“DEVELOPMENT OF A SUSTAINABLE PULLULAN-BASED ANTIMICROBIAL COATING SYSTEM”

A Project Report Submitted to

Anekant Education Society's

Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati

(Autonomous)

Under

'Seed Money for Teacher Scheme' 2023-24

Submitted by

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CERTIFICATE

This is to certify that the final report on a research project Seed Money for Teacher Scheme 2023-24, entitled **"Development of A Sustainable Pullulan-Based Antimicrobial Coating System"** is a record of bonafide research work carried out by Principal investigator Mrs. Komal Ramchandra Jagtap and Co- Investigator Ms. Kajal Mahadev Gaikwad Assistant Professors, Department of Microbiology, Anekant Education Society's Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati (Autonomous) Dist. Pune, Maharashtra. A copy of the final report of Research Project has been kept in the library of College and an executive summary of the report has been posted on the website of the College.

Principal

Dr. Avinash S. Jagtap

TULJARAM CHATURCHAND COLLEGE BARAMATI

DECLARATION

I hereby declare that the project report entitled "**Development of A Sustainable Pullulan-Based Antimicrobial Coating System"** completed and written by me under the financial support of Seed Money for Teacher Scheme 2023-24, at Tuljaram Chaturchand College of Arts, Science and Commerce (Autonomous), Baramati Dist. Pune, Maharashtra. has not been previously published or formed the basis of any degree, diploma, research project or any other similar title.

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(Mrs.Komal R. Jagtap)

TULJARAM CHATURCHAND COLLEGE * BARAMATI

Anekant Education Society's

Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati

(Autonomous)

REPORT FOR RESEARCH PROJECT

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ABSTRACT

According to the Grand View Research Report the estimated market size of antimicrobial coatings is $5.4 billion by 2024. The COVID-19 pandemic and increasing antimicrobial resistance has accelerated the growth of the antimicrobial coatings market. Biopolymer-based antimicrobial coatings are innovative solutions. Pullulan is one of the widely used biopolymers in food, pharmaceutical, agricultural, cosmetics and chemical industries. In this study, *Aureobasidium pullulans* CBS 584.75 strain were isolated from the blackened patches on wet wall. Its morphological, biochemical, and molecular characterizations were done. The pullulan production was successfully achieved and precipitated using ethanol. The modification in pullulan were done by depositing silver and copper nanoparticles and by carboxymethylationreactions. The modifications were characterized by Fourier Transform Infrared (FTIR), UV visible spectroscopic analysis and Scanning Electron Microscopy (SEM). In order to form antimicrobial coating system, their antimicrobial susceptibility were tested. The modifications in polymer pullulan shows antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella spp*, and *Pseudomonas aeroginosa*. The degree of inhibition was measured by the average of inhibition zones. The results showed that there were significant difference between the antimicrobial effect of pullulan and modified pullulans.

Overall the study aims for escalating threat of antimicrobial resistance, to harness the potential of pullulan as a base material for developing antimicrobial systems and to explore various modification strategies to enhance the antimicrobial properties of pullulan. This development and utilization of effective antimicrobial systems may play a crucial role in healthcare, food safety, sanitation, and disease prevention.

Keywords

Pullulan, Carboxymethylation, FT-IR, SEM, Antimicrobial susceptibility testing.

1. INTRODUCTION

An exopolysaccharide pullulan is produced extracellularly by a polymorphic, omnipresent fungus, *Aureobasidium pullulans* which looks like a yeast. It is a water-soluble homopolysaccharide, it was found to be a high molecular weight polymer. Its structural formula is [α-D-Glucopyranosyl-(1→4)-α-D-Glucopyranosyl-(1→4)-α-DGlucopyranosyl-(1→6)]n. It is unbranched linear polymer with maltotriose repeating units. (Singh *et al*., 2012).The presence of both α-(1→6) and α-(1→4) glycosidic linkages are responsible for the structural flexibility, elasticity, and enhanced solubility. This offers moldability and spinnability to polymer. Pullulan is a dry, amorphous, whitish powder. It is flavorless, tasteless, and odorless. The FDA of US has recognized pullulan as generally regarded as safe (Singh *et al*., 2021). Many studies have reported it as a non-carcinogenic, nontoxic, and edible natural polymer (Raychaudhuri *et al*., 2020; Trinetta *et al*., 2016). This polymer shows good thermal stability can decompose at 250–280°C (Farris *et al*.,2012). Pullulan shows high solubility in water, dilute alkali dimethyl sulfoxide and formamide, and insoluble in organic solvents of alcohols (Farris *et al*., 2014; Yangilar *et al*.,2013). It forms a transparent, colorless and viscous solution having superior adhesivity as a binder. It can form thin film with oxygen barrier and moisture retention ability (Falsafi *et al*., 2023. These versatile properties of pullulan make it the best sustainable polymer for various applications.

Traditional antimicrobial coatings often rely on synthetic polymers derived from petrochemicals. It may contains various isothiazolinone, zinc pyrithione, quaternary ammonium compounds, mercury and lead, they can have detrimental effects on ecosystems when they leach into water systems or accumulate in the environment (Jaishankar M. *et al*., 2014, Wuana R.A. *et al*., 2011). Pullulan gum is very similar to petroleum derived synthetic polymers. It found highly biocompatible and biodegradable. So this is a great replacement for the non biodegradable polymers. Increasing antimicrobial resistance to commonly used disinfectants, drugs, sanitizers and antiseptics is evolved as a concerned point of public health. Pullulan and its modifications based coatings provide an alternative strategy to combat microbial growth, reducing the selective pressure for the development of antimicrobial resistance.

2. OBJECTIVES

1. Isolation, characterization and identification of pullulan producing strain.

2. Production and characterization of pullulan.

3. Modifications in structure of pullulan.

4. Antimicrobial susceptibility testing.

3. MATERIALS AND METHOD

Isolation of *Aureobasidium pullulans* CBS 584.75 strain

The blackened patches from wet wall were scrapped with the help of sterilized blades and collected in sterile polythene bags. And inoculated into minimal salt medium containing 1gm of $(NH_4)_2HPO_4$; 0.5gm of NaCl; 0.05gm of $MgSO_4$ and 10µg/ml of chloramphenicol and pH 5.5. And incubated for 48 hours on an orbital shaker incubator at 120 rpm (Lotrakul *et al.*,2009). After incubation, 10µl of the supernatant was spread plate technique on sterile Yeast extract Malt extract media (Yeast extract 0.3gm, Malt extract 0.3gm, Peptone 0.5gm, Dextrose 1gm, Distilled water 100ml, pH 5 to 6 and Agar 4gm) and incubated at room temperature for four days (Pollock *et al.,* 1992). Pure cultures were maintained on YEME medium and Minimal Salt agar medium.

Characterization of *Aureobasidium pullulans* CBS 584.75 strain

1) Morphological characterization

Colony Characteristics were observed on a daily basis. Morphological changes were recorded by microscopic observation under electron microscope.

2) Biochemical characterization

Carbon utilization was checked for fructose, glucose, maltose, sucrose, lactose, starch and methyl-a- glucoside. Ammonium nitrate, ammonium sulfate, sodium nitrate, peptone, L-asparagine and yeast extract checked for nitrogen assimilation. The selected isolates were evaluated for tolerance to high salt concentrations by growing them on sterile media containing 2%, 5%, and 10% NaCl concentrations. Urease production and citrate assimilation were conducted(Takahashi *et al.,*1981 & Singh *et al.,* 2010).

3) Molecular identification

The phylogenetic analysis is predicated on a single gene sequence, which has a total length 1335bp of 16SrRNA gene and a closest type strain found in the database (Felsenstein *et al.,*1985; Kimura *et al.,*1980; Saitou *et al.,*1987 &Tamura *et al.,* 2013).

Production of pullulan

For production, 1% of the inoculum containing $250x10^7$cells/ml used. A media of 2gm dextrose, 0.06gm ammonium sulfate (NH4)2SO4, 0.5 gm of dipotassium hydrogen orthophosphate (K2HPO4), 0.04 gm of yeast extract 0.1 gm of sodium chloride (NaCl), 1 ml of chloramphenicol solution, 100 ml of autoclaved distilled water, and pH 5 used. Incubated for 28 hours at 25°C (Gaikwad *et al.,* 2022).

Extraction and Estimation of pullulan

The production broth was centrifuged at 10,000g for 20 minutes. The cell-free supernatant separated and mixed with double volume of ice-chilled ethanol and incubated at 5°C for 24 hours to precipitate pullulan. After incubation broth centrifuged at 2,500g for 20 minutes at 4°C, the resulting precipitates were collected. Then it was washed with acetone and deionized water, and then dried at 50°C (Thirumavalavan *et al.,*2008 & Kachhawa *et al.,* 2003).The precipitated pullulan was dried and amount of pullulan quantified in terms of grams per litre of fermented broth (Charles *et al.,* 2014 & Cotelli *et al.,*2020).

Characterization of pullulan

Folin-Lowry method used for the estimation of protein content in pullulan (Lowry *et al.,*1951) while the sugar content in pullulan was quantified by using the phenol sulfuric acid method (Dubois *et al.,*1956).

Modification in structure of pullulan

Chemical modification by adding functional groups

1) Carboxymethylation : Carboxymethylation of pullulan is done by dissolving 0.1g pullulan in distilled water 1ml followed by the addition of 0.01g of potassium hydroxide (potassium hydroxide/sugar unit = 1.0 mmol/mmol) solution and 1.26 mL of acrylic acid (acrylic acid/sugar unit = 1.0 mmol/mmol).This reaction allowed to proceed at 50°C for 4 hours, after which the solution cooled to room temperature and dropped into 5 mL of ethanol. A yellow-brown viscous product obtained. The product then dissolved in 4 mL of distilled water, and filtered. (Ying Wu *et al*., 2021)

Modification by deposition of nanoparticles

1) Silver nanoparticle : 0.1 gm of pullulan dissolved in 5ml of deionized water and stirred at 40℃. 0.01 mg/mL silver nitrate (AgNO3) added to this solution and the mixture stirred continuously for 5 hours at room temperature. To obtain AgNPs@Pull, the metal ion-absorbed pullulan solution heated to 70℃ and shaken for 5 hours at 150 rpm to reduce Ag(I) or Au(III) into zerovalent metallic forms. On heating, the color of Pull-Ag solutions turned into light brown indicated the formation of metallic Ag NPs. (Dionísion *et al*., 2013)

2) **Copper oxide nanoparticle :** A copper sulphate solution prepared by dissolving 0.2g of CuSO4.5H2O 5ml of water (4% weight/volume) and stirred for 10 minutes to achieve homogeneity. A pullulan solution then prepared by heating 0.5g of polysaccharide powder in 5ml of water (10% weight/volume) at 90℃ and stirring at 800 rpm. The prepared CuSO4 solution mixed with the polysaccharide solution and stirred homogeneously for about 10 minutes. A control solution of copper sulphate without pullulan also prepared. (Ahmad *et al* .,2021 & Mahmood *et al*., 2018)

Characterization of pullulan and modified pullulan

1) Fourier transform infrared (FT-IR) spectroscopy

The pullulan and modified pullulanwas characterized through Fourier transform infrared spectroscopy (FT-IR)(Shang *et al.*, 2013). A test sample prepared by mixing 2 mg of pullulan with 200 mg of KBr. A 3mm diameter having compact disks was prepared. And scanning were done within the range of 4,000-500cm^{-1} with 4 cm^{-1} resolution, using 32 scans.

2) UV-Visible spectroscopy

The formation of silver and copper nanoparticles studied by UV-Visible spectroscopy.

3) Scanning Electron Microscope (SEM)

The surface morphology determined by using scanning electron microscopy (SEM) (David *et al*.,2013).

Antimicrobial activity testing

Antimicrobial assay of test samples was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. Daoud *et al*., 2015). To check sterility testing Muller Hinton agar plates were prepared and incubated at 37 o C for 24 hrs. Plates were considered sterile and Used for antimicrobial susceptibility testing if there was no growth after 24 hrs. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards (The McFarland standards were prepared by combination of 9.95 ml 1% H2SO4 acid with 0.05 ml of a 1.175% aqueous solution Barium chloride) giving a final inoculum of 1.5 × 108 CFU/ml. MHA plate was lawn cultured with standardized microbial culture broth. Test sample of 50 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO).The external surface of each plates was divided into three part one disc containing extract one for positive control and remainder for negative control. To achieve uniform turbidity, 0.1 ml suspension was spread on sterile surface of Muller Hinton agar plates . It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. DMSO at a concentration of 10% was employed as a negative control. The test were done in triplicates.Common Pathogens used for antimicrobial susceptibility testing: -1) *Escherichia coli*, 2) *Bacillus subtilis*, 3) *Salmonella typhimurium*, 4) *Pseudomonas aeruginosa.*

4. RESULT AND DISCUSSION

Isolation of *Aureobasidium pullulans* CBS 584.75 strain

On 4 days incubation at 37ºC, the colonies observed fully grown on Sabouraud dextrose agar. Initially, colonies are whitish creamy, and smooth, after 3-4 days colonies become yellowish-green. And after prolonged incubation colonies became black due to the melanin pigment production.

Figure 1. Site of sample collection

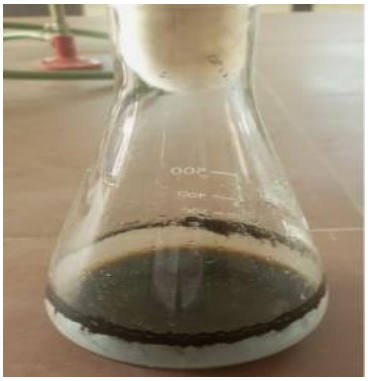


Figure 2. Enrichment broth for isolation of *Aureobasidium pullulans* CBS 584.75 strain

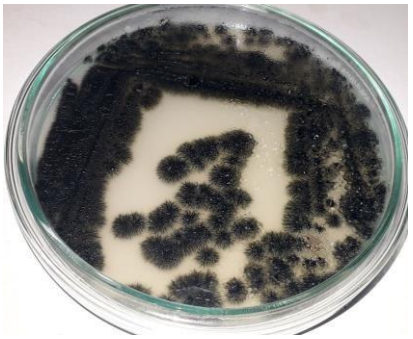


Figure 3.Isolation of *Aureobasidium pullulans* CBS 584.75 strain on Sabouraud dextrose agar medium.

Characterization of *Aureobasidium pullulans* CBS 584.75 strain

1) Morphological Characterization

Aureobasidium pullulans CBS 584.75 strains showed polymorphic growth. After incubation of 24 hours the colonies were observed whitish and smooth. Later on slimy, mycelial colonies became brown to black due to the production of melanin.

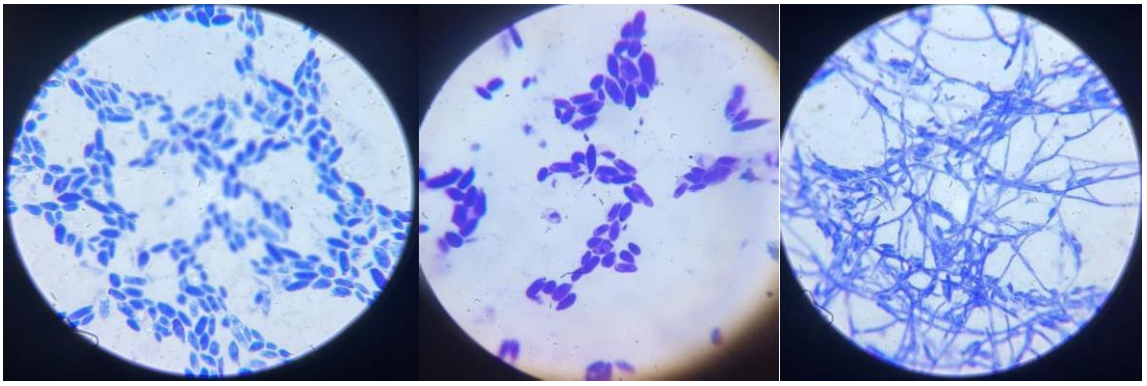


Figure 4. Microscopic observation of colony of *Aureobasidium pullulans* CBS 584.75 strain

2) Biochemical Characterization

Aureobasidium pullulans CBS 584.75 strain was tested for the utilization of different sugars and nitrogen sources and also checked for enzyme synthesis shown in Table 1. It was found that it can utilize fructose, glucose, maltose, sucrose, lactose, and mannitol. Not assimilate methanol, the same results obtained from the data given by Takahashi et al. 1981 Amylase, it was tested by ability to form clear zone around the colony, after addition of iodine. Peptone, ammonium nitrate, sodium nitrates, sodium nitrite, asparagine were used as a sole nitrogen sources and checked for assimilation. And also tested for high salt tolerance, it was found that the isolated strain able grow at 10% NaCl and temperature upto 45°C. pH values 3.0 to 11.0 were found suitable for the growth (Takahashi *et al.*,1981& Singh *et al.*,2010).

Table No.1. Biochemical characterization : Sugar Assimilation; Nitrogen source assimilation; Temperature; Growth with NaCl; pH; Production of enzymes.

1. Sugar Assimilation								
Glucose +	Fructose +	Galactose +	Sucrose +	Maltose +	Lactose +	Ribose +	Starch +	Mannitol -
2. Nitrogen source Assimilation								
Ammonium sulfate +	Ammonium nitrate +	Sodium nitrate +	Sodium nitrite +	L- asparagine +	Yeast extract +			
3. Temperature								
25 +	30 +	35 +	40 +	45 W	50 –			
4. Growth with NaCl								
1.0 +	2.5 +	5 +	7.5 +	10 +	12.5 –			
5. pH								
3 +	5 +	7 +	9 +	11 +				
6. Production of enzymes								
Gelatinase +	Protease +	Amylase +	Cellulose +	Xylanase +	Urease +			

3) Molecular Characterization

Further identification was carried out by 18SrRNA sequencing. The phylogenetic relationship of is shown in figure 5.

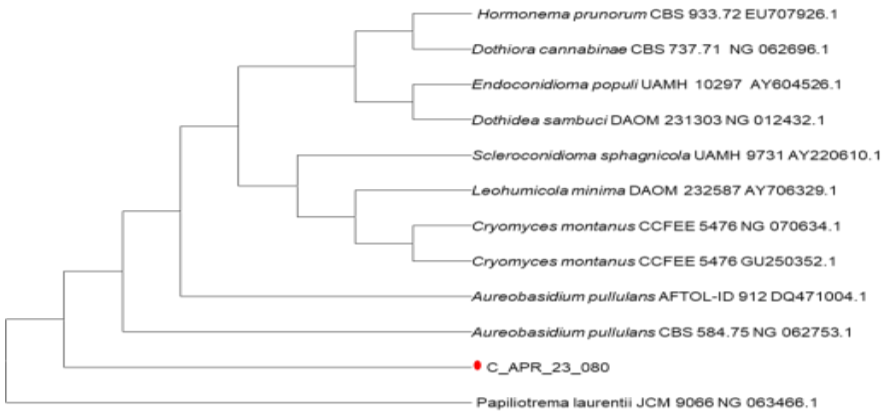


Figure 5. Phylogenetic analysis on the basis of 16S rRNA gene comparison

A phylogenetic analysis done on the basis of 16S rRNA gene comparison. Found that strain BS placed in clade with the species *Aureobasidium pullulans* CBS 584.75 strain. And shows 100 to 99% pairwise similarity. Using GenBank database, From the results of phylogenetic tree and 1335 bp sequence of 16SrRNA gene and pairwise similarity it was concluded that the this isolate might be the genus of *Aureobasidium*(Felsenstein*et al.*,1985; Kimura*et al.*, 1980& Saitou *et al.*,1987).

Production, extraction and estimation of pullulan

On production, the dry weight estimation was done. Total 3.5gm/lit of pullulan were obtained by precipitating with alcohol.

Chemical analysis of exopolysaccharide pullulan

For the total estimation of carbohydrate and protein content dried pullulan was used. The content of total carbohydrate found 4.5+- 0.05g/100ml by phenol sulfuric acid method. The content of protein measured by the Folinlowry method and it found 0.4+- 0.005mg/100ml.

Modification in structure of pullulan

Chemical modification by adding functional groups

1) Carboxymethylation

After incubation for 4 hours at 50°C a yellow-brown viscous product was obtained. This showed the completion of carboxymethylation of pullulan. The product then dissolved in 4 mL of distilled water, and filtered to obtain clear purified product.

Modification by deposition of nanoparticles

1) Silver nanoparticle

On reduction the color of Pull-Ag solutions turned into light brown indicated the formation of metallic silver nanoparticles. Heated to 70°C dnd obtained dry powder form by Pull-AgNP.

2) Copper oxide nanoparticles

After reaction stirring at 800 rpm, a colour change observed indicated the formation of copper nanoparticles. Dry product obtained by heating at 90°C.

Characterization of pullulan and modified pullulan

1. Fourier transform infrared (FT-IR) spectroscopy of pullulan

In the present study, FTIR spectroscopic analysis of the obtained pullulan was conducted and then compared with that of the standard pullulan sigma. Mujdeci*et al.,* 2024 obtained the FT-IR absorbance spectra in the region of 4000–400 cm−1 same as in the present study. They obtained the peaks of the –OH groups in the sugars and C–H bonds at 3000cm−1 and 2900 cm−1. Also Hamidi*et al.*,2019; Hilares*et al.*,2017 & Choudhury *et al.*,2011 found that the mostly observed –OH i.e. hydroxyl groups in EPS shows strong absorption at 3433.87 cm−1.Here As in the Figure 3, a strong absorption peak of the hydroxyl group observed at 3714cm^{-1}, the C-H bond on the carbohydrate chain showed absorption peak at 1288cm^{-1}. The absorption peak at 933 cm^{-1} and 678cm^{-1} shows shows α-1,4 & α-1,6 glycosidic linkages. It confirms that the obtained EPS is pullulan.

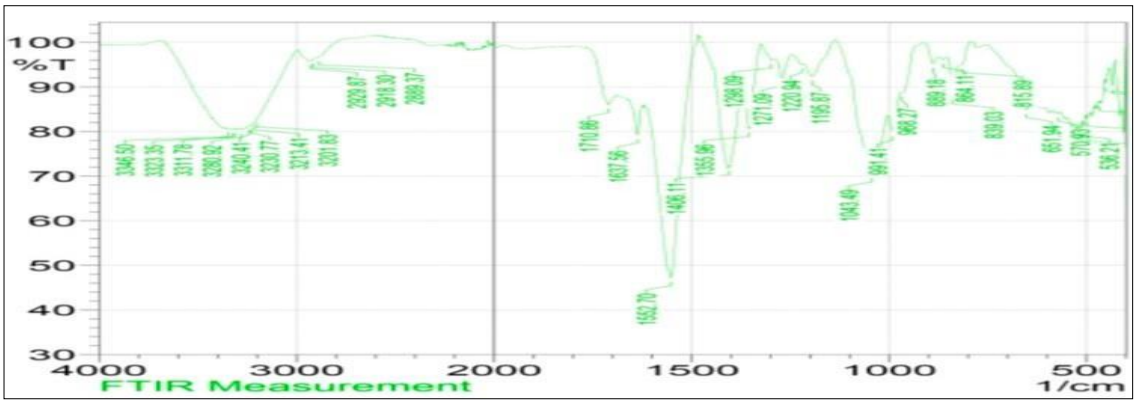
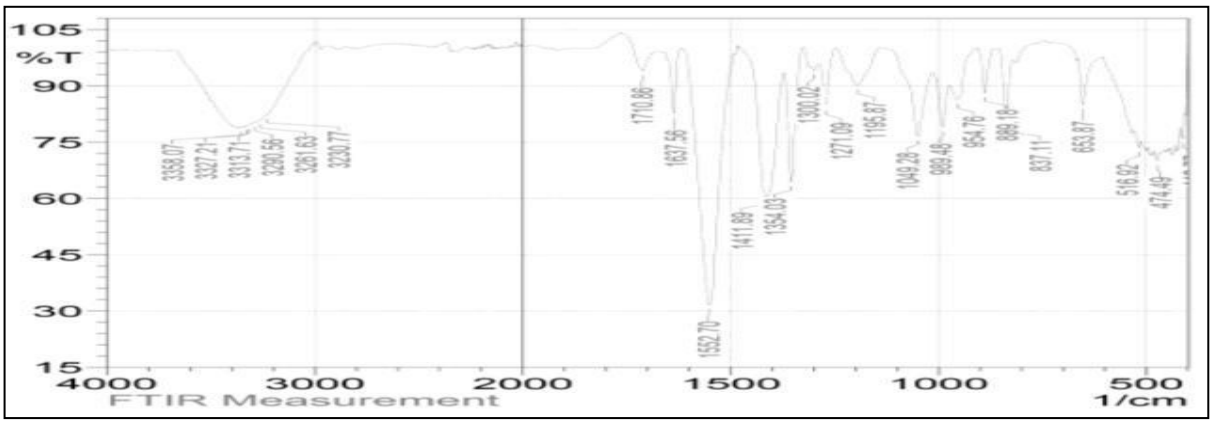


Figure 6. FTIR Spectrum of obtained pullulan from *Aureobasidium pullulans* CBS 584.75 strain

Fourier transform infrared (FT-IR) spectroscopy of modified pullulan

2. **Fourier transform infrared (FT-IR) spectroscopy Carboxymethylated pullulan**

A. Control



A. Carboxymethylated pullulan

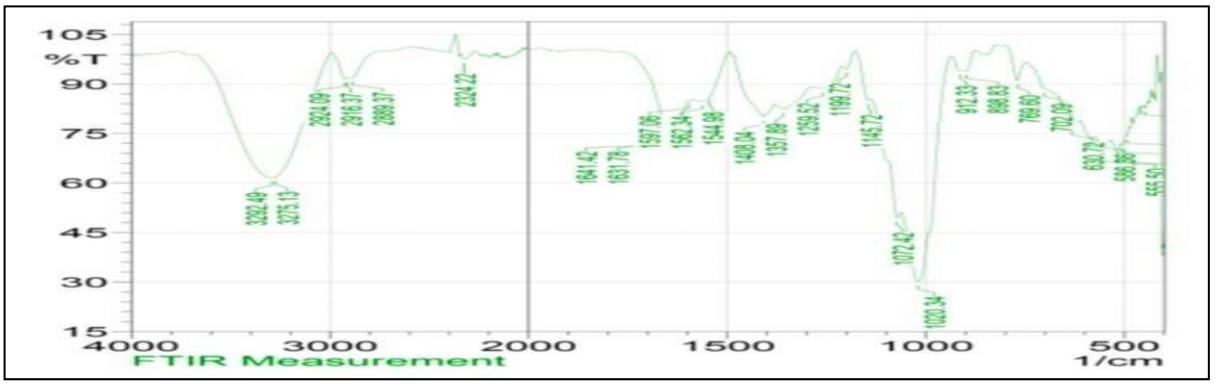
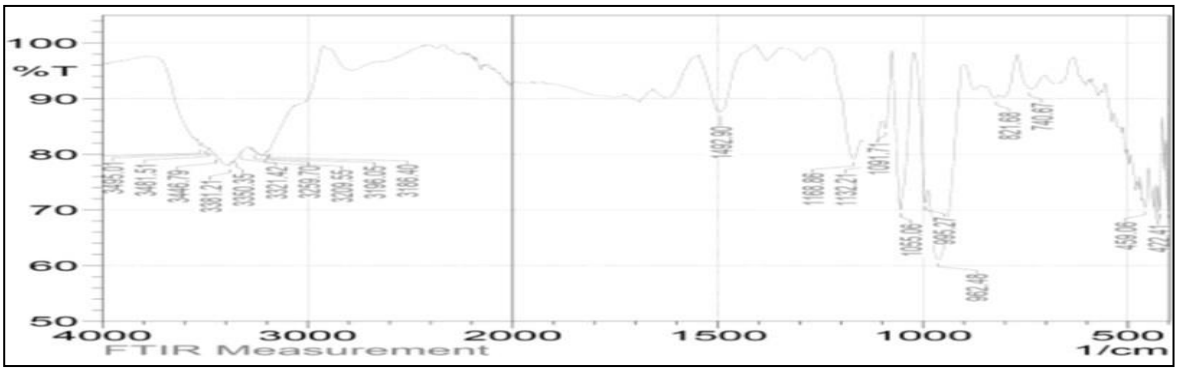


Figure7.Fourier transform infrared (FT-IR) spectroscopy: A. Control, B. Carboxymethylated pullulan

FT-IR analyses have been running to provide the structure of CMP. Figure 8 shows the FT-IR spectra of pullulan and CMP, and explain that, the characteristic absorption bands for pul- lulan are at 3432, 2929, 1654, 1125, 1032 1642 and 860 cm, which they relative to O-H C-H, O-C-C, C-O-Cand C-O stretching, C-O- bending and D-glucosidic bands respec-tively. Furthermore, the characteristic absorption bands of CMP appears at 3350cm stretching for -OH of anhy- droglucose units, 2920 cm for C-Hgroups, 1590 cm for asymmetric carboxylate group (COO) and at 1416 cm (symmetric carboxylate group (COO). At 1087 cm, a characteristic stretching peak attributed to ether bond in pullulan have been observed. In addition, there are three peaks for D-glucosidic which they appear at 857, 757 and 932 cm .

3. Fourier transform infrared (FT-IR) spectroscopy of Silver nanoparticles deposited pullulan

A. Control



B. Silver nanoparticles deposited pullulan

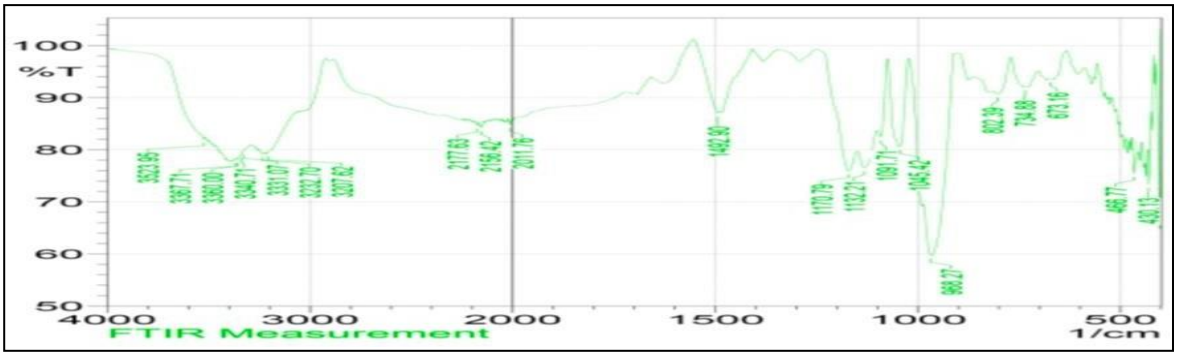
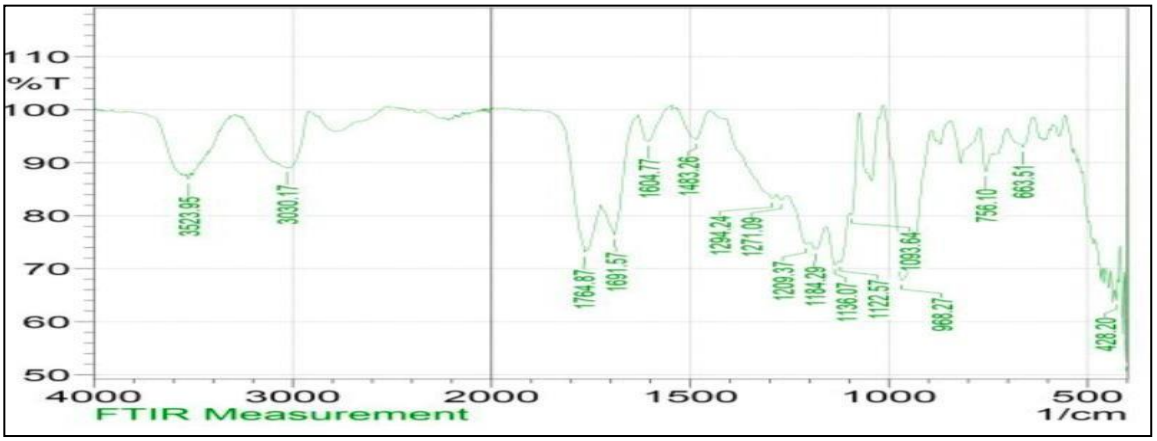


Figure 8. Fourier transform infrared (FT-IR) spectroscopy : A Control, B Silver nanoparticles deposited pullulan

The FTIR spectrum of AgO shows peaks at 1631.78, 1641.42 cm¹ revealed the formation AgO as shown in Figure 8 A. A broad peak noticed at 3352.28, 3332.99, 3300.20, 3277.06 cm¹ attributed to O-H stretching of the moisture content.

3. Fourier transform infrared (FT-IR) spectroscopy of Copper nanoparticles deposited pullulan

A. Control



B. Copper nanoparticles deposited pullulan

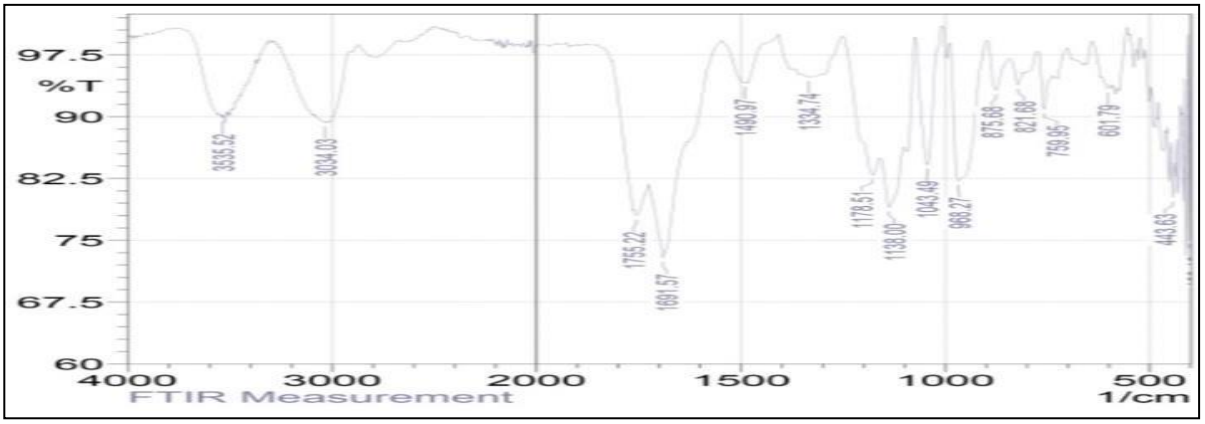


Figure 9. Fourier transform infrared (FT-IR) spectroscopy : A Control, B Copper nanoparticles deposited pullulan

The FTIR spectrum of CuO shows peaks at 437.84, 563 cm¹ revealed the formation CuO as shown in Figure la. A broad peak noticed at 3356.14, 3323.35, 3292.49, 3277.06, 3253.91, 3238.48, 3226.91 cm¹ attributed to O-H stretching of the moisture content. A peak obtained at

1089 cm¹ (BEND) attributed to the C-O bond of hydroxyl moiety .The peak noticed at 1211 cm¹ resembles to CH-O-CH stretching in all nanocomposites are shown by respective absorption peaks as presented in figure.

4) UV-Visible spectroscopy of Pullulan deposited Silver nanoparticle and copper nanoparticle

(A) **(B)**

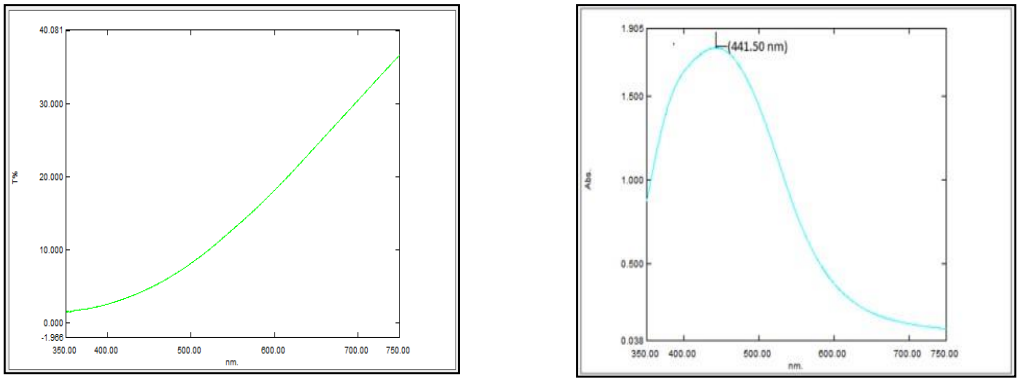


Fig. 10. UV-Vis spectra of silver nanoparticles synthesized.

(A) The spectra recorded for similar mixture excepting that no period of incubation was given.

(B) $AgNo_3$(1 mM) solution was mixed with pullulan and UV-Visible spectra was recorded after incubation.

(A) **(B)**

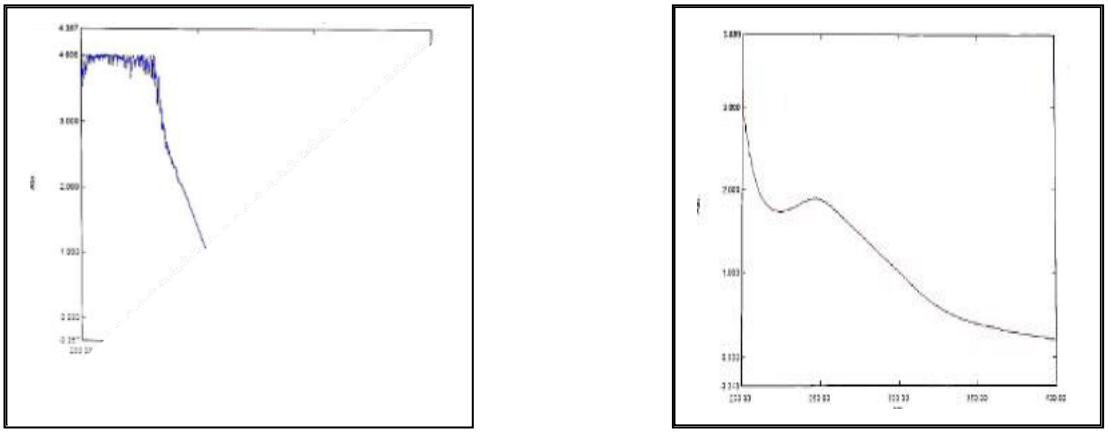


Fig. 11. UV-Vis spectra of copper nanoparticles synthesized.(A)The spectra recorded for similar mixture excepting that no period of incubation was given. (B) CuSO4(1 mM) solution was mixed with pullulan and UV-Visible spectra was recorded after incubation.

The formation of silver nanoparticles was studied by UV-Visible spectroscopy. The silver nanoparticles absorb radiation in the visible region of electromagnetic spectrum due to excitation of surface plasmon vibration giving silver nanoparticles striking colours in various medium. Fig. 11 A and B showed absence of peak at zero hrs and characteristic absorption at 441 nm for the reaction mixture containing, pullulan incubated with AgNO3for 24 hrs respectively.

Antimicrobial activity testing

Antimicrobial activity of native pullulan and the chemically modified pullulan by carboxymethylation and deposited by silver nanoparticles, copper nanoparticle were tested against *Escherichia coli, Bacillus subtilis, Salmonella typhimurium* and *Pseudomonas aeroginosa.*Pullulan alone has no inhibitory effect on E. coli but shows inhibitory effects on *B. subtilis, S. typhimurium, and P. aeruginosa*. Carboxymethylated pullulan (CP) generally has higher inhibitory effects compared to pullulan alone on all tested bacteria. Carboxymethylated pullulan shows similar or slightly enhanced effects on *B. subtilis* but reduced effects on *S. typhimurium* compared to control of carboxymethylation alone.These findings suggest that pullulan, especially when modified, can modulate antimicrobial activity, potentially enhancing or reducing bacterial susceptibility to antibiotics depending on the bacterial strain.

Pullulan demonstrates varying degrees of inhibition against the different bacterial strains, with a moderate inhibitory effect observed against *Escherichia coli* and *Bacillus subtilis*. Additionally, it exhibits a slightly more potent inhibition against *Salmonella typhimurium* and *Pseudomonas aeruginosa* compared to its effect on *Escherichia coli* and *Bacillus subtilis*. Control of AgNP shows a inhibition pattern akin to pullulan against *Escherichia coli* and *Bacillus subtilis* but exhibits improved inhibition against *Salmonella typhimurium* and *Pseudomonas aeruginosa* compared to pullulan. Combination of pullulan and AgNP displays heightened inhibition relative to pullulan and control AgNP across all bacterial strains, especially remarkable against *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Control of CuNP demonstrates an inhibition pattern similar to control AgNP, with slightly superior inhibition against *Escherichia coli* and *Bacillus subtilis*. Combination of pullulan and CuNP presents significantly enhanced inhibition against all bacterial strains, notably against *Escherichia coli* and *Salmonella typhimurium.*

Thus Pullulan with either AgNP or CuNP demonstrates improved antimicrobial activity compared to pullulan alone or the respective nanoparticle controls, with Pullulan + CuNP showing the highest level of inhibition across all tested bacterial strains.

Antimicrobial activity of carboxymethylated pullulan

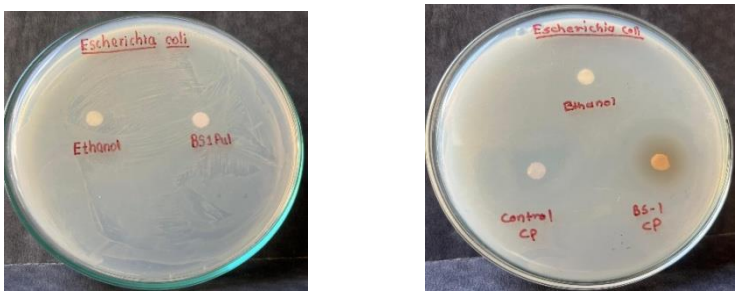


Fig. 12. Antimicrobial activity of pullulan and carboxymethylated pullulan against *Escherichia coli*

Fig. 13 Antimicrobial activity of pullulan and carboxymethylated pullulan against *Bacillus subtilis*

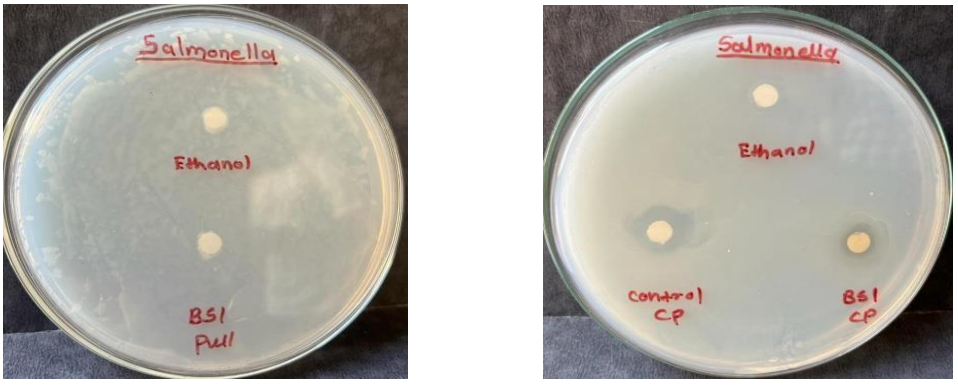


Fig.14 Antimicrobial activity of pullulan and carboxymethylated pullulan against *Salmonella typhimurium*

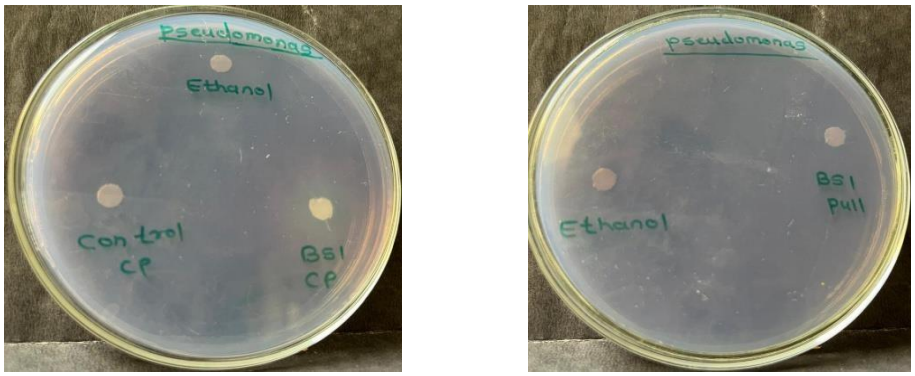


Fig.15 Antimicrobial activity of pullulan and carboxymethylated pullulan against *Pseudomonas aeroginosa*

Antimicrobial activity of silver nanoparticles deposited pullulan

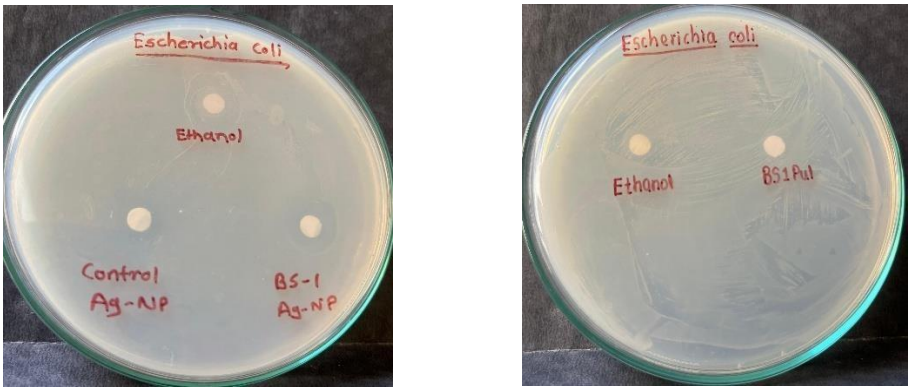


Fig. 16 Antimicrobial activity of pullulan and silver nanoparticles deposited pullulan against *Escherichia coli*

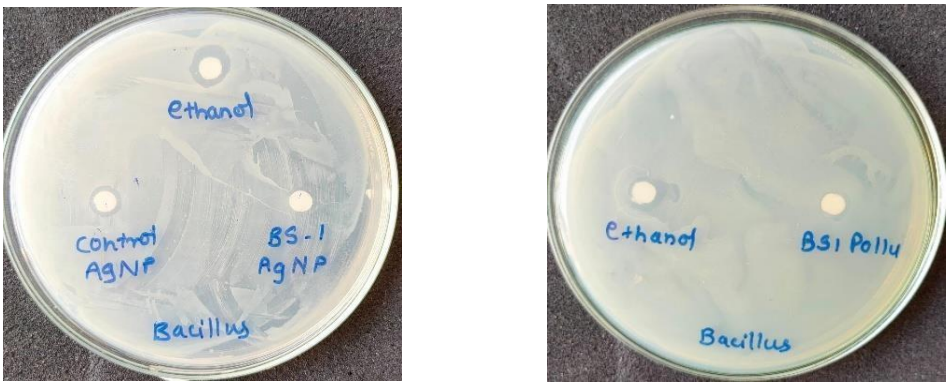


Fig. 17 Antimicrobial activity of pullulan and silver nanoparticles deposited pullulan against *Bacillus subtilis*

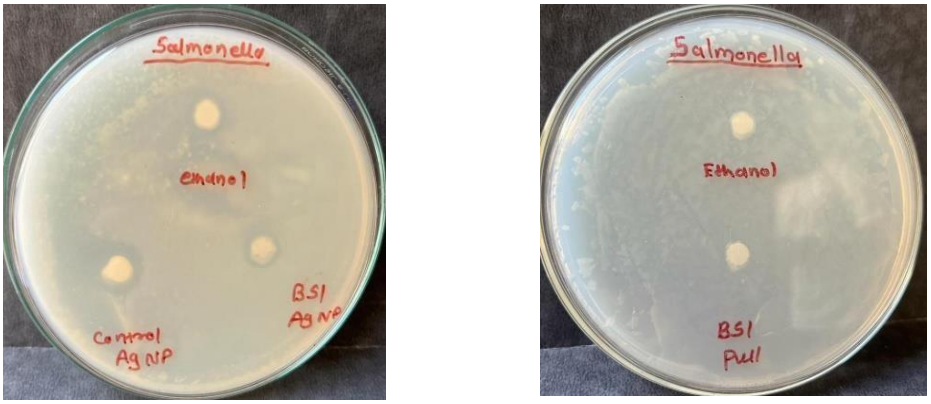


Fig.18 Antimicrobial activity of pullulan and silver nanoparticles deposited pullulan against *Salmonella typhimurium*

Fig.19 Antimicrobial activity of pullulan and silver nanoparticles deposited pullulan against *Pseudomonas aeroginosa*

Antimicrobial activity of copper nanoparticles deposited pullulan

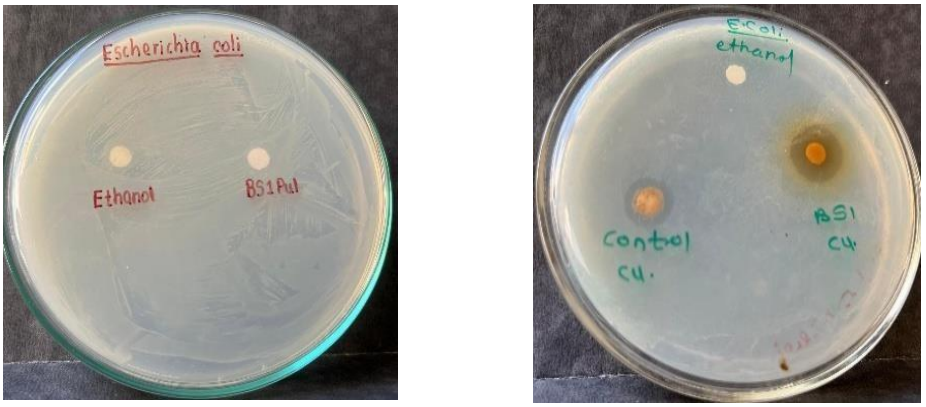


Fig. 20. Antimicrobial activity of pullulan and copper nanoparticles deposited pullulan against *Escherichia coli*

Fig. 21 Antimicrobial activity of pullulan and copper nanoparticles deposited pullulan against *Bacillus subtilis*

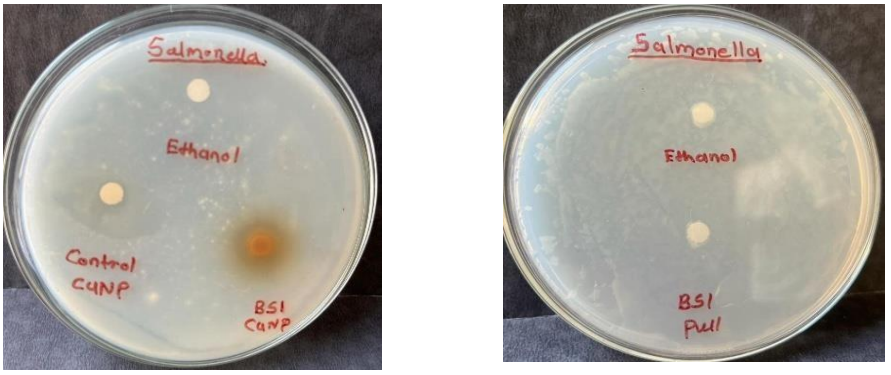


Fig.22Antimicrobial activity of pullulan and copper nanoparticles deposited pullulan against *Salmonella typhimurium*

Fig.23 Antimicrobial activity of pullulan and copper nanoparticles deposited pullulan against *Pseudomonas aeroginosa*

Scanning Electron Microscopy (SEM) Analysis

Surface morphological analysis were done by using Scanning Electron Microscopy (SEM) Analysis. It showed the formation of entire thin film.

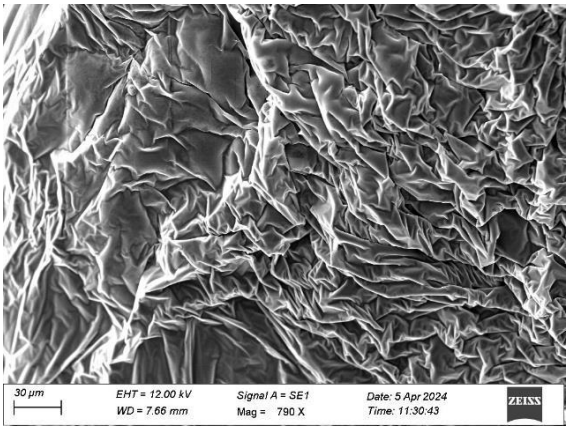
Fig.24. Scanning Electron Microscopy (SEM) Analysis

A. Scanning electron micrograph of pullulan coating.

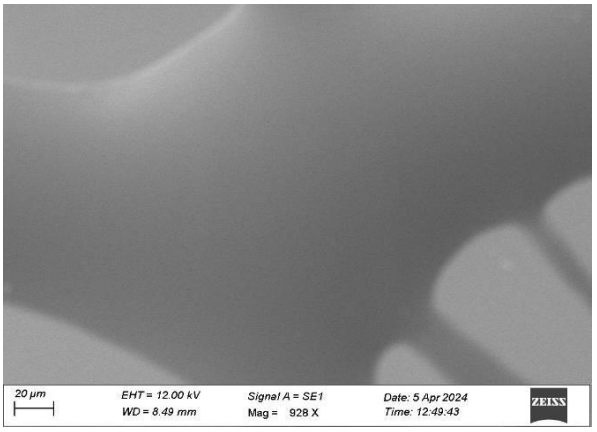
B. Scanning electron micrograph of Carboxymethylated pullulan coating

C. Scanning electron micrograph of silver nanoparticles deposited pullulan coating

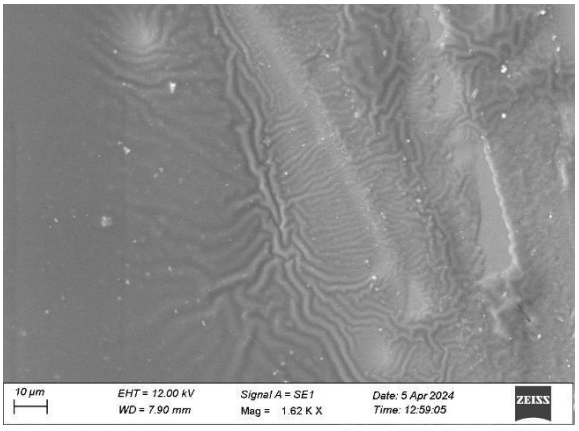
D. Scanning electron micrograph of copper nanoparticles deposited pullulan coating



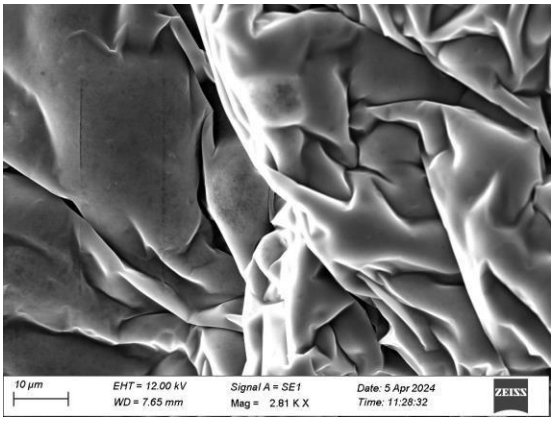
A



B



C



D

CONCLUSION

CONCLUSION

The present study suggests that *Aureobasidium pullulans* CBS 584.75 strain isolated from the wet walls surfaces can be a rich source of pullulan. The pullulan deposited with silver, copper nanoparticles and carboxymethylated pullulan showed good antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella spp*, and *Pseudomonas aeroginosa*. Thus can be effectively used in formulation of in antimicrobial coating system. The study suggests that, pullulan-based antimicrobial coatings will be significant for public health, environmental sustainability, and economic well-being. This will align the global shift towards sustainable materials and processes, meeting consumer demands for eco-friendly and socially responsible products.

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