ISSN: 2349 - 4891

International
Journal of Recent Research and Applied Studies
(Multidisciplinary Open Access Refereed e-Journal)

Saccharomyces cerevisiae Yeast Derived Gold Nanoparticles Synthesis, Characterization and Their Antimicrobial Activity

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Received 12th September 2016, Accepted 13th October 2016

Abstract

The yeast mediated synthesis and characterization of gold nanoparticles (AuNPs) were prepared and it also challenged against certain bacterial and fungal strains. In this present study, biosynthesized AuNPs were confirmed by analyzing the excitation due to the applied electromagnetic field of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 540 nm and the peak was observed between 500-600 nm. The SEM absorption of the product was recorded as synthesis of nanoparticles spherical in structure of about 90 nm in diameter. The EDS revealed the presence of pure gold nanoparticles in higher percentages. DLS-zeta potential showed negative charge (-30.5) which indicated that the sample is moderately stable at room temperature. The S. cerevisiae biomass extracts, in HAuCl4 peaks were observed at recorded in the region between 4000 and 400 cm-1. They include 3434 cm-1, 2363 and 2079.16 cm-1, 1637 cm-1 which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. In XRD, peaks were observed at 2θ of 38, 44, 65 and 77 are corresponding to the Bragg's reflections such as (111), (200), (220) and (311). The sample was most effective against Salmonella typhimurium NCIM 2501 (B5) while smaller effect was noticed from Micrococcus luteus NCIM 2871 (B4) in the bacterial division. But in fungi, which was effective against Trichophyton rubrum MTCC 3272 (F4) whereas smaller effect was observed in Candida albicans MTCC 1637 (F1).

Keywords: Gold nanoparticles (AuNPs), *Saccharomyces cerevisiae*, Antimicrobial activity, Yeast mycelia.

Introduction

The modern nanotechnology exploits the novel nanoscale properties of nanoparticles which has led to the production of a vast amount of engineered nanoparticles (NPs). Engineered NPs are deliberately manufactured by human activities to serve for special purposes and they are different from both incidental NPs that are produced as a side product of human activity, for example, from industrial processes or transport and from natural NPs, for example, humic substances, produced from weathering, microbial action or chemical hydrolysis (Shankar et al., 2004). Many different techniques have been developed and employed to generate metal nanoparticles, including gold nanoparticles. Those techniques for preparing nanoparticles have advanced rapidly over the recent decades and continue to evolve leading to more and improved control over the size and shape of the particles generated. Two fundamentally different approaches towards the controlled generation of nanostructures have evolved irrespective of the field or discipline (Ahmad *et al*. 2003).

The bottom up method (the chemical approach), where the atoms (produced from reduction of ions) are assembled to generate nanostructures, and the opposite approach, the top down method, also known as the physical method, where material is removed from the bulk material through grinding, milling, chemical methods or volatilisation of solid material followed by condensation of the vapour components, leaving only the desired nanostructures. Both approaches can be implemented in either gas, liquid, supercritical fluids, solid states, or in vacuum. Most of the manufacturers are interested in the ability to control one or more of the following aspects of the nanoparticles: a) particle size b) particle shape c) size distribution 16 d) particle composition and e) degree of particle agglomeration.

An important aspect of both approaches is the stabilisation of the particles to avoid aggregation and coalescence (Koziara et al., 2003). Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes ranging from 1-100 nm at this nanoscale, AuNps possess different physicochemical characteristics when compared to the bulk gold (Anita et al., 2011). The primary advantage of biological route is the ability, in theory to manipulate the properties of the nanoparticles by gaining control over the mechanism that determines their size and shape (Mazumdar et al., 1999); Misra et al., 2001). Many microorganisms, both unicellular and multicellular are known to produce inorganic materials either intracellularly or

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extracellularly (Mosmann, and Tim, 1983) often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. A number of different genera of fungi have been investigated in this regard and it has been shown that fungi are extremely good candidates in the synthesis of gold (Pattanayak and Sunita, 2008) or gold (Rahman et al., 2004). In this study, to find the successful synthesis of yeast *Saccharomyces cerevisiae* mediated gold nanoparticles (AuNPs) by using of simple, less cost effective biosynthesis method and also find its medicinal applications.

Materials and Methods

The preparation of *Saccharomyces cerevisiae* (which is purchased from bakery) biomass was grown in 500 ml Erlenmeyer flask containing 100 ml of sterile Malt Extract Glucose Yeast Extract Peptone (MGYP) broth. Incubation was with shaking (200 rpm) at 35°C for 4 days. The flasks were removed from the shaker and placed at 5 to 10°C, to let the mycelium settle. For synthesis of gold nanoparticles, the mycelial mass was separated from the sterile distilled water by centrifugation (1500 rpm) for 10 min; the mycelial pellets were weighed and used for the synthesis of gold nanoparticles. Preparation of metal stock solutions 333.79 g of $HAuCl_4$ in 1000 ml of distilled water were used to obtain the 10 M. Exposure of biomass to metal solutions Five grams of wet biomass were exposed to 50 ml of a sterilized aqueous solution of HAuCl4 at varying concentrations in 250 ml Erlenmeyer flasks and the flasks placed on a shaker at 200 rpm and incubated at 35°C for 4 days. After Incubation the color was changed and turbid was occurred, that indicated the presence of gold nanoparticles in the culture.

UV-vis Spectroscopy

The gold nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the Perkin Elmer spectrophotometer at a resolution of 1 nm (from 300 to 700 nm) in 2 ml quartz cuvette with 1 cm path length (Vignesh et al., 2014).

Scanning electron microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDS)

Scanning electron microscope (SEM) analysis the employed to characterization of size, shape and morphologies of formed nanoparticle. SEM gives high-resolution images of the surface of a sample is desired. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The Morphological characterization of the samples was done using JEOLJSM 5800 for SEM and EDS analysis.

Dynamic light scattering (DLS)

The DLS technique uses light to determine the size of particles in a solution. Light at a given frequency is sent through the solution from a laser. When the light interacts with the moving particles in the solution and is scattered, the frequency of the light is also changed. This change of light frequency is directly related to the size of the particles in the solution. The DLS is capable of measuring particles in the size range from a few nano-meters to a few micrometers. It is therefore applicable for determining the size of nanoparticles.

Fourier transmission infrared (FTIR) spectroscopy

FTIR is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It used to analysis of possible bio molecule and also bonding interaction between themselves. The FTIR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. The characterization of functional groups on the surface of AuNPs by *S. cerevisiae* biomass extract extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm−1 range at a resolution of 4 cm−1. The sample were prepared by dispersing the AuNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc.

X-ray diffraction

The XRD is a technique used to study phase composition of a sample, crystal structure, texture or orientation and is also determining the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis (Pandiyarajan et al., 2013).

Antimicrobial activity

The test sample was challenged against certain microbial strains (procured from MTCC and NCIM, India) for antimicrobial sensitivity using the disc diffusion method (Vignesh et al., 2013, Vignesh et al. 2012a, 2012b). The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholarae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1) and *Cryptococcus sp*. MTCC 7076 (F2). *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4), A sterile cotton swab was used to inoculate the bacterial and fungal suspension on surface of MHA and PDA agar plates (Vignesh et al., 2015a, 2015b). The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used (Beevi et al., 2012). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus) (Lakshmi praba et al., 2013). After incubation, the zone of inhibition was measured with ruler. All the trial was performed thrice and mean values were presented.

Results and Discussions

Biosynthesis of Au nanoparticles

The *S. cerevisiae* biomass aqueous extract solution and Gold chloride solutions were prepared separately. A quantity of 1.5 ml of *S. cerevisiae* biomass extract was mixed with 30 ml of 10-3 M of Gold chloride for the synthesis of gold nano particles. During gold nanoparticles synthesis, the change of color from pale yellow to dark pink colour suggested the formation of gold nanoparticles.

UV- Vis spectrum analysis

Biosynthesized AuNPs were confirmed by analyzing the excitation due to the applied electromagnetic field of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 540 nm and the peak was observed between 500-600 nm. The Figure 1 shows the UV absorption peaks of *S. cerevisiae* derived AuNps. The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions (Trease and Evans, 1972).

Scanning electron microscope (SEM)

The SEM absorption of the product was recorded as synthesis of nanoparticles spherical in structure of about 90 nm in diameter (Figure 2). The SEM image showing gold nanoparticles synthesized using *S. cerevisiae* extract confirmed the development of gold nanostructures.

Energy dispersive spectroscopy (EDS)

The EDS revealed the presence of pure gold (Figure 3) nanoparticles in higher percentages. Gold peak is higher than other peak. As EDS equipment works at low vacuum (1-270 pa) it allows to observe non-conducting samples without the need to cover them with a thin conductive film, and consequently no evidence of noise by the coating material (Valientic et al. 1995).

Dynamic light scattering of particle size and zeta potential analyses

Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly disparity index of particles in a colloidal suspension. The Figure 4 shows the particle size of the synthesized nanoparticle sample. After analyzing data, it was found that Au nanoparticles size were in the range of 50-100 nm. The highest fraction of Au-NP present in the solution was of 85 nm. From the plot it was evident that the solution was consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis. Zeta potential measures the potential stability of the particles in the colloidal suspension. Gold nanoparticles generally carry a negative charge. The synthesized gold nanoparticles from the *S. cerevisiae* biomass extract showed negative charge and were stable at room temperature. DLS-zeta potential showed negative charge (-30.5) which indicated that the sample is moderately stable at room temperature (Figure 5).

Fourier transform infra-red spectroscopy

FTIR gives the information about functional groups present in the synthesized gold nanoparticles for understanding their transformation from simple inorganic $HAuCl_4$ to elemental gold by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the bio-fabrication of gold nanoparticles mediated by the *S. cerevisiae* biomass extracts (Figure 6). The *S. cerevisiae* biomass extracts, in HAuCl4 peaks were observed at recorded in the region between 4000 and 400 cm-1. They include 3434 cm-1, 2363 and 2079.16 cm-1, 1637 cm-1 which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. These carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the bio-fabrication of the nanoparticles by the action of the protein or phytochemicals (Vetrichelvan Jegadeesan, 2002). The Figure 6 clearly illustrates the bio-fabrication of the AuNPs by the action of the secondary metabolites in *S. cerevisiae*.

X-ray diffraction

The XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesized nanoparticles particles. The XRD pattern of synthesized particles were analyzed and found peak profile of relevant particles. In this result, peaks were observed at 2θ of 38, 44, 65 and 77 are corresponding to the Bragg's reflections such as (111), (200), (220) and (311). Other peaks were also observed along with the main peaks. These compounds might be reason for the formation of other peaks (Figure 7).

Antimicrobial studies

The antimicrobial activity assay is AuNPs sample was challenged against various NCIM and MTCC microbes using the disc diffusion method. The test concentrations (15 and 30 µL/disc) produce zone on MHA and PDA plates for bacteria and fungi, respectively. The sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4) in the bacterial division. But in fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Candida albicans* MTCC 1637 (F1). The higher (30µL/disc) concentration got larger zone effect than the small (15µL/disc) concentration against certain microorganisms (Table 1). However, silver particles used in this study are negatively charged (Stoimenov et al., 2002; Sondi et al., 2003; Hamouda et al., 2000). While the mechanism of the interaction between these particles and the constituents of the outer membrane of microorganisms is unfortunately still unresolved, it

would appear that, despite their negative surface charge, they somehow interact with "building elements" of the bacterial membrane, causing structural changes and degradation and finally, cell death.

Conclusion

We developed an eco-friendly, simple and efficient method for the synthesis of gold nanoparticles using *S. cerevisiae*. The AuNPs are usually produced by the addition of a reducing agent to a solution of chloroaurate ions ($AuCl_4^-$), causing reduction of the gold ions and aggregation of the Au atoms into AuNPs. Different organic compounds are usually added to form a protective layer on the surface of the AuNPs, thus preventing their aggregation into larger particles. The AuNPs showed good biocompatibility and good stability for over 4 weeks. Therefore, they can be used for imaging and drug-delivery applications in the human body. According to the above reports, we conformed that this AuNPs may act an alternative nano-antibiotics in near future.

Acknowledgments

The author thank the Biospark Biotechnological Research Center (BBRC), Tiruchirappalli, Tamil Nadu, India for nanoparticle studies and also thank her mentor Dr. S. Vignesh, Head, Department of Microbiology, Thanthai Hans Roever College (Autonomous), Affiliated to Bharathidasan University, Perambalur – 621 220, Tamil Nadu, India for his valuable support and corrected this manuscript.

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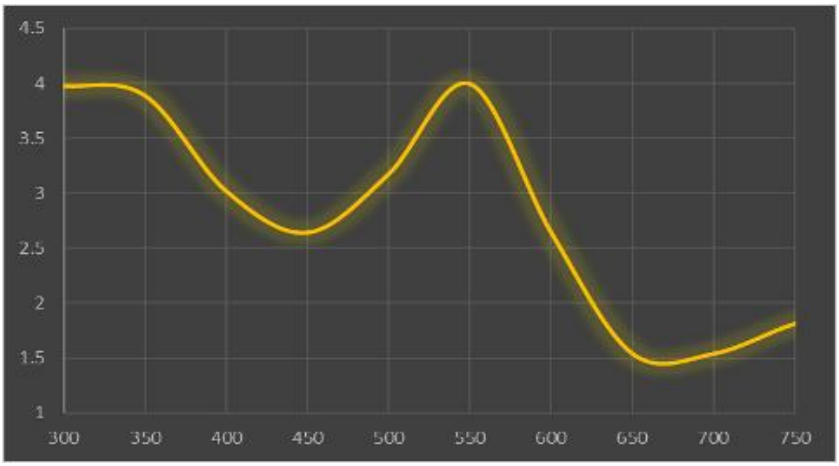

Figure I. Uv-Vis Characterization of AuNPs

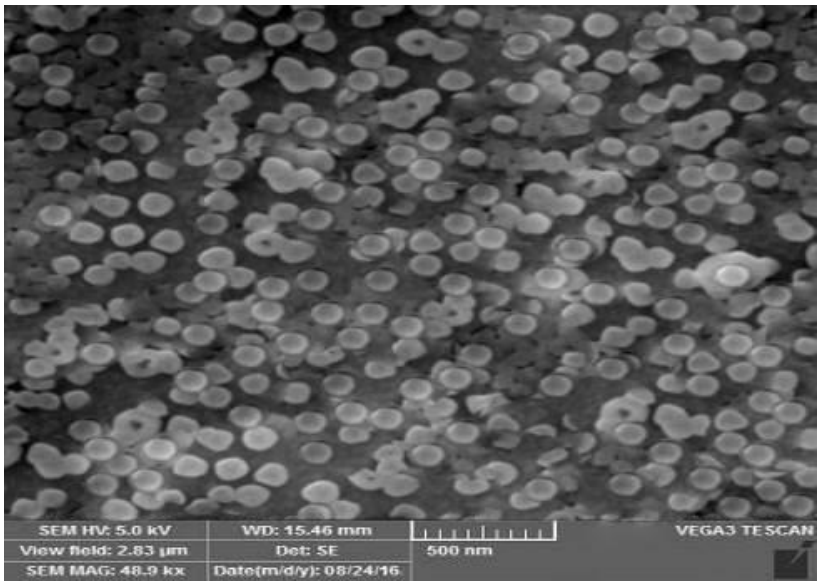

Figure II. SEM analysis of AuNPs

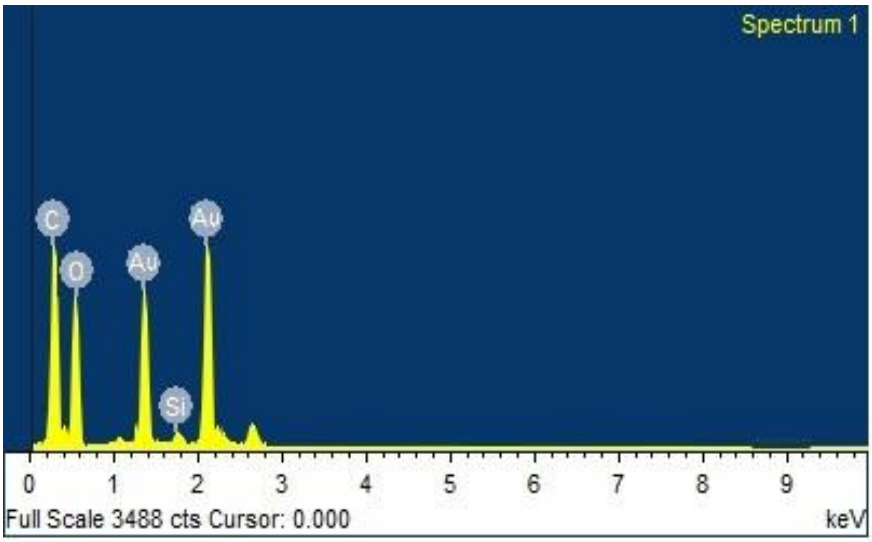

Figure III. EDS characterization of AuNPs

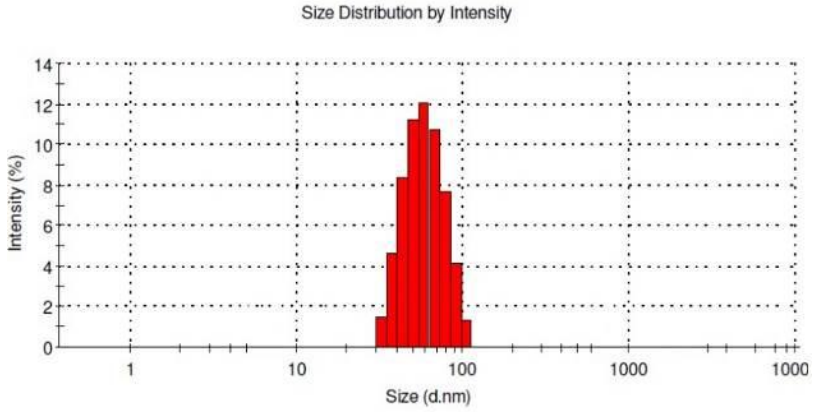

Figure IV. DLS-Size distribution of AuNPs

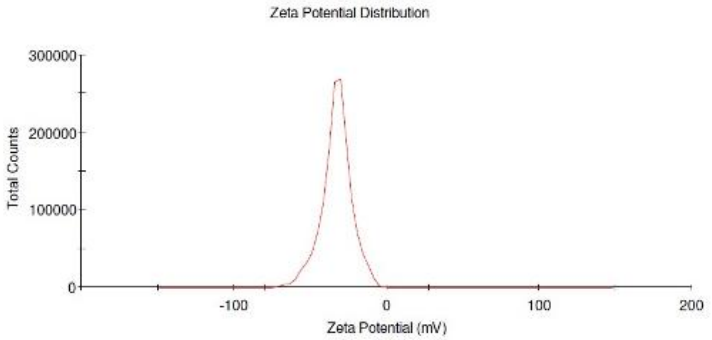

Figure V. DLS-Zeta potential of AuNPs

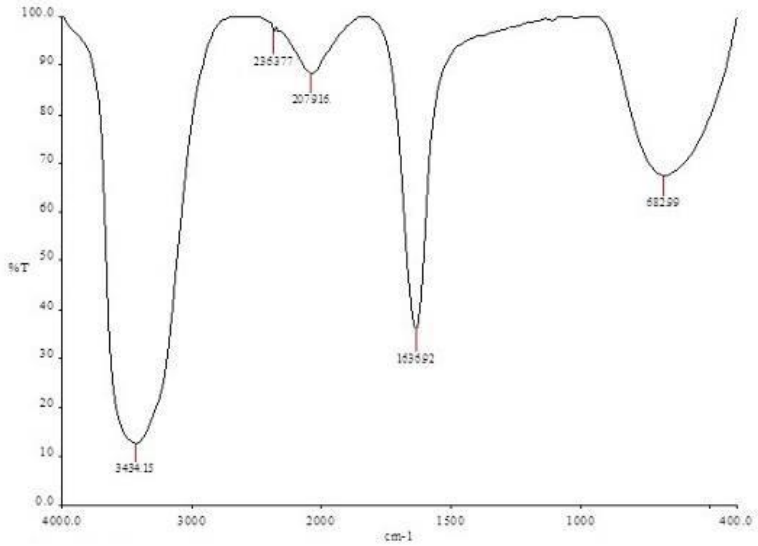

Figure VI. FTIR characterization of AuNPs

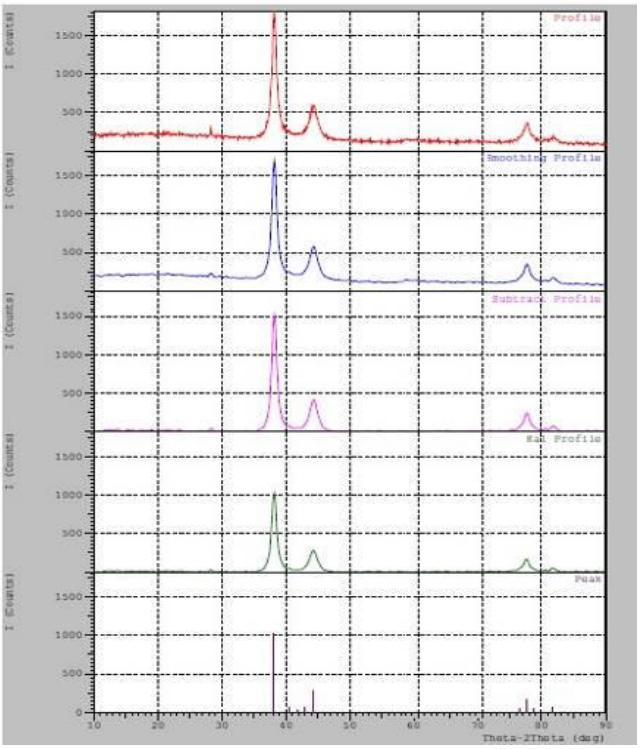

Figure VII. XRD characterization of AuNPs

Table I. Antimicrobial activity of *S. cerevisiae* derived AuNps

S.No	Test Microorganisms		AuNPs µL/disc		PC
Bacteria			15	30	10 mcg
1.	*Aeromonas liquefaciens*	B1	11	12	14
2.	*Enterococcus fecalis*	B2	13	14	8
3.	*Klebsiella pneumoniae*	B3	12	13	28
4.	*Micrococcus luteus*	B4	14	16	38
5.	*Salmonella typhimurium*	B5	11	13	0
6.	*Vibrio cholarae*	B6	13	14	16
Fungi					
7.	*Candida albicans*	F1	12	13	10
8.	*Cryptococcus* sp.	F2	12	16	9
9.	*Microsporum canis*	F3	11	15	9
10.	*Trichophyton rubrum*	F4	13	15	7

PC - Positive Control (Using antibiotic disc)
Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)