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Novel eco-friendly synthesis of titanium oxide nanoparticles by using *Planomicrobium* sp. and its antimicrobial evaluation

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ABSTRACT

Synthesis of TiO_2 nanoparticles using optimized biomass of Planomicrobium sp., which is isolated from melted ice. Herein, the TiO_2 exhibited maximum absorbance peak at 400 nm in UV Vis spectroscopy. The XRD spectrum shows the nature of the nanoparticles and the crystalline size of the nanoparticles measured using Debye-Scherer equation was used to particle size range from 8.89 nm. SEM image shows the shape of the nanoparticles. The FTIR confirmed the existence of protein as the stabilizing agent of the TiO_2 particles. The nanoparticles exhibit antibacterial microbial activity against B.Subtilis, K.planticola, and A.niger.The ecofridely cost effective method was involving in this synthesis of nanoparticles.

Keywords: *Planomicrobium sp.*, TiO_2 nanoparticles, SEM, FTIR, Antimicrobial activity

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INTRODUCTION

Nanotechnologies concern the expansion of experimental processes for the synthesis of nanoparticles with unusual physicochemical and optoelectronic properties, medical digagnostics, biomedical imaging, chemical and biochemical sensing, nanomedicine and nanoelectronics. [1, 2]. Various chemical and physical methods are involving for the synthesis of nanoparticles [3, 4]. The chemical and physical synthesis of nanoparticles is expensive and often involves the use of toxic, hazardous chemicals which may pose environmental risks [5]. Biological methods have been put ahead to be advantageous over other synthetic methods as they are cost effective and do not involve the use of toxic chemicals, high pressure, energy and temperatures [6, 7]. The Nanoparticle are synthesized using various biosources such as bacteria, fungi, yeast, plant extract; Synthesis using bio-organisms is compatible with the green chemistry principles: the bio-organism is eco-friendly as are the reducing agent employed and the capping agent of the reaction [8]. Various classes of gram positive bacteria and Gram negative bacteria contain known to adsorb and capture up heavy metal ions. Advantages of using bacterial system consist of easy handling and they can be manipulated hereditarily without much difficulty [9-11]. Also, a bacterial system could prove to be an excellent polydispersity and stability alternative for the plant extract and fungal nanoparticles synthesis [11]. The biological method of nanoparticles synthesis is conventional to chemical and biological methods for the synthesis of in a clean, non-toxic, ecologically sound and environment-friendly manner [1].A number of organisms can grow and survive in the high ion at high metal ion concentration due to their resistance to the metal. The mechanisms involve for the synthesis of nanoparticle are: efflux systems, alteration of solubility and toxicity via reduction or oxidation,

biosorption, bioaccumulation, extra-cellular complexation or precipitation of metals and lack of specific metal transport systems [12, 13].

Titanium dioxide (TiO_2) is a material of great significance in many fields, e.g., photo catalysis, solar cell devices, gas sensors, and biomaterials [14]. The non- toxic and biocompatible properties of Titania find its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries [15, 16]. TiO_2 catalysts have been confirmed to be excellent and efficient photocatalysts for the degradation and inhibition of numerous toxic environmental contaminants. Various applications of titanium dioxide include air and water cleaning and surface cleaning [17]. Titanium is recommended for desalinization plants because of its strong resistance to corrosion from sea water. In medical applications the titanium pins are due to because of their non-reactive nature when contacting bone and flesh [18]. The TiO_2 nanoparticles are synthesized using various methods such as sol gel, hydrothermal, flame combustion, solvothermal, fungal mediated biosynthesis etc. The production of silver nanoparticles within the periplasmic surface of *Pseudomonas stutzeri* and the formation of gold nanoparticles using *Salmonella typhi* [11] and *C. limone* [12]. The plant sources are also involving in nanoparticles synthesis. Recently the microorganisms such as *Lactobacillus sp.* and *Saccharomyces cerevisae* are used for the synthesis of Titanium dioxide nanoparticles [15].

In this work, we have developed TiO_2 nanoparticles using *Planomicrobium sp*, the synthesized nanoparticles were characterized using XRD which revealed the crystalline structure of the nanoparticles, Scanning electron microscope involved for analysis the shape of the nanoparticles. The FTIR was used for characterized the functional group and antimicrobial activity of TiO_2 nanocolloids are also compared.

MATERIALS AND METHODS

Bacterial strain and growth conditions

Ice cream samples were collected from milk market. They were serially diluted and spread on nutrient agar plates. The plates were then incubated at 27ºC for 1week. The isolated microorganism was identifying by morphologically and biochemical characterized as *Planomicrobium sp.,*. In this study, we used *Planomicrobium sp* produce the green pigment pyoverdin.

Synthesis of TiO_2 nanoparticles using *Planomicrobium sp*

Planomicrobium sp., culture was allowed to grow for 24 hrs in distilled water containing suitable nutrient sources for 24 hrs. Then 25 ml was taken and diluted four times by adding 75 ml of sterile distilled water containing nutrients. The diluted culture solution was again allowed to grow for 24 hrs. Followed by, 20 ml of 0.025g of TiO_2 was added to the culture solution and it was heated on steam bath up to 50° C for 10–20 min, then white colour deposition starts to appear at the bottom of the flask. The culture solution was cooled and allowed to incubate for 24 hrs at room temperature. After 12 to 48 hrs the culture solution was observed to have white colour deposited at the bottom of the flask.

Characterization of TiO_2 nanoparticles

The UV spectra of the samples were measured by UV–visible spectrophotometer. The absorption spectra were taken at different time intervals. The supernatant treated with TiO_2 was air dried and used for analysis. Scanning electron microscope (HITACHI Model S-3000H) was recorded by focusing on clusters of particles, and it shows the morphology of the nanoparticles. To check phase formation and purity, powder XRD patterns were recorded using an X-ray diffractometer (X'per PRO model) using CuKα radiation, at the 40 keV in the 2Θ range of 10-80. The FTIR (MAKE – BRUKER Optik GmbH MODEL No - TENSOR 27), the sample was mixed with KBr and then pressed into thin pellet. Infrared spectra were measured at the wavelength in the range of 400-4000 cm^{-1}. It shows the functional group of the TiO_2 nanoparticles.

Antibacterial activity of TiO_2 Nanoparticles

The antibacterial effect of TiO_2 nanoparticles were examined by disc diffusion analyse against a gram positive bacteria *Bacillus substilis* (3053) and gram negative bacteria *Klebseilla planticola* (2727). The different concentration of TiO_2 nanoparticles were added in sterile discs and allowed few minutes in hot air oven to dry. After that the dried discs were gently placed in the Muller Hinton medium and incubate it at 24 hrs incubation. Then the discs were containing different concentration of TiO_2 nanoparticles (50 µl, 100 µl and 200 µl) placed into the Petri plates. After incubation at 37°C for 24 hours, the zone of inhibition the against nanoparticles were observed

Antifungal activity of TiO_2Nanoparticles

The antifungal activity of TiO_2 nanoparticles was carried out against *Aspergillus niger* were grown in Rose Bengal medium with different concentration of TiO_2 nanoparticles (100 µl, 200 µl, 300 µl and 400 µl),the placed Petri plates at room temperature. After 48 hours of incubation the plates were observed.

RESULTS AND DISCUSSION

Isolation and Identification

In this study used strain was isolated from ice cream. The isolates were morphologically and biochemically characterized as *Planomicrobium* sp (Figure 1). It is gram positive, rod shaped and non spore forming bacteria to identify and maintain at Gene bank Acme Progen Biotech (India) Pvt.Ltd, Salem. *Planomicrobium sp.,* is psychrotolerant and yellow to orange pigmented bacteria [19].

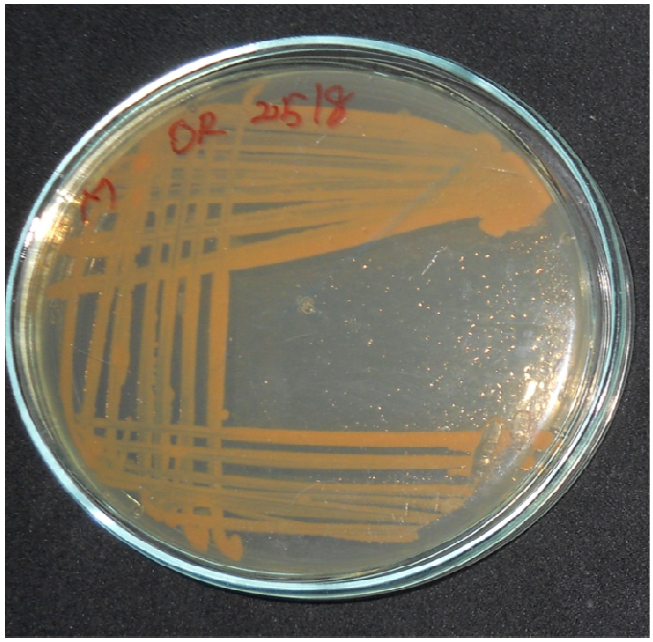

Fig 1: *Planomicrobium* Sp

UV-is spectrophotometer

A study on biosynthesis of TiO_2 nanoparticle by the culture broth of *Planomicrobium sp.*, was carried out in this work. The culture broth incubated with TiO_2 Fig. 2a showed a color change from yellow to dark white. Fig. 2b shows that control there is no color change could be observed in culture broth without addition of TiO_2. The TiO_2 were synthesized extracellularly from *Planomicrobium sp.*,. In Fig. 2 shows UV–Visible spectrum the absorption band at about 400 nm in the broad peak and the maximum synthesis can be observed at 24 hours from the beginning of 4 hour to 24 hour the absorption were increased, at 48 hours of incubation it starts to reduce the production. The similar result could be observed with biological synthesis of TiO_2 nanoparticles [20].

X-ray Diffractometer

The X-ray diffraction was used to confirm the crystalline nature of the synthesized nanoparticles. The Fig. 3 shows the XRD patterns of TiO_2 nanoparticles and it reveals four intense peaks in the whole spectrum of 2θ values ranging from 20° to 80°. A comparison of XRD spectrum with the TiO_2 particles formed in our experiments was in the form of nanocrystals. The peaks at 2θ values of 25.37º, 37.85 º, 48.09 º, 53.92 º, 55.10 º and 62.77 º corresponding to 101, 004, 200, 105, 211 and 204. This indicates conform clearly that good nanoparticles assisted by bacterium *Planomicrobium sp.*, are composed of pure crystalline TiO_2. The XRD spectra pure crystalline structures of TiO_2 nanoparticle have been published by the Joint Committee on Powder Diffraction Standards (file no. 84 -1285). the broadening of Bragg's peaks indicates the format The mean size of the as-prepared TiO_2 nanocrystals was measured

by using the Scherer equation of the full width of half maximum (FWHM) of ion of nanoparticle. The 101, refraction peak using the following equation:

D= K λ / β Cos θ

The equation uses the mention peak width at angle θ, where K is a shape factor, β is the width of the XRD peak at half height and λ is the wavelength. The mean size of the biosynthesized TiO_2 crystalline size was found to be 8.89 nm.

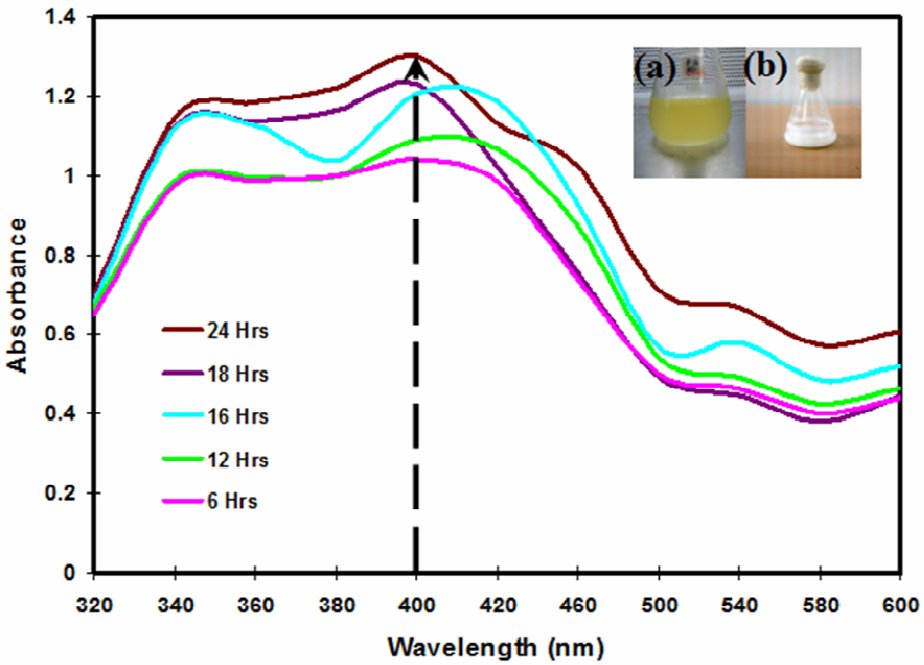

Fig 2: shows the absorption spectrum of TiO_2 nanoparticles synthesis and (a) In control without addition of TiO_2 (b) The colour changes and nanoparticles formation with addition of TiO_2

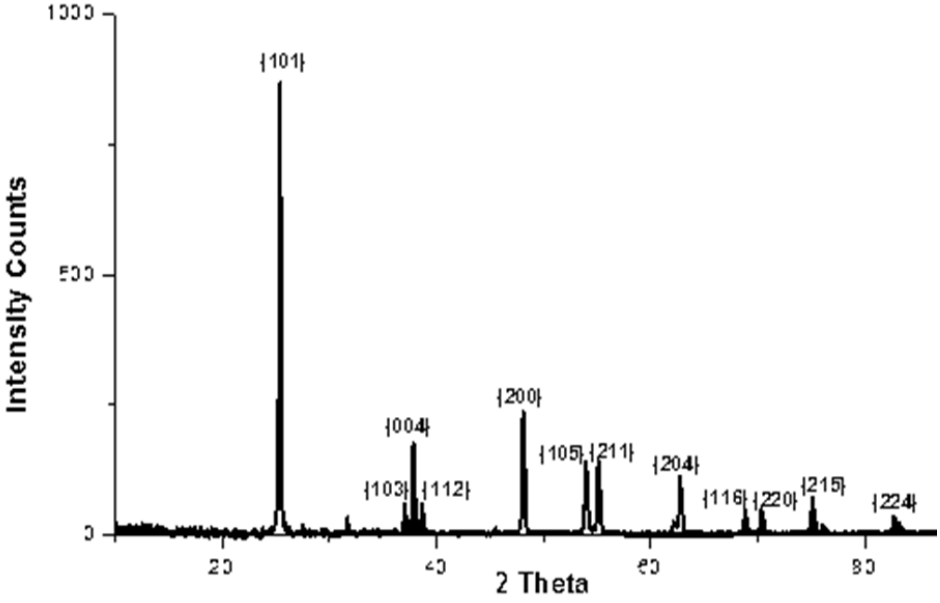

Figure 3: XRD pattern of TiO_2 nanoparticle

Scanning Electron Microscope

The Fig. 4 shows Scanning electron microscope images of TiO_2 nanoparticles. The TiO_2 nanoparticles were viewed at different magnification like 10,000X and the particles was approximately in the range of 100 to 500 nm (Scale bar 500 nm). The SEM image clearly indicates the particles were agglomerated and they formed irregular Shape. Few particles with were spherical in shape. Similar result of the TiO_2 nanoparticles shape was reported by using *Lactobacillus sp.*, [15].

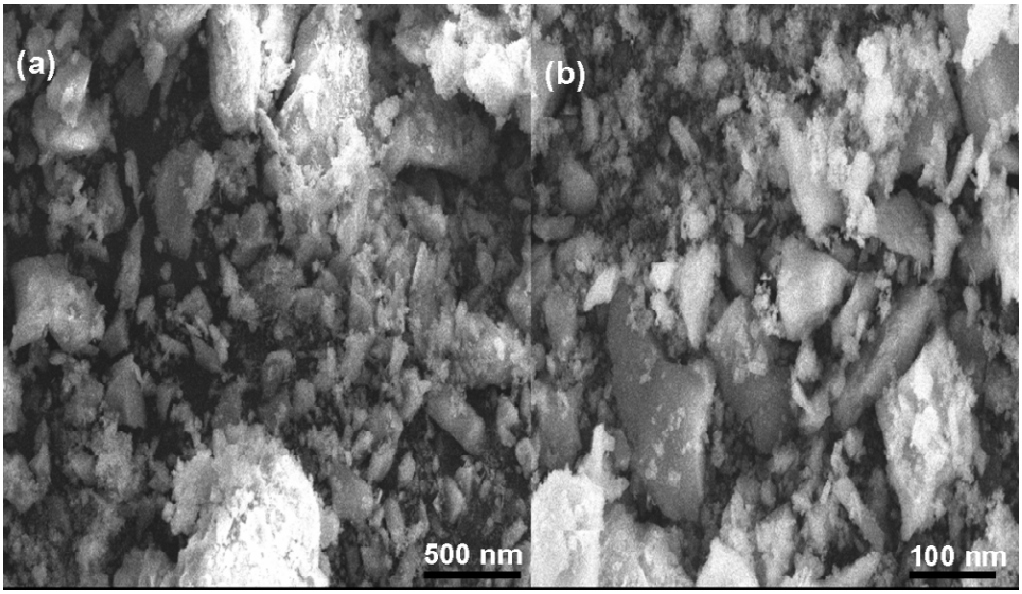

Figure 4: Shape of the TiO_2 Nanoparticles shown Scanning Electron Microscope (a and b)

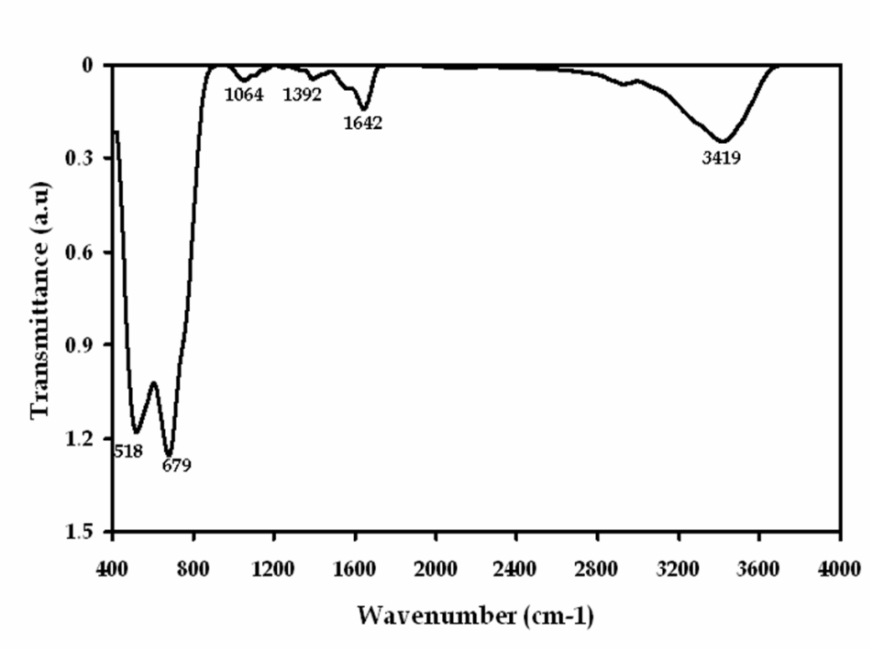

Figure 5: FTIR spectrum of the Tio_2 nanoparticles

FTIR

The Fig. 5, Table 1 shows the FT-IR spectrum data which will shown the results the peak at 518cm^{-1} indicates the Ti-O stretching vibrations , 1642 cm^{-1}, 1392 cm^{-1},and 1054 cm-1 had the slight peak level, 679 cm^{-1} O-H stretching

vibrations and bands revealed N-H stretching vibrations of primary and secondary amines respectively. 3419 cm^{-1} are identified as the phenol groups arise due to the O-H stretching vibrations. 1642 cm^{-1} can be assigned to the -C=C- stretching vibrations and 1392 cm^{-1} indicated C-H in plane bending vibrations of alkenes respectively. The band at 1064 cm^{-1} corresponds to the C-N stretching vibrations of aliphatic amines respectively. It has confirmed that the amines linkages of proteins have the stronger ability to bind metal, so that the proteins may possibly form a coat covering the metal nanoparticles to prevent agglomeration of the particles and stabilizing in the medium. Similar result of synthesized TiO_2 nanoparticles by using *Bacillus subtilis* either through the amines residues in the proteins and lipids [20].

Table 1: Detection of various functional groups by FTIR from *planomicrobium sp*

S. No	Group frequency cm^{-1} of the sample	Functional group assignment
1	518	Ti-O stretching vibrations
2	679	OH group, N-H stretching Vibrations 1º & 2º amines
3	1064	C-N stretching vibrations, Aliphatic amines
4	1392	alkenes - C-H bending vibrations
5	1642	-C=C- stretching vibrations
6	3419	phenol groups - O-H stretching vibrations

Antimicrobial activity of TiO_2 nanoparticles

Antibacterial activity of TiO_2 nanoparticles

Table 2 shows the antibacterial activity of TiO_2 nanoparticles were carried out by disc diffusion method against *Bacillus subtilis* (3053) and *Klebsiella platicola* (2727) purchased from (MTCC Mumbai India). The various concentrations of TiO_2 nanoparticles 50 µl, 100 µl and 200 µl. The formation of zone around the TiO_2 nanoparticles integrated discs clearly moved the antibacterial property of TiO_2 nanoparticles. The clear zones of inhibition while the standard antibiotic like Kanamycin. The inhibition of zone increased while the concentration of TiO_2 nanoparticles increased. The zone of ihibiton based on the average description, strong activities of 14 ± 0.33 and 17 ± 0.32 deduced *K.planticola* and *B.subtilis*, respectively. The mean of three replicates of zone of inhibition (mm) around disc with *planmicrobium sp.*, mediated TiO_2 nanoparticles is presented in the Table 2. The concentration of ppm level for 0.1 ppm, 0.2 ppm and 0.4 ppm. The differential sensitivity of Gram-negative and Gram-positive bacteria towards nanoparticles may be depends upon their cell outer layer attribute and their interaction with the charged TiO_2 nanoparticles. It was observed that the negative charge on the cell surface of Gram-negative bacteria was higher than that the Gram-positive bacteria. Moreover, the cell barrier of Gram-negative bacteria consists of an outer membrane collected of lipids and proteins which perform as a barrier and present effective protection against antibacterial agents. But the cell barriers of Gram-positive bacteria do not consist of a cell surface. TiO_2 synthesized were highly opportune bacteria, hence a great potential in biomedical applications and cosmetics [21].

Table 2: Grown inhibitions of *(a) Klebsiella planticola (b) Bacillus subtilis* the presence of TiO_2 nano particles

Concentration of TiO_2 nanoparticles	Zone of inhibition (mm in diameter)		
	B.subtilis	***K.planticola***	**ppm**
50 µl	9.6 ± 0.33	8 ± 0.33	0.1
100 µl	13 ± 0.33	11 ± 0.33	0.2
200µl	17 ± 0.32	14 ± 0.33	0.4
Kanamycin (control)	18 ± 0.66	22 ± 1.001	-

± *Standard deviation*

Antifungal activity of TiO_2 nanoparticles

The antifungal activity of TiO_2 nanoparticle was carried out against *Aspergillus niger* in Rose Bengal agar medium (Purchase from Microbiology Department of Sri Paramakalyani College, Alwarkurichi). The various concentrations of nanoparticles 100 µl, 200 µl, 300 µl and 400 µlmixed with medium and poured in Petri plates. The fungus were inoculated into the nanoparticles and then incubated for 48 hours at room temperature. After 48 hours, the plates show the susceptible growth of fungus and its exhibits the fungal growth was decreased at the increased concentration of TiO_2 nanoparticles. The positive control was maintained and the maximum growth inhibition was achieved at the concentration of 1ml of TiO_2 nanoparticles. In that figure 6 (a) is the positive control containing only medium with fungi and 0.1 ml, 0.5 ml and 1 ml of nanoparticles containing agar having the different level of fungal growth in figurers 6(b), 6(c) and 6(d) respectively. The figure 6(d) having low level of fungal growth because of higher amount of nanoparticles it shows the antifungal capacity of the nanoparticles.

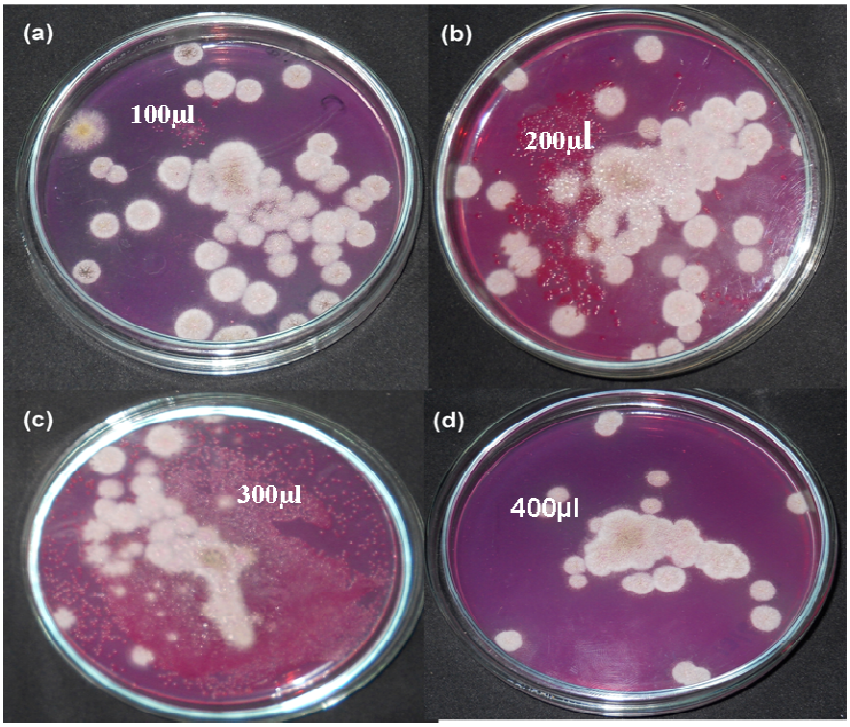

Figure 6: Growth of fungus *Aspergillus niger* on different concentration of TiO_2 nanoparticles containing medium.

CONCLUSION

In the present investigation, the TiO_2 nanoparticles was synthesized by using *Planomicrobium sp.*, which was isolated from contaminated ice cream and identified by biochemical test. The synthesis of Tio_2 nanoparticles was confirmed by colour change of the liquid medium from yellow to intense dark white in colour and it exhibited at maximum absorbance at 380 nm play a prominent role in reduction Tio_2 to TiO_2 nanoparticles. The X- ray diffractometer showed the crystalline nature of Nanoparticles. The possibility of protein as stabilizing material in TiO_2 nanoparticles is exposed by the FTIR analysis. The morphology of the TiO_2 nanoparticles observed using scanning electron microscope. The antibacterial activity of TiO_2 nanoparticles were carried out using various concentrations against *Bacillus subtilis* (3053), *Klebsiella planticola* (2727) and maximum was observed at 200 µl. The biosynthesis of nanoparticles is non-toxic, ecofriendly. The biologically synthesized nanoparticles are used for inhibit the growth of the pathogenic microorganisms, cosmetics, paints and food packaging material, etc.

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