITS) region are used widely as molecular markers for fungi, through the exploitation of both conserved and variable regions, and a large number of sequences are available in the data bank [11]. Various PCR primer sets targeting 18S rDNA and ITS region are available for assessing fungal diversity in soil DNA.

Chitosan can be produced in higher quantities by mass culturing the fungi by employing large scale fermentation techniques. Since supplies of seafood waste are seasonable and variable, the use of alternative sources for chitosan, mainly fungi, is of major benefit. Besides it is an ecofriendly and cost effective technique as compared to the chemical methods used for extraction of chitosan from crustaceans. By optimising the culture conditions, better quality chitosan can be produced which can be used for medical or food applications.

Natarajan *et al*., 2001 reported that the maximum biomass for the three fungal strains *Aspergillus niger* MTCC 872 (19.8 g/L), *Aspergillus niger* MTCC 2208 (20.8 g/L) and *Aspergillus niger* MTCC 1785 (17.3 g/L) was seen in the runs performed with the Growth Medium III at 120 hrs [12].

Thayza *et al*., 2007 reported the best yield of chitin and chitosan per 1 g of biomass from *C. elegans* (UCP 542) are obtained using yam bean medium and Sabouraud sucrose medium for chitin 400.9 mg/g and 261.7 mg/g and chitosan 58.9 mg/g and 34.9 mg/g, respectively [13].*C. elegans* (UCP 542) grown in malt medium with glucose 2% during 96 hrs, shows average biomass production of 4.8 g/L. This result is similar to the reported by Synowiecki and Al-Khatteb (1997) which obtained a yield biomass of *Mucor rouxii* grown in yeast extract and glucose 2% medium, for 48 hrs, to the 4 g, per litre of medium[14].

The characterization of chitin and chitosan obtained from *C. elegans* in yam bean medium by infrared spectra are similar to those reported in the literature [15]. The most significant parts of chitin and chitosan spectra are those showing the amide bands at approximately 1665, 1555, 1313 cm^{-1} , which could be assigned to the C = O stretching, the N-H deformation in the CONH plane and the CN bond stretching plus CH wagging. In a similar way, chitin from *C. elegans* shows bands in the amide II region, which was 1153, 1378 and 1558 cm^{-1}.

The UV-VIS spectra of Ag NPs in suspension and spectra of the supernatants of the postreaction mixtures in which various amounts of chitin reacted with the AgNPs was reported. Vinh *et al* 2013 reported peak at 390.5 nm which representative of the spherical Chitin AgNP's used in their work [16]. Honary *et al* (2011) reported similar results with peaks in the range of 400-420 nm which is typical of surface Plasmon band indicating formation of Silver nanocomposites with Chitosan [17].

Sowmya *et al* (2010) reported that SEM images of the β-chitin/ nBGC composite scaffold were found to have porous structures with smooth surface morphology. The pore size of β-chitin/nBGC composite scaffold is in the range of 10-20 μm [18]. Bin Ahmad *et al* (2012) stated that the SEM image of Ag/Cts BNC's in their work showed show layered surfaces with small flakes [19]. Similar results were suggested by Sowmya et al (2010) for the EDS spectrum of β-chitin/nBGC composite scaffold shows the peaks of Ca, P, Si and O. The atomic ratio of Si:Ca:P:O was found to be 29:13:8:48 wt %. The EDX spectra for the CS Ag BNCs had confirmed the presence of elemental compounds in the CS and Ag NPs without any impurity peaks. All the samples tested for EDX were coated with gold to prevent the accumulation of static electric fields during imaging. The Ag BNCs film morphologies were dependent on several factors including polymer solubility, solvent evaporation, total thickness, molecular weight and surface composition.

Koide S.S., (1998) stated that chitin and chitosan *in vitro* showed antibacterial and anti-yeast activities [20]. One of chitosan derivatives, i.e., N-carboxybutyl chitosan, was tested against 298 cultures of different pathogenic microorganisms that showed bacteriostatic and bactericidal 16 activities, and there were marked morphological alterations in treated microorganisms when examined by electron microscopy according to Muzzarelli R, *et al*., 1990 [21].

Vihn *et al.,* 2013 suggested that For Ag NP/chitin composites, spatial restriction due to the chitin was expected to prevent or weaken the interaction between microorganisms and Ag NPs.When Ag NPs adsorbed onto chitin particles interact with microorganisms, the interaction may increase with the increasing number of Ag NPs in the composites. This is supported by the present experimental results showing a relative relationship between antimicrobial activity and the amount of AgNPs added to the cultures as Ag NP/chitin composites [16].

A study of the mechanism of the reaction process of silver nitrate with chitosan by using FTIR also showed possible interaction between silver salts and chitosan molecules, which may account for the reduction of Ag ions and stabilization of silver-chitosan nanoparticles. Thus the attachment of silver to the nitrogen atoms in chitosan reduced the vibration intensity of the N-H bond due to increased molecule weight after silver binding.

Vanaja et al, 2014 reported similar results for degradation of methylene blue by nanoparticles synthesized from *Morinda tinctoria*. Absorption peak for methylene blue dye was centered at 660 nm in visible region which diminished and finally it disappeared which indicates that the dye had been degraded. The percentage of degradation efficiency of silver nanoparticles was calculated as 95.3% at 72h. The degradation percentage was increased as increasing the exposure time of dye silver nanoparticles complex in sunlight [22].

CONCLUSION

Fungi are abundantly available sources for the production of industrially important secondary metabolites. These results present an economically viable methodology for production of the polysaccharides-Chitin and Chitosan from marine fungi. Further results have been provided for cost effective synthesis of bionanocomposites from both the polysaccharides which have potential application as antibiotics and in bioremediation.

ACKNOWLEDGEMENT

The authors are very grateful to Centre for Bioscience and Nanoscience Research (CBNR), Coimbatore, Tamil Nadu, India, SAIF Cochin for analysis of SNPs.

REFERENCE

1. Deshpande MV. 1986. *Enzymatic degradation of chitin and its biological applications*. *J. Sci. Ind. Res.* 1986. *45,* 273-281.
2. Nicol S., *Life after death for empty shells*. *New Sci.* 1991, 129: 46-48.
3. Wang SL., Hwang JR. *Microbial reclamation of shellfish waste for the production of chitinase*. *J. Enzyme Microbiol. Technol*. 2001, 28, 376-382.
4. Sudarshan N R., Hoover DG, Knorr D. *Antibacterial action of chitosan. Food Biotechnol.* 1992, 6, 257-272.
5. Moore G.K., Roberts G.A.F. *In: Proceedings 1st International Conference on Chitin/Chitosan (Eds. Muzzarelli R.A.A. & Pariser E.R.). MIT Sea Grant Report* 78-7, p 421.1977
6. Ng C.H., Hein S., Chandrkrachang S., Stevens W.F. *Evaluation of an improved acid hydrolysis-HPLC assay for the acetyl content in chitin and chitosan*. *Journal of Biomedical Materials Research Part B-Applied Biomaterials.* 2006 76, 155-160.
7. S. Sharma., N. Ahmad., A. Prakash., V. N. Singh., A. K. Ghoash., B. R. Mehta.2010. *Synthesis of crystalline AG nanoparticles, (Ag NPs) from microorganisms*. *Materials Sciences and Applications.* 2010, 1, 1–7.
8. Buitron C.L., Quezada M.,Moreno G. *Aerobic degradation of the azo dye acid red 151 in a sequencing batch bio-filter. Bioresource Technol.*2004. *92,* 143–149.
9. Sokmen M., Allen D.W., Akkas F., Kartal N. and Acar F. *Photodegradation of some Dyes using Ag-loaded titanium dioxide. Water Air Soil Pollut.* 2001. 132:153–163.
10. Franco, Luciana de Oliveira., Maia, Rita de Cássia Gomes., Porto., Ana Lúcia F., Messias, Arminda Sacconi., Fukushima, Kazutaka., Campos Takaki, Galba Maria. *Heavy metal biosorption by chitin and chitosan isolated from Cunninghamella elegans (IFM 46109). Brazilian Journal of Microbiology*. 2004. *35,* 243-247.
11. Anderson I C., Cairney JWG**.**, *Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environmental Microbiology*. 2004. *6,* 769-779.
12. Nadarajah, K., Kader, J., Mazmira, Mohd., Paul, D.C. *Production of chitosan by fungi. Pakistan Journal of Biological Sciences.* 2001. 4:263-265.
13. Thayza Christina Montenegro Stamford., Tânia Lucia Montenegro Stamford., Newton Pereira Stamford., Benicio de Barros Neto., Galba Maria de Campos-Takaki., *Growth of Cunninghamella elegans UCP 542 and production of chitin and chitosan using yam bean medium ,Electronic Journal of Biotechnology,* 2007*,* 10, 61-68.
14. Synowiecki, Józef., Al-Khatteb., Nadia Ali Abdul. *Mycelia of Mucor rouxii as a source of chitin and chitosan. Food Chemistry*, 1997, 60, 605-610.
15. Franco., Luciana de Oliveira., Stamford, Thayza Christina Montenegro., Stamford, Newton Pereira., Campos-Takaki, Galba Maria de. *Cunningamella elegans (IFM 46109) como fonte de quitina e quitosana*. *Revista Analytica*, 2005. *4*, 40-44.
16. Vinh Quang Nguyen., Masayuki Ishihara., Shingo Nakamura., Hidemi Hattori., Takeshi Ono., Yasushi Miyahira., and Takemi Matsui. ,*Interaction of Silver Nanoparticles and Chitin Powder with Different Sizes and Surface Structures: The Correlation with Antimicrobial Activities*, *Journal of Nanomaterials*, Volume 2013, Article ID 467534.
17. S Honary ., K Ghajar., P Khazaeli., P Shalchian. *Preparation, Characterization and Antibacterial Properties of Silver-Chitosan Nanocomposites Using Different Molecular Weight Grades of Chitosan*, *Tropical Journal of Pharmaceutical Research.* 2011,10 ,69-74.
18. S. Sowmyaa., P.T. Sudheesh Kumara., K.P. Chennazhia., S.V. Naira., H. Tamurab., R. Jayakumara.2011. *Biocompatible β-chitin Hydrogel/Nanobioactive Glass Ceramic Nanocomposite Scaffolds for Periodontal Bone Regeneration*, *Trends Biomater. Artif. Organs.*2011. 25(1):1-11
19. Mansor Bin Ahmad., Jenn Jye Lim., Kamyar Shameli., Nor Azowa Ibrahim., Mei Yen Tay., Buong Woei Chien. *Antibacterial activity of silver bionanocomposites synthesized by chemical reduction route. Chemistry Central Journal.*2012. 6:101.
20. Koide, S. S., *Chitin-Chitosan: Properties, Benefits and Risks Nutrition Research.* 1998.*18,* 1091-1101.
21. Muzzarelli R.A.A. *Chitosan-based dietary foods*. *Carbohydrate Polymers* 1996.29: 309-316.
22. M. Vanaja., K. Paulkumar., M. Baburaja., S. Rajeshkumar., G. Gnanajobitha., C. Malarkodi., M. Sivakavinesan., G. Annadurai. *Degradation of Methylene Blue Using Biologically Synthesized Silver Nanoparticles, Bioinorganic Chemistry and Applications* Volume 2014, Article ID 742346, 8 pages.