Nanotherapeutic drug synthesized by way of a microbial pathway targeting *Staphylococcus aureus*

Abstract:

Staphylococcus aureus (*S. aureus*) is one of the most common human pathogens causing various infectious diseases. Further, its ability to form biofilm and the emergence of antibiotic resistance strains has made it difficult to treat the infection. A nanoparticle-based therapeutic approach is an emerging area to treat *S. aureus* infection. Among the different methods to synthesize nanoparticles (NPs), the use of microorganisms to fabricate metal nanoparticles with the antibacterial property against *S. aureus* has been investigated by several studies. The microbial approach is cost-effective, eco-friendly, and devoid of toxic byproducts produced in other methods of nanoparticles formation. The review details the use of bacteria, fungi, yeast, algae, and lichens for producing nanoparticles of various metals, such as silver, gold, zinc, copper, iron, cerium, etc., of varying sizes and shapes and their effective use against *S. aureus*. The present review focuses on the reports of microbial-fabricated nanoparticles as therapeutic agents for treating *S. aureus* infection.

Keywords: Staphylococcus aureus, microbial synthesis, nanoparticles, antibacterial

Introduction

Due to pathogenic microorganisms and related infectious diseases, the modern world faces a difficult situation. *Staphylococcus aureus*, a Gram-positive bacteria belonging to the genus *Staphylococcus*, is one of the most aggressive and commonly found human pathogens (Tong et al., 2015). Although the substantial population infected with *S. aureus* generally remain asymptomatic, with bacteria commonly present on the skin or mucosal surface (Sakr et al., 2018), it

can gain access to the bloodstream through any cut present in the skin surface (McCaig et al., 2006) and consequently can cause debilitating infections like necrotizing fasciitis, impetigo, pyomyositis, *S. aureus* bacteremia, mediastinitis, osteomyelitis, septic arthritis, meningitis, infective endocarditis, etc. and thus affecting various tissues like blood, muscle, and skin and vital internal organs like bone, brain, lungs, hearts, etc. (David and Daum, 2017; Lowy, 1998; Tong et al., 2015). The problem is further made complicated by the emergence of drug-resistant strains of *S. aureus*, such as Methicillin-resistant Staphylococcus aureus (MRSA) that is resistant to the beta-lactam class of antibiotics, for example, methicillin, oxacillin, carbapenems, nafcillin, and cephalosporins (Rao et al., 2019; VanEperen and Segreti, 2016). The MRSA can be classified based on genotypic characteristics into either hospital-acquired MRSA (HA-MRSA), isolated from patients admitted to healthcare facilities such as nursing homes and hospitals, or community-acquired MRSA (CA-MRSA), found in the community with no previous history of contact with healthcare environment (David and Daum, 2010; Lindsay, 2013; Otto, 2013). The infections due to *S. aureus* affect many people and cause a significant number of death annually in the United States (Kourtis et al., 2019). Similarly, MRSA has also spread to other parts of the world. It is frequently isolated from different geographical regions, including East Asia, South Asia, the Middle East, Europe, and North Africa (Lakhundi and Zhang, 2018). The therapeutic approach against MRSA generally involves the application of a non-beta-lactam class of antibiotics such as vancomycin, daptomycin, delafloxacin (Bassetti and Righi, 2015). Vancomycin, a glycopeptide antibiotic, was initially used to treat MRSA strains. However, later new strains of *S. aureus* that were resistant to it were clinically isolated from different parts of the world; these strains were named vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (Appelbaum, 2006). Recently, a research group has isolated MRSA strains showing resistance towards delafloxacin, a fluoroquinolone antibiotic commonly used to treat MRSA infection (Iregui et al., 2019). The *S. aureus* strains resistant to delafloxacin were found in community-acquired MRSA and MRSA

isolated from healthcare-related infections, thus indicating the gravity of the problem caused by *S. aureus* (Iregui et al., 2019).

Similarly, other approved antibiotics, such as daptomycin, a lipopeptide antibiotic, also have witnessed resistance emerging against them by *S. aureus* (Stefani et al., 2015). Also, according to a report published by World Health Organization (2014), antibiotic-resistant strains pose a serious health risk to the population, a threat to food security, and a hindrance to the development of a country. Such antibiotic resistance strains have made the use of any conventional antibiotics obsolete and limited the efficacy of another therapeutic approach. In the face of such overwhelming odds, new therapeutic tools must be added to the shrinking arsenal of antibacterial agents in the fight against *S. aureus* infection.

Nanomedicine entails the application of nanotechnology in the medical field for screening, diagnosis, and therapy of the disease (Freitas, 2005; Fülöp et al., 2012). Nanomedicine has effectively emerged as a potential therapeutic alternative for leading cancer and other non-infectious diseases (Dube, 2019). However, nanotechnology for treating infectious diseases is continuously growing (Huh and Kwon, 2011; Blecher et al., 2011). Nanomaterials, specifically nanoparticles, are efficacious against various infectious diseases (Khan et al., 2016). The nanoparticles are small-sized particles generally between 1 and 100 nm (Kim et al., 2010). The nanoparticles that have been utilized in research studies against *S. aureus* are generally synthesized via three different methods: chemical, microbial-synthesized, and plant-synthesized. Chemical methods are inherently energy-intensive, involve complex methodology, and produce byproducts injurious to the environment (Li et al., 2011). The chemical method was in vogue a few years ago. However, it has given way to the more environment-friendly methods that utilize either microbial extract or biomass or plant extract as reducing and capping agents for the synthesis of nanoparticles.

On the other hand, although plant-mediated nanoparticles synthesis is time-efficient and straightforward, it generally produces polydispersed nanoparticles due to the presence of

phytochemicals, such as polyphenols, proteins, flavonoids, terpenoids, etc. (Ovais et al., 2016; Salunke et al., 2014). Further, the availability of a plant is hinged on certain factors, like the geography of the region and season of the year, that in turn affect the phytochemical profile of a plant extract to be used for the biogenic synthesis of nanoparticles (Singh et al., 2013). In comparison, the microbial route of nanoparticles synthesis is devoid of any such requirement, and the experimental conditions (temperature, pH, pressure, humidity, etc.) can be easily varied to control the size and shape of nanoparticles. The microbial methods take advantage of microorganisms like bacteria, actinomycetes, cyanobacteria, fungi, yeast, and algae to fabricate nanoparticles. The microbial-mediated synthesis of metallic nanoparticles can be either extracellular or intracellular, depending on the location where the formation of the nanoparticles takes place (Singh et al., 2013; Golinska et al., 2014). The extracellular synthesis has an advantage over the intracellular mode of synthesis because it does not require downstream processing to recover nanoparticles from within the confine of the bacterial cell wall (Iravani, 2014; Singh et al., 2016D). A distinct advantage of nanoparticles synthesis using microbial methods is that its production can be increased by scaling up microbial biomass via fermentation techniques (Moon et al., 2010).

Moreover, nature is abundant with various microbes containing diverse biomolecules that can reduce nanoparticle synthesis (Zhang et al., 2011; Li et al., 2011). The microbes-based synthesis of green nanoparticles involves bioreduction of the ionic state of metal (e.g., Au3+, Ag+) to the elemental form of the metal (e.g., Au0, Ag0) by biomolecules and enzymes found in microorganisms (Kharissova et al., 2013; Iravani, 2014). Further, the microbial biomass can not only be used to prepare metal NPs but can also be utilized for fabricating metal oxide NPs and bi-, tri-, or multi-metallic NPs. Given such advantages, it is no surprise that several research groups have successfully synthesized metallic nanoparticles with antibacterial potential against *S. aureus* from microorganisms.

Bacterial-fabricated nanoparticles against *S. aureus*

Bacteria is an excellent source for nanoparticle synthesis because of their ability to acclimatize to the surrounding environment, their ready availability, and contain biomolecules that can reduce metal ions into their corresponding nanoparticles and modify the surface of NPs functionalization (Ovais et al., 2018). Many bacterial species have been utilized to synthesize metal nanoparticles with toxic properties toward *S. aureus*. Bacteria belonging to many phyla, especially Actinobacteria, have been explored to synthesize nanoparticles showing antibacterial activities toward *S. aureus* in Table-1. Examples of bacteria belonging to the phylum Actinobacteria that have been studied for the synthesis of metal NPs to control the growth of *S. aureus* (Table-1). In one such study, Subashini and Kannabiran (2013) synthesized silver NPs from *Streptomyces* sp. and reported their antimicrobial activity against *S. aureus*. Similarly, in another study, biogenic silver NPs were synthesized from *Streptomyces aureofaciens*, an actinomycete, and found to be effective at a concentration as low as 50 μg/mL against *S. aureus* (Sundarmoorthi et al., 2011). Manikprabhu and group (2016) prepared spherical silver NPs with a size range of 4-50 nm using actinobacteria *Sinomonas mesophila* MPKL 26 in a sunlight-mediated green synthesis. They reported good antibacterial activity for the nanoparticles against the multi-drug-resistant strain of S. aureus. Similarly, Raja and John (2017) fabricated spherical silver NPs of 80 nm size from a marine *Micromonospora* sp., actinobacteria, and clinically-isolated drug-resistant S. aureus.

Although most of the studies using Actinobacteria for the nanoparticles synthesis fabricate silver NPs, there are reports that synthesized gold NPs (Balagurunathan et al., 2011; Shanmugasundaram et al., 2017; Jafari et al., 2018), zinc NPs (Rajamanickam et al., 2012), selenium NPs (Shaaban et al., 2018), and metal oxide NPs (Shaaban et al., 2018; Nabila and Kannabiran, 2018; Hassan et al., 2019), with antibacterial activities against *S. aureus*. For example, Balagurunathan et al. (2011) reported intracellular biosynthesis of gold NPs by *Streptomyces viridogens* strain HM10, isolated from Himalayan mountain soil, and the biogenic gold NPs demonstrated antimicrobial action against *S. aureus*.

In addition to actinobacteria, other bacterial species have also been reported for the biogenic synthesis of metallic NPs showing antibacterial properties against *S. aureus*. These include species belonging to the genera in Table-2. Nanda and Saravanan (2009) reported the extracellular synthesis of silver NPs of the size range 160 to 180 nm in a cost-effective process by utilizing the wild strain of *S. aureus* itself. Further, the authors showed that the synthesized silver NPs interfere with the cell wall synthesis of *S. aureus* and inhibit its growth (Nanda and Saravanan, 2009). In the same way, *S. aureus* and its drug-resistant strain MRSA were utilized to synthesize selenium NPs having antibacterial properties toward *S. aureus* (Cruz et al., 2018). The authors noted that the selenium NPs synthesized from *S. aureus* and *E. coli* had their highest antibacterial activities against the microbe *S. aureus* and *E. coli*, respectively. The authors proposed an attractive theory that the metallic NPs synthesized from a particular bacteria are generally most effective towards the same bacterial species from which they were synthesized (Cruz et al., 2018).

Similarly, Srivastava and Mukhopadhyay (2015) synthesized spherical-shaped selenium nanoparticles (SeNPs) with a 40–120 nm size range from non-pathogenic bacteria *Ralstonia eutropha. They reported* a very high (up to 99%) reduction in the growth of *S. aureus* in the presence of selenium NPs. Moreover, biogenic SeNPs exhibited higher efficiency, indicated by a lower MIC value of 100 µg/mL than conventional antibiotic ampicillin, with a MIC value of 250 µg/mL, against *S. aureus* (Srivastava and Mukhopadhyay, 2015). Selenium NPs synthesized by many other bacteria such as *Stenotrophomonas maltophilia* SeITE02 (Zonaro et al., 2015), *Enterococcus faecalis* (Shoeibi and Mashreghi, 2017), *Staphylococcus aureus* (Cruz et al., 2018), MRSA (Cruz et al., 2018), *Escherichia coli* (Cruz et al., 2018), and *Pseudomonas aeruginosa* (Cruz et al., 2018) also have been shown to inhibit the growth of *S. aureus*. Similarly, *Bacillus* sp. is also reported for the biosynthesis of selenium NPs that can kill *S. aureus* bacteria. For instance, Shakibaie and others (2015) synthesized selenium and selenium oxide NPs from MSh-1 strain of *Bacillus. They showed* that the nanoparticles inhibit biofilm formation by

clinically-isolated *S. aureus*, including other human pathogens. Coating medical implant devices with microbial-synthesized nanoparticles can be a novel approach to remove the possibility of *S. aureus* infection. For example, Sonkusre and Cameotra (2015) coated polystyrene, glass, and catheter surfaces with SeNPs synthesized from bacteria *Bacillus licheniformis* JS2 and found that the coated surface can inhibit the biofilm formation by *S. aureus.*

Bacteria can also synthesize nanoparticles of different morphology that act against *S. aureus*. Dhandapani and group (2014) grew ZnO nanocrystals of various shapes, such as spherical nanoflower, on the cotton fabric surface by successfully utilizing activated ammonia synthesized from ureolytic bacteria Serratia ureilytica. The authors further reported that the cotton fabrics loaded with ZnO NPs showed good antibacterial activity against *S. aureus*. Singh et al. (2015) obtained anisotropic silver NPs of various shapes such as nano bar, pentagonal, spherical, icosahedral, hexagonal, truncated triangle, and triangular shapes, with particle size ranging between 30 and 100 nm from *Bhargavaea indica* in an extracellular synthesis process. Further, the synthesized silver NPs exhibited antibacterial activity against *S. aureus*. They improved commercial antibiotics' antimicrobial activity, including lincomycin, vancomycin, novobiocin, penicillin G, cycloheximide, and rifampicin against *S. aureus* (Singh et al., 2015).

To combat *S. aureus*, bacteria-synthesized metal oxide and multi-metal nanoparticles have also been synthesized successfully from bacteria. For example, Taran et al. (2017) synthesized ZnO and TiO_2 NPs from the bacteria *Halomonas elongata* IBRC-M 1021 and reported the antibacterial activity of ZnO NPs against *S. aureus*. In another study, the bismuth oxide nanoparticles, i.e., Bi2O3, an oxide of non-toxic metal bismuth, were synthesized by Dalvand and others (2018) from the bacteria *Bacillus licheniformis* PTCC1320. The cube-shaped Bi2O3 NPs with sizes in the range of 26 to 62 nm were found to inhibit the growth of *S. aureus* in a concentration-dependent manner (Dalvand et al., 2018). Similarly, Ramasamy et al. (2016) prepared bimetallic gold-silver (Au-Ag) NPs from *Shewanella oneidensis* MR-1. They found that the bimetallic NPs are capable of inhibiting

the growth and inhibiting the biofilm formation of S. aureus. Besides metal oxides, cadmium sulfide (CdS) and zinc sulfide (ZnS) nanoparticles have been synthesized from bacteria and possess suitable antibacterial activities against *S. aureus*. For example, Malarkodi and group (2014) synthesized spherical-shaped CdS and ZnS NPs, with sizes 10 to 25 and 65 nm, from *Klebsiella pneumoniae* (strain MAA) extracellularly. Further, the authors found excellent antibacterial activities for CdS and ZnS NPs against *S. aureus* in a concentration-dependent manner. Similarly, CDs NPs with an average size of 6.7 ± 2.4 nm obtained from the cell-free extract of bacteria *Pseudomonas chlororaphis* CHR05 also showed excellent antibacterial properties toward *S. aureus* (Ashengroph et al., 2019).

Cyanobacteria, alternatively known as blue-green algae, are particular bacteria capable of deriving energy through photosynthesis. Recently, aqueous extracts of cyanobacteria *Trichodesmium erythraeum* were utilized to synthesize silver NPs with the cube-shaped and average size of 26.5 nm active against the tetracycline-resistant strain of *strain S. aureus* (Sathishkumar et al., 2019). The silver NPs with sizes in the range of 40 to 80 nm biosynthesized from cell biomass of cyanobacteria *Microcoleus* sp. isolated from mangrove acted as an excellent antibacterial agent toward *S. aureus* (Sudha et al., 2013). Uma Suganya et al. (2015) used protein extracted from cyanobacteria *Spirulina platensis* as a reducing and stabilizing agent to synthesize spherical-shaped gold NPs with sizes ranging from 2 to 8 nm. They reported the dose-dependent killing of *S. aureus* due to the piercing of its thick peptidoglycan layer by gold NPs. Similarly, an aqueous extract of *Spirulina platensis* was used by Sharma et al. (2015) for the extracellular synthesis of anti-*S. aureus* silver NPs. The extracts of cyanobacteria *Leptolyngbya* JSC-1 were used to fabricate silver and gold NPs with antibacterial potential toward *S. aureus* (Zada et al., 2018A; Zada et al., 2018B).

One crucial thing in the microbial fabrication of NPS is that the bacteria for the synthesis of nanoparticle that acts against *S. aureus* must be carefully chosen because nanoparticles of a metal

synthesized from two different bacteria may behave differently against *S. aureus*. For example, Wang and group (2016A) synthesized gold NPs from the bacteria *Microbacterium resistance. They found* no antibacterial activity for nanoparticles against *S. aureus*. In contrast, gold NPs synthesized from *Deinococcus radiodurans* (Li et al., 2016) and *Pseudomonas fluorescens* 417 (Syed et al., 2016) could inhibit the growth of *S. aureus*.

Several studies have investigated the reduction of metal ions into nanoparticles by bacterial extract and concluded that bacterial enzymes like reductase, proteins, etc., are responsible for metal ion reduction (Iravani, S., 2014). Although bacteria have been used frequently to synthesize nanoparticles for inhibiting and killing *S. aureus*, bacterial-mediated synthesis entails a time-consuming optimization step and complex nanoparticles extraction and purification step (Narayanan and Sakthivel, 2010; Iravani, 2014).

Fungi-fabricated nanoparticles against *S. aureus*

Fungi are another microorganism that has been used to synthesize effective metal nanoparticles versus *S. aureus* (Yadav et al., 2015; Khan et al., 2018A; Guilger-Casagrande and Lima, 2019). In comparison to the bacteria, NPs synthesis from fungi has advantages like tolerating high metal concentrations. They can endure higher flow pressure and agitation in bioreactors, the easier downstream processing, faster growth rate and easier maintenance, and excellent production of extracellular enzymes that can be used as a reducing agent (Mohanpuria et al., 2008; Narayanan and Sakthivel, 2010; Saha et al., 2010; Gudikandula and Maringanti, 2016; Feroze et al., 2020). Silver nanoparticles with antibacterial properties toward *S. aureus* have been synthesized from the various fungal genus in Table-3.

In a study, authors isolated filamentous fungi (*Aspergillus terreus* SP5, *Paecilomyces lilacinus* SF1, and *Fusarium* sp. MP5) from the soil of high altitude and cold climatic regions of eastern Himalayan. They used them to synthesize silver NPs that exhibited antimicrobial activity against *S. aureus* MTCC96 (Devi and Joshi, 2012). Furthermore, the mycosynthesized silver NPs

showed a synergistic effect with antibiotics like chloramphenicol, ciprofloxacin, erythromycin, and methicillin against *S. aureus* MTCC96 (Devi and Joshi, 2012). Likewise, Gudikandula et al. (2017) isolated multiple strains of *Basidiomycetes* from a forest region. Among these two strains, *Ganoderma enigmatic* and *Trametes ljubarskyi* were utilized to synthesize silver NPs that showed antibacterial activity against *S. aureus*. Similarly, Chan and Don (2012) employed two white-rot fungi, *Schizophyllum commune* and *Pycnoporus sanguineus*, for the biosynthesis of silver NPs and examined their potential to inhibit the growth of S. aureus. Silver NPs synthesized from *S. commune* were shown to be most effective against *S. aureus*, as observed by its zone of inhibition of about 2.0 cm (Chan and Don, 2012). Similarly, in another study, blight-causing pathogenic fungus *Cryphonectria* sp. was isolated from the stems of chestnut and was used for the extracellular synthesis of silver NPs (Dar et al., 2013). These silver NPs exhibited higher antibacterial activity than AgNO3 and conventional antibiotic streptomycin against *S. aureus* (Dar et al., 2013). Gudikandula and Maringant (2016) reported that fungus *Pycnoporus* sp. (HE792771) synthesized silver NPs showed more excellent antibacterial activity, with a higher zone of inhibition in the well-diffusion assay than chemically synthesized silver NPs toward S. aureus. Some studies used fungi synthesized metal NPs in combination with conventional antibiotics and reported synergistic antibacterial effect towards *S. aureus* at a lower concentration than when both metal NPs and antibiotics were used individually (Fayaz et al., 2010; Raheman et al., 2011; Devi and Joshi, 2012). Such a therapeutic approach enhances the efficacy of chemical antibiotics by reducing its minimum inhibitory concentration significantly against S. aureus.

Metal nanoparticles other than silver have also been synthesized from fungi and shown to be an excellent antibacterial agent to treat *S. aureus* infection. For example, gold NPs synthesized from marine endophytic fungus *Cladosporium cladosporioides* isolated from seaweed *Sargassum wightii* were found to inhibit the growth of *S. aureus* by disrupting its cell membrane (Hulikere M et al., 2017). Further, as per the authors, the bioreduction of gold metal salts to nanoparticles was

mediated by NADPH-dependent reductase and phenolic compounds present in the aqueous extract of the fungus (Hulikere M et al., 2017). In another study, authors fabricated gold NPs with spherical shape and size ranging from 10.3 to 38.7 nm from an aqueous extract of oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer reported its growth inhibition property against *S. aureus* (Bawadekji et al., 2018). Mohamed and group (2015) employed *Alternaria alternata* fungi to synthesize cubic-shaped iron oxide NPs with antibacterial potential toward *S. aureus*.

Similarly, Sidkey et al. (2016) fabricated iron NPs intracellularly and extracellularly from *Aspergillus foetidus*. The size of the iron NPS ranges from 31.53 to 61.94 nm and 80 to 370 nm for intra- and extracellular particles, respectively, and both for both types of nanoparticles showed the ability to inhibit the growth of S. aureus (Sidkey et al., 2016). Munusamy and group (2014) fabricated spherical-shaped cerium oxide NPs of size 5 to 20 nm from the precursor cerium chloride heptahydrate by using culture filtrate of fungi *Curvularia lunata* and further reported the antibacterial activity of synthesized nanoparticles versus *S. aureus*. Alrabadi et al. (2017) synthesized magnesium oxide NPs extracellularly by employing fungi *Trichoderma viride*. Moreover, reported better antibacterial activity for the green NPs than the antibiotic amoxicillin against *S. aureus*.

Similarly, Ganesan and others (2020) utilized an aqueous extract of fungus belonging to *Periconium* sp. To synthesize ZnO NPs with hexagonal shape and size of 40 nm and found excellent antibacterial activity for the nanoparticles toward *S. aureus* in a concentration-dependent manner. In an exciting study, the authors used the fungus *Aspergillus welwitschia* to fabricate oval and spherical shaped tellurium NPs that exhibited antibacterial activity against MRSA; S. *aureus* was resistant toward the tellurium NPs (Abo Elsoud et al., 2018). Recently, some fungal strains such as *Aureobasidium pullulans*, *Mortierella humilis*, *Trichoderma harzianum*, and *Phoma glomerata* were utilized to biosynthesize selenium and tellurium NPs (Liang et al., 2019). Although

authors did not study the antibacterial properties of fungal-synthesized selenium and tellurium NPs, these can be an excellent prospect for nanotherapeutic against *S. aureus* infection (Liang et al., 2019).

Yeast-fabricated nanoparticles against *S. aureus*

Yeast is another crucial category of organisms that have been used for the green synthesis of nanoparticles exhibiting anti-*S. aureus* activity. Like fungi, yeast also possesses more advantages than bacteria, such as fast growth rate, simple nutrient requirement, adept in producing enzymes in high amounts, etc. (Dinesh et al., 2011). Few studies have applied yeast to synthesize metal nanoparticles to counter the growth of *S. aureus*. For example, Dinesh and group (2011) employed *Candida* sp. VITDKGB, a marine yeast, was collected from Nicobar Islands, India, to synthesize silver NPs of 87 nm size and reported the excellent antibacterial activity of NPS against *S. aureus*. Mishra et al. (2011) synthesized near-spherical silver and gold NPs in the size range of 10–20 nm and 50–70 nm, respectively, from the yeast *Candida guilliermondii*. They found both types of nanoparticles to be active against *S. aureus*. In a similar study, silver and gold NPs of the average size of 30 nm and 5 nm respectively were synthesized using cell-free extract of fungus *Candida albicans*. They inhibited the growth of *S. aureus* (Ahmad et al., 2013). *Candida albicans* was also utilized in another study to synthesize silver NPs having an average size in the range of 20-8 nm and of various shapes such as spherical, rod-like, decahedral, triangular, and platelet-like exhibited antibacterial activity against *S. aureus* in agar disc diffusion test (Rahimi et al., 2016). Bhat and group (2015) synthesized silver NPs utilizing *Candida albicans* and investigated their antibacterial effect when used alone and combined with the antibiotic ciprofloxacin on *S. aureus*. The authors found antibacterial activity for silver NPs when used alone. They observed an increase in antibacterial activity of the antibiotic when used in combination with the silver NPs against *S. aureus* (Bhat et al., 2015). Jalal and group (2018) isolated yeast *Candida glabrata* from oropharyngeal mucosa of human immunodeficiency virus patients and synthesized

silver NPs. The synthesized silver NPs were spherical with size within the range of 2–15 nm and could inhibit the growth of *S. aureus* (Jalal et al., 2018). Waghmare et al. (2018) employed *Candida utilize* for the extracellular biosynthesis of silver NPs that was a spherical shape with size in the range of 20–80 nm and reported the bactericidal activity of silver NPs against *S. aureus* via agar disc diffusion assay. Eugenio and group (2016) biosynthesized silver NPs and AgCl NPs using yeast *Candida lusitaniae* isolated from the gut of termites and reported strong growth inhibitory potential of nanoparticles toward *S. aureus*. The yeast *Kluyveromyces marxianus* was employed for the bioproduction of silver NPs of spherical shape with a size range between 3 and 12 nm, and the obtained silver NPs showed antibacterial activity against the drug-resistant strain of *S. aureus* (Ashour et al., 2014). Badhusha and Mohideen (2016) biosynthesized silver NPs of different sizes and shapes by controlling pH from *Saccharomyces cerevisiae*; the synthesized silver NPs were toxic to the *S. aureus* growth in well diffusion test.

Metal nanoparticles other than silver metals have also been synthesized from yeast and investigated for their efficacy against *S. aureus*. To give an example, Moghaddam et al. (2017) used ZnO NPs of hexagonal wurtzite structure with size in the range of 10–61 nm synthesized from the yeast *Pichia kudriavzevii* and reported the effectiveness of green ZnO NPs to inhibit the growth of *S. aureus*. Similarly, Chauhan et al. (2014) reported extracellular biosynthesis of ZnO NPs from *Pichia fermentans* JA2 isolated from spoiled fruit pulp and observed zone of inhibition against *S. aureus* in disc diffusion assay. Peiris and group (2018) biosynthesized spherical-shaped titanium dioxide NPs (TiO_2NPs) with an average size of 6.7±2.2 nm using Baker's yeast, i.e., Saccharomyces cerevisiae. Further, the authors found that the combination of green TiO_2NPs and sunlight is an excellent antibacterial agent toward *S. aureus* (Peiris et al., 2018). In another study, Venkat Kumar et al., 2019) synthesized cadmium sulfide nanoparticles (CdS NPS) of spherical shape and in size range 50-60 nm using *Candida albicans* and noted that the CdS NPs are capable of inhibiting the growth of *S. aureus* in a concentration-dependent manner.

Algae-fabricated nanoparticles against *S. aureus*

Algae are the photosynthetic eukaryotic organisms. That belong to a diverse group containing both unicellular and multicellular organisms. Algae are rich in biomolecules like carbohydrates, protein, fats, nucleic acids; pigments like carotenoids, chlorophylls, and phycobilins; and important secondary metabolites like alkaloids, terpenes, polyphenols, etc. (Michalak and Chojnacka, 2015). These natural chemicals can be an excellent reducing and stabilizing agent for the synthesis of nanoparticles. Algae, both microalgae and macroalgae have been employed in the eco-friendly and cost-effective green synthesis of metal NPS with the ability to kill *S. aureus* bacteria. To illustrate, Aziz et al. (2015) used a freshwater green algae *Chlorella pyrenoidosa. They reported* successful biosynthesis of silver NPs with the size distribution of 8±2 nm showing antibacterial activity against *S. aureus.* According to the authors, the silver NPs capped with protein disrupted the *S. aureus* cell membrane, reached inside the cells, and caused the production of active oxygen species, thus killing the bacteria (Aziz et al., 2015). Similarly, good antibacterial activity against *S. aureus* was exhibited by silver NPs, of spherical and triangular shape and size in the range of 5 to 25 nm, synthesized from marine green algae *Caulerpa racemosa* isolated from the South-East coast of India (Kathiraven et al., 2015). Green algae *Caulerpa serrulata* was used by Aboelfetoh et al. (2017) to synthesize silver NPs with spherical shape and an average size of 10±2 nm to check the growth of *S. aureus*. The green algae such as *Chlorella Vulgaris* (Annamalai and Nallamuthu, 2016; Soleimani and Habibi-Pirkoohi, 2017), *Enteromorpha flexuosa* (wulfen) J. Agardh (Yousefzadi et al., 2014), *Spirogyra* sp (Pinjarkar et al., 2016; Salari et al., 2016), *Urospora* sp. (Suriya et al., 2012), *Pithophora oedogonia* (Mont.) Wittrock (Sinha et al., 2015), *Enteromorpha compressa* (Ramkumar et al., 2017) have also been reported for the synthesis of silver NPs with toxicity towards *S. aureus*. El-Rafie and group (2013) extracted water-soluble polysaccharides from red (*Pterocladia capillaries*, *Jania rubins*), green (*Ulva facial*), and brown (*Colpmenia sinus*) algae to reduce and stabilize silver ions for the preparation of silver NPs. The

functionalized NPs were found to be stable for an extended period, and cotton fibers immobilized with the nanoparticles showed potential to be used as an antiseptic wound dressing (El-Rafie et al., 2013). Silver NPs synthesized from brown marine weed *Sargassum wightii* Greville isolated from the infected silkworm *Bombyx mori* L. A strong zone of inhibition was obtained against *S. aureus* (Govindaraju et al., 2009). Similarly, other species of genus *Sargassum*, such as *S. cinereum*, *S. ilicifolium*, *S. wightii*, have also been utilized to synthesize silver NPs having the antibacterial potential to prevent *S. aureus* growth (Mohandass et al., 2013; Kumar et al., 2012A; Shanmugam et al., 2013). Further, another brown alga like *Ecklonia cava* (Venkatesan et al., 2016) and *Turbinaria ornata* (Krishnan et al., 2015) have also emerged as efficient vehicles for the anti-*S. aureus* silver NPs synthesis. Pugazhendhi et al. (2018), using marine red algae *Gelidium amansii* synthesized spherical-shaped silver NPs with size in the range 27-54 nm and reported its detrimental effect on *S. aureus* bacteria. Ethanolic extract of *Acanthophora specific*, marine red algae served as a capping and reducing agent in the formation of cubic-shaped silver NPs, with sizes ranging between 33 and 81 nm, that was destructive to *S. aureus* (Ibraheem et al., 2016). Similarly, Kumar et al. (2012B) also used *Acanthophora specifera* to fabricate silver NPs. However, in this case, the authors obtained spherical-shaped NPs, of 48 nm size that inhibited the biofilm formation by *S. aureus*. De Aragão and group (2019) extracted a polysaccharide from red algae *Gracilaria birdie*. They used it as a reducing and stabilizing agent for the synthesis of silver NPs to inhibit *S. aureus* growth. Aqueous extract of red algae *Amphiroa fragilissima* (Sajidha and Lakshmi, 2016) prepared silver NPs with effective antibacterial activity toward *S. aureus*. Silver chloride NPs with an average diameter of 9.8±5.7 nm prepared from *Chlorella Vulgaris* reduce the viability of *S. aureus* up to 98% in a dose-dependent manner (da Silva Ferreira et al., 2017).

Ramakritinan et al. (2013) synthesized silver, gold, and bimetallic silver-goldNPs (in three different ratios: 1:1, 1:3, and 3:1) from marine red alga *Gracilaria* sp. and found silver NPs and bimetallic NPs with Ag: Au ratio of 1:3 to be most effective against *S. aureus*. Similarly, the

aqueous extract of marine algae *Gracilaria corticata* was used as a reducing agent to prepare gold NPs that showed good antibacterial activity against *S. aureus* in well diffusion test (Naveena and Prakash, 2013). Abdel-Raouf et al. (2017) prepared gold NPs with the size distribution of 3.85–77.13 nm and distinct shapes like a spherical rod, truncated, triangular, hexagonal from the ethanol extract of red algae *Galaxaura elongate* and reported the efficacy of fabricated NPs toward both MRSA and *S. aureus*. In another study, an aqueous extract of green microalga *Chlorella Vulgaris* was utilized to prepare spherical-shaped gold NPs, with sizes ranging between 2 and 10 nm, that was found to be toxic to *S. aureus* in agar well diffusion assay (Annamalai and Nallamuthu, 2015). Gold NPs of various shapes like grain, triangular, and spherical were fabricated from diatom *Nitzschia* found to inhibit the growth of *S. aureus* (Borase et al., 2017). Diatom-fabricated gold NPs further enhanced the antibacterial effects of commercial antibiotics penicillin and streptomycin synergistically against *S. aureus* (Borase et al., 2017). Polydispersed gold NPs fabricated from marine red algae *Kappaphycus alvarezii* inhibited the growth of S. aureus in the disc diffusion assay (Rajasulochana et al., 2012). Abboud et al. (2014) reported the synthesis of majorly spherical-shaped copper oxide NPs (mixture of cuprous and cupric oxide NPs) of 5–45 nm dimension using brown algae *Bifurcaria bifurcata*. They showed the toxicity of nanoparticles to *S. aureus*. On the other hand, Arya et al. (2018) utilized green algae *Botryococcus braunii* to synthesize a mixture of cuprous and cupric oxide NPs. The green copper oxide NPs had cubical and spherical with an elongated shape and were an effective nanotherapeutic agent against *S. aureus (*Arya et al., 2018). Ishwarya and group (2018) prepared spherical ZnO NPs of 40-50 nm size by utilizing hot water extracts of marine seaweed *Sargassum wightii*. They recorded the anti-biofilm activity of ZnO NPs against *S. aureus*.

Lichen-fabricated nanoparticles against *S. aureus*

Lichens are the symbiosis between a fungus and an alga living mutually beneficial relationships. Lichens possess several secondary metabolites, antioxidants, etc., that can be used as reducing agents

for nanoparticle synthesis. There are very few studies using lichens to synthesize nanoparticles and their use as antibacterial nanotherapeutic against *S. aureus*. For example, Alavi et al. (2019) used an aqueous extract of lichen *Protoparmeliopsis muralis* to synthesize nanoparticles of various metals such as Ag, Cu, Fe_3O_4, TiO_2, and ZnO. The synthesized metal NPs were all in a spherical shape, and the average size was 33.49±22.91, 253.97±57.2, 307±154, 133.32±35.33, and 178.06±49.97 nm for Ag, Cu, Fe_3O_4, TiO_2, and ZnO NPs, respectively. Moreover, all metallic NPs were found to possess bacteriostatic and bactericidal properties against *S. aureus,* and the antibacterial activities of nanoparticles were in the following order: Ag > ZnO > Fe_3O_4 > Cu > TiO_2 (Alavi et al., 2019). Similarly, another lichen *Usnea longissima* was used to form spherical shaped silver NPs of 9.40–11.23 nm size, and the nanoparticles exhibited antibacterial activity toward *S. aureus* (Siddiqi et al., 2018). Din and group (2015) prepared silver NPs of 13 nm size from an aqueous extract of the lichen *Ramalina dumeticola* and reported the nanoparticles to be effective against MRSA.

Conclusion

Nanomaterials have spawned a great deal of attention from the scientific community. Similarly, interest in *S. aureus* is also increasing because it is a common pathogen causing severe infections and the growing emergence of new drug-resistant strains. It is no surprise that metal nanoparticles have emerged as a great prospect to treat infectious diseases in these scenarios. Although a chemical process also produces metal nanoparticles, it is energy-intensive, cost-intensive, and environmentally harmful due to the generation of toxic materials. The synthesis of metal nanoparticles from the microorganism to treat *S. aureus* infection is an excellent alternative to the problem. The abundance and diversity of the microbial world present us with an excellent opportunity for nanoparticle synthesis that is environmentally benign and economically cost-effective. This review discussed the production of metal nanoparticles using various microbes, such as bacteria, fungi, yeast, and lichens, and their application to combat the growth of pathogenic bacteria *S. aureus*, including its resistant strains. Nanoparticles of silver metal have been most commonly synthesized from microorganisms to

treat *S. aureus*. Further, nanoparticles of metals like gold, zinc, iron, titanium, copper, metal oxides, and multi-metals have also been synthesized from microbes by a substantial number of studies to combat the *S. aureus.* Though the use of microbial-fabricated metal nanoparticles as the bacteriostatic and bactericidal agent against *S. aureus* is shown to be effective by many studies, the research into their benefit and toxicity to humans must be appropriately investigated further so that they can be effectively used in practical clinical settings.

Figures and Legends:

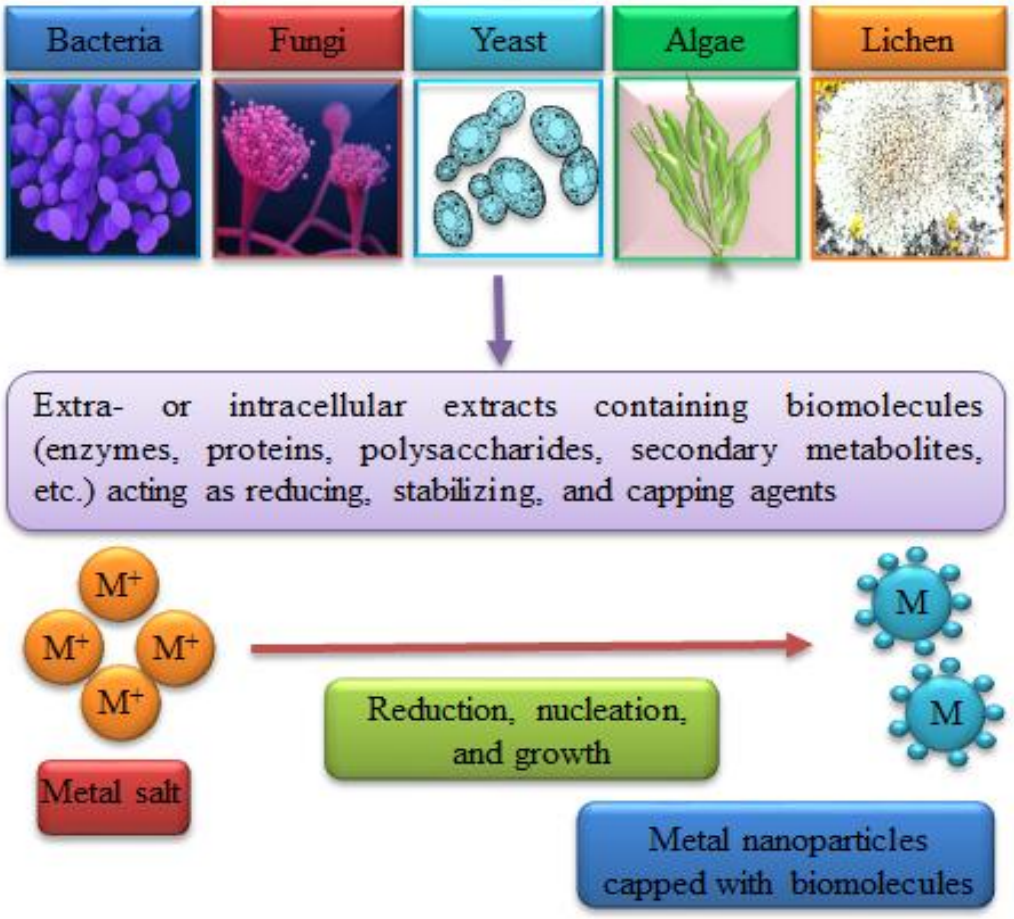

Figure 1: *Synthesis of metal nanoparticles from microorganisms. The lichen image is taken from* [*http://www.stridvall.se/lichens/gallery/Protoparmeliopsis/AAAA1582?full=1*](http://www.stridvall.se/lichens/gallery/Protoparmeliopsis/AAAA1582?full=1).

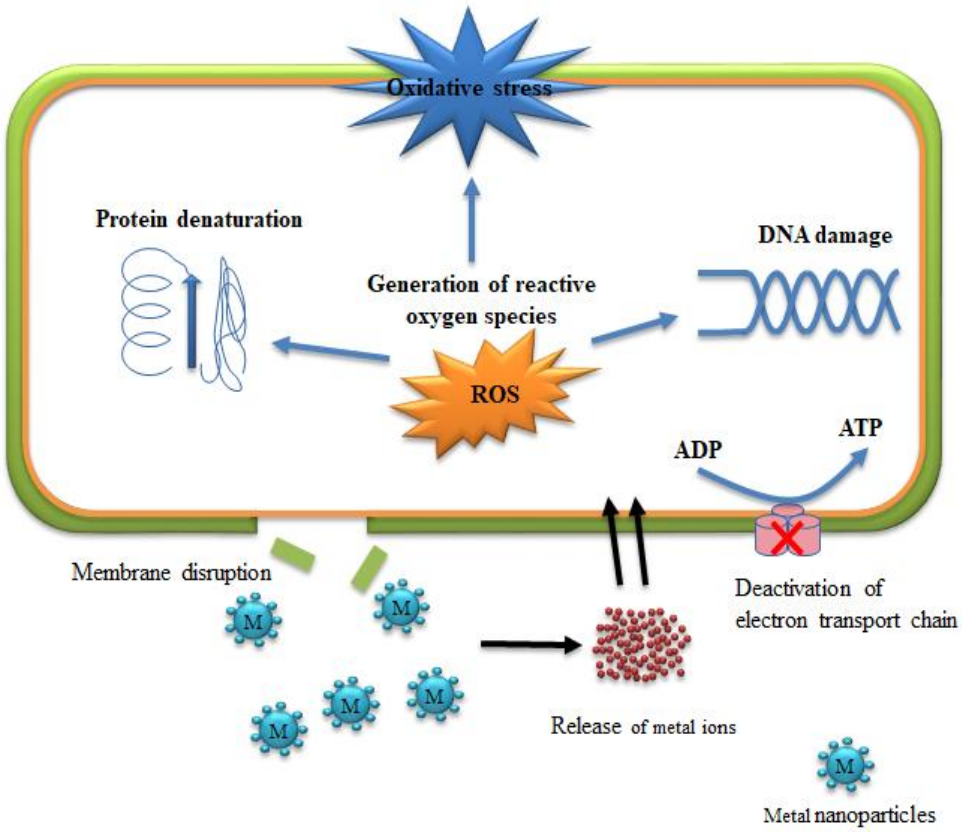

Figure 2: *Mode of antibacterial action of microbial-synthesized metal nanoparticles against S. aureus*

Table1: *Antibacterial activities toward* S. aureus strains

S.No.	Actinobacteria sp.	References
1.	*Streptomyces aureofaciens* MTCC 356	Sundarmoorthi et al., 2011
2.	*Streptomyces* sp. VITBT7	Subashini and Kannabiran, 2013
3.	*Streptomyces* sp. JAR1	Chauhan et al., 2013
4.	*Nocardiopsis* sp. MBRC-1	Manivasagan et al., 2013
5.	*Streptomyces enissocaesilis*	Shaaban et al., 2018
6.	*Streptomyces* sp. SSHH-1E	El-Naggar et al., 2016
7.	*Kocuria rosea* BS-1	Kumar and Sujitha, 2014
8.	*Nocardiopsis valliformis*	Rathod et al., 2016
9.	*Streptomyces viridogens* strain HM10	Balagurunathan et al., 2011
10.	*Streptomyces* sp. B5	Shanmugasundaram et al., 2017
11.	*Micrococcus yunnanensis* strain J2	Jafari et al., 2018
12.	*Streptomyces rochei MHM13*	Abd-Elnaby et al., 2016
13.	Actinobacteria SH11 strain	Wypij et al., 2017
14.	*Streptomyces xinghaiensis* OF1	Wypij et al., 2018
15.	*Corynebacterium glutamicum*	Gowramma et al., 2014
16.	*Streptacidiphilus durhamensis*	Buszewski et al., 2018
17.	*Streptomyces* sp. Al-Dhabi-87	Al-Dhabi et al., 2018
18.	*Actinomycetes* VITBN4	Nabila and Kannabiran, 2018
19.	*Streptomyces zaomyceticus* Oc-5	Hassan et al., 2019
20.	*Streptomyces pseudogriseolus* Acv-11	Hassan et al., 2019

Table 2: *Metallic NPs showing antibacterial properties against S. aureus.*

S.No.	genera	References
1.	*Alcaligenes*	Divya et al., 2019
2.	*Aeromonas*	Singh et al., 2016A
3.	*Bacillus*	Velmurugan et al., 2014; Ghiuță et al., 2018; Saravanan et al., 2018B; Sunkar and Nachiyar, 2012; Rehman et al., 2019; Shivashankarappa and Sanjay, 2015; Elbeshehy et al., 2015; Khiralla and El-Deeb, 2015; Dalvand et al., 2018; Piacenza et al., 2017; Deljou and Goudarzi, 2016; Zare et al., 2012; Shakibaie et al., 2015; Abdallah et al., 2019),
4.	*Delftia*	Shakibaie et al., 2019
5.	*Enterococcus*	Shoeibi and Mashreghi, 2017
6.	*Deinococcus*	Li Sundarmoorthi et al., 2016
7.	*Escherichia*	Cruz et al., 2018
8.	*Halococcus*	Srivastava et al., 2015
9.	*Halomonas*	Taran et al., 2018
10.	*Kinneretia*	Singh et al., 2016B
11.	*Klebsiella*	Shahverdi et al., 2007; Malarkodi et al., 2014
12.	*Lysinibacillus*	Bhatia et al., 2016
13.	*Novosphingobium*	Du et al., 2016
14.	*Ochrobactrum*	Thomas et al., 2014; Zonaro et al., 2
15.	*Pantoea*	Monowar et al., 2018
16.	*Pseudomonas*	Cruz et al., 2018; Barsainya and Singh, 2018; Ashengroph et al., 2019; Syed et al., 2016; Punjabi et al., 2018; Shakibaie et al., 2017; Gopinath et al., 2017; Banerjee et al., 2019; Pandey et al., 2018; Baker et al., 2015
17.	*Ralstonia*	Srivastava and Mukhopadhyay, 2015
18.	*Serratia*	Dhandapani et al., 2014
19.	*Shewanella*	Vaigankar et al., 2018; Ramasamy et al., 2016)
20.	*Sporosarcina*	Singh et al., 2016E; Rahimi et al., 2018
21.	*Staphylococcus*	Nanda and Saravanan, 2009; Rauf et al., 2017; Cruz et al., 2018
22.	*Stenotrophomonas*	Zonaro et al., 2015; Cremonini et al., 2018
23.	*Thermoactinomyces*	Deepa et al., 2013
24.	*Weissella*	Singh et al., 2016C

Table 3: *Silver nanoparticles by means of antibacterial properties toward S. aureus, synthesized from the a number of fungal genus*

S.No.	Fungus *genus*	References
1.	*Agaricus*	Mirunalini et al., 2012; ul-Haq et al., 2015; Sriramulu and Sumathi, 2017
2.	*Alternaria*	Ibrahim and Hassan, 2016; Shaheen and Abd El Aty, 2018; Singh et al., 2017
3.	*Amylomyces*	Musarrat et al., 2010
4.	*Aspergillus*	Nayak and Anitha, 2014; Rodrigues et al., 2013; Saravanan and Nanda, 2010; Bharathidasan and Panneerselvam, 2012; Naqvi et al., 2013; Rajakumar et al., 2012; Barapatre et al.; 2016; Fatima et al., 2016; Shahzad et al., 2019; Sagar and Ashok, 2016; Kathiresan et al., 2010; Ottoni et al., 2017; Binupriya et al., 2010; Nanda et al., 2018; Balakumaran et al., 2016; Li et al., 2012; Devi and Joshi, 2012; Netala et al., 2016; Khan et al., 2018B
5.	*Beauveria*	Prabakaran et al., 2016
6.	*Bionectria*	Rodrigues et al., 2013
7.	*Calocybe*	Mirunalini et al., 2012
8.	*Chaetomium*	Singh et al., 2018
9.	*Colletotrichum*	Azmath et al., 2016
10.	Cordyceps	Wang et al., 2016B
11.	*Cryphonectria*	Dar et al., 2013
12.	*Emericella*	Barapatre et al., 2016
13.	*Fusarium*	Ingle et al., 2008; Bawskar et al., 2015; Gholami-Shabani et al., 2014; Joshi et al., 2013; Husseiny et al., 2015; Mekkawy et al., 2017
14.	*Ganoderma*	Gudikandula et al., 2017; Jogaiah et al., 2017; Mohanta et al., 2016; Sriramulu and Sumathi, 2017; Mirunalini et al., 2012
15.	*Guignardia*	Balakumaran et al., 2015
16.	*Macrophomina*	Joshi et al., 2013
17.	*Monascus*	El-Baz et al., 2016
18.	*Mucor*	Aziz et al., 2016
19.	*Nigrospora*	Shaheen and Abd El Aty, 2018; Muhsin and Hachim, 2014
20.	*Penicillium*	Singh et al., 2014; Ma et al., 2017; Sarsar et al., 2015; Hamad, 2018; Shaheen and Abd El Aty, 2018; Majeed et al., 2016; Nayak et al., 2018; Bharathidasan and Panneerselvam, 2012; Feroze et al., 2020
21.	*Pleurotus*	Kaur et al., 2018; Debnath et al., 2019; Al-Bahrani et al., 2017; Devika et al., 2012; Mirunalini et al., 2012; Nithya and Raghunathan, 2009; Vigneshwaran et al., 2007
22.	*Paecilomyces*	Devi and Joshi, 2012
23.	*Pestalotia*	Raheman et al., 2011
24.	*Phenerochaete*	Saravanan et al., 2018A
25.	*Phomosis*	Bharathidasan and Panneerselvam, 2012
26.	*Pycnoporus*	Chan and Don, 2012; Gudikandula and Maringanti, 2016
27.	*Rhizopus*	Ottoni et al., 2017
28.	*Schizophyllum*	Chan and Don, 2012; Gudikandula et al., 2015
29.	*Sclerotinia*	Saxena et al., 2016
30.	*Scopulariopsis*	Hamad, 2018
31.	*Trametes*	Gudikandula et al., 2017
32.	*Trichoderma*	Kumari et al., 2017; Fayaz et al., 2010; Saravanakumar and Wang, 2018; Ottoni et al., 2017; Ahluwalia et al., 2014

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