Chapter 14
Anti-quorum Sensing Properties of Mushrooms

Zdenka Bedlovičová and Imrich Strapáč

Abstract The increasing resistance of pathogens to conventionally used antibiotics forces the scientific community to look for new ways of treatment for infectious diseases. It is the pressing need to find out the new sources of effective drugs. Nature is an unlimited source of substances with health-promoting properties. The discovery of new drugs, in general, is based on current knowledge of natural medicine, chemistry, and biology with the combination of modern technologies. Mushrooms are appreciated as naturally occurring source of compounds with nutritional, chemical, and medicinal qualities, so they can be used for the development of natural medicines and as a source of anti-infective agents. Quorum sensing is known as an intercellular mechanism of communication between microbes and is very important for life of microorganisms and population growth. These facts have made quorum sensing inhibition an interesting target in the development of the new antibacterial drugs. Mushrooms offer a variety of chemical compounds inhibiting quorum sensing such as flavonoids, phenolics, quinones, terpenoids, or vitamins and polysaccharides. The aim of this chapter is to summarize the results in the field of quorum sensing inhibition by mushrooms as a response in fighting with the microbial resistance.

Keywords Mushrooms · Quorum sensing · Microbial resistance · Antimicrobial activity

Abbreviations

AHL	Acyl homoserine lactone
AIPs	Autoinducing peptides
AIs	Autoinducers
GC	Gas chromatography
LC	Liquid chromatography

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MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
QQ	Quorum quenching
QS	Quorum sensing
QSI	Quorum sensing inhibition

1 Introduction

The increasing resistance in pathogens is a relevant reason to find out the new anti-infective agents. The researchers are forced to identify new chemical structures to develop novel drugs to treat microbial infections. Diseases caused by microorganisms (viruses, bacteria, fungi) are relatively common cause of mortality of patients worldwide (in the EU, there were 33,100 deaths during the years 2011–2012; in the United States, 50,000 people per year die on MRSA (methicillin-resistant *Staphylococcus aureus*) infections), but the alarming situation is in developing countries (Asfour 2018; Cassini et al. 2019; Chokshi et al. 2019).

Bacterial cells are capable of social interactions including quorum sensing (QS) as intercellular communication possibility. QS controls variety of extracellular functions, such as virulence, biofilm production, nutrient scavenging, and population growth. Inhibition of quorum sensing (QS) as the way of communication between bacterial cells then plays a noble target for developing new antibiotics and biocides (Asif and Acharya 2012; Azimi et al. 2020). Inhibition of QS can be executed by interfering with signalling pathways and/or intercepting with the signal molecules of quorum sensing (Zhang and Dong 2004; Rasmussen and Givskov 2006; Williams 2007). Naturally occurred chemical compounds represent a promising way to develop antibacterial drugs based on the QS disruption, for example, flavonoids and phenolics have been studied as inhibitors of virulence factors production and biofilm generation (Nazzaro et al. 2013).

In that context, the mushrooms are good candidate to be a source of bioactive compounds with anti-QS properties. They represent a valuable resource of bioactive compounds such as proteins, saccharides, fatty acids, vitamins, phenolic compounds, flavonoids, carotenoids, terpenes, lycopenes, anthraquinones, and minerals, indicating antioxidant, antimicrobial, antitumour, antiviral, and otherwise beneficial properties (Borchers et al. 2004; Obi et al. 2009; Bedlovičová et al. 2016; Lee-Hoon et al. 2020).

The aim of this chapter is to briefly introduce the readers into quorum sensing, quorum quenching, and capability of mushrooms to inhibit this intercellular communication.

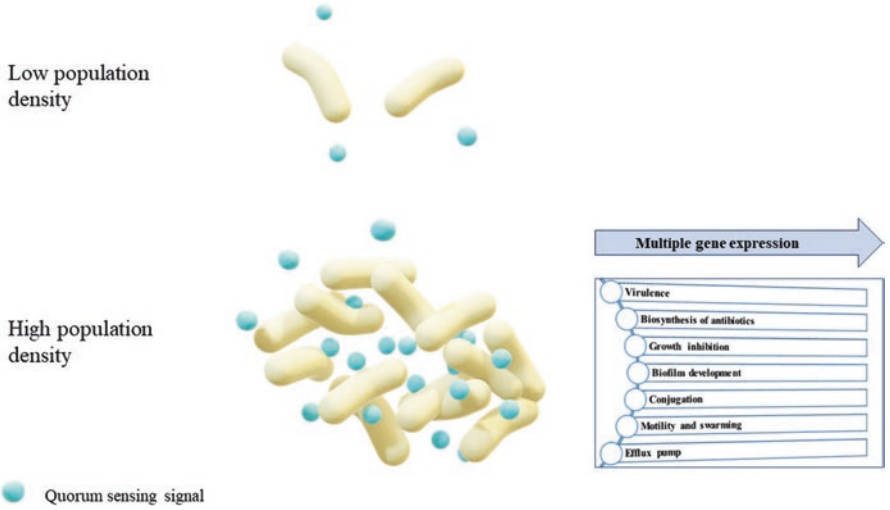

Fig. 14.1 The quorum sensing: at low cell density, the AIs are produced at essential concentrations, but when the cell density is increased, the signal molecules are produced at increasing concentrations to reach the quorum (threshold density) of cells. At this stage, the gene expression leads to accumulation of signals followed by population growth to induct of quorum sensing-dependent genes and to switch on QS-controlled features. These features differ between bacteria species

2 Quorum Sensing

The quorum sensing (QS), or cell-to-cell communication, is understood as social interaction of bacterial cells. Bacteria are able to co-operate and sense the information from other cells in the population to coordinate activities of every single cell when they reach a quorum (threshold concentration). This process is usually achieved through formation of small signal molecules (autoinducers) which are responsible for gene expression regulation and then controlling density of bacterial cell population. When the sufficient bacteria cell concentration is reached, the density of population increases, the synthesis of autoinducers (AIs) rises in the environment leading to threshold concentration of AIs followed by activation of repress target genes (Fig. 14.1) (Williams 2007; Deep et al. 2011; Wu and Luo 2021). Thus, mechanism of quorum sensing is based on the biosynthesis, release, and uptake of autoinducers accumulated in the environment.

Autoinducers regulate the expression of genes in another bacterial cells leading to control of bacterial responses, including variety of physiological processes such as virulence, formation of biofilm, antibiotics biosynthesis, etc. (Asfour 2018). The signal molecules are divided into three main groups. The first is a group of *N*-acyl homoserine lactones (AHLs) synthesized by Gram-negative bacteria to control density of population; the second class of AIs are oligopeptides (autoinducing peptides, or AIPs) consisting of 5–34 amino acids produced by Gram-positive bacteria for intercellular communication, and finally, the third main group of signalling

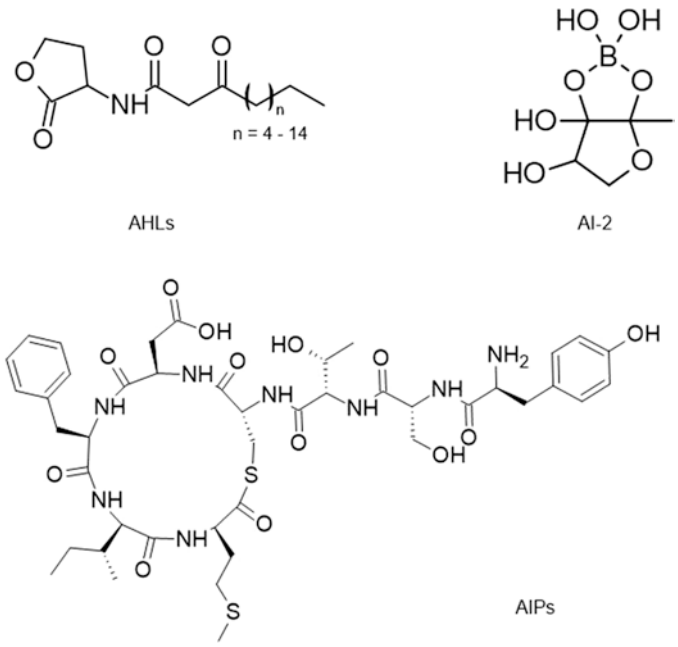

Fig. 14.2 The chemical structures of signalling molecules AHLs, AIPs, and AI-2

molecules are AI-2 (identified as a furanosylborate diester produced by members of the proteins of LuxS family) generated by both Gram-negative and Gram-positive bacterial cells for communications between different species (Xavier and Bassler 2003; Azimi et al. 2020) (Fig. 14.2).

According to the type of bacteria, various mechanisms of quorum sensing are proceeded. In Gram-positive bacteria, the precursors of autoinducing peptides are modified and transported by ATP-binding complex into extracellular environment. As the concentration of AIPs achieves the threshold level, the kinase protein is activated and the response-controlling protein is phosphorylated. Finally, this protein interacts with the target leading to the QS gene regulation. On the other side, in Gram-negative bacteria, signalling molecules directly diffuse into extracellular matrix. Signal molecules are accumulated and bind to the receptor and then form AI-receptor complex. This complex is ultimately bound to the target promoter leading to the QS gene regulation (Asfour 2018). It is necessary to mention that the concentration of signalling molecule increases with the bacterial cell population growth, but when the concentration reaches a certain level, molecules are diffused back into the intracellular matrix to regulate specific genes, for example, biofilm formation, production of antibiotics, or virulence factors (Finch et al. 1998; Zaki et al. 2013).

3 Compounds Inhibiting Quorum Sensing

A broad spectrum of compounds inhibiting QS has been reported. Several mechanisms of quorum sensing inhibition (referred as quorum quenching) were identified: (a) inhibition of the signal molecules (autoinducers) synthesis; (b) degradation of AIs by enzymes; (c) scavenging the signal molecules by antibodies and macromolecules; (d) competition with AIs in binding to receptor; (e) interfering with the binding of AIs to gene promoters leading to inhibition of gene expression (Kato et al. 2007; Morohoshi et al. 2007; Kalia and Purohit 2011; Kalia et al. 2014; Glamočlija et al. 2015a; Paluch et al. 2020).

3.1 Quorum Quenching

Quorum quenching is defined as inhibition mechanism of quorum sensing process. In general, it serves as effective help in inhibition of microbial communication, mainly when standard antibiotics and anti-infectives are inefficient due to resistance of microorganisms.

Quorum quenching as mechanism of disruption of the bacterial communication can decrease or definitely inhibit the virulence factors, for example, production of pyocyanin in *Pseudomonas aeruginosa* or violacein in *Chromobacterium violaceum* (Morohoshi et al. 2008, 2010; Mion et al. 2021).

Production of pyocyanin can be avoided by various compounds, for example, quaternary ammonium salts containing lipophilic alkyl chains (Piecuch et al. 2016), quinolin-2(1*H*)-ones (Morkunas et al. 2016), heterocycles including aminopyridine (Miller et al. 2015), or thiazolidine-2,4-diones (Froes et al. 2020). Violacein biosynthesis may be reduced by furanones (Morohoshi et al. 2007), secondary metabolites of *Halobacillus salinus* (Teasdale et al. 2009), maniwamycins (Fukumoto et al. 2016), etc.

The QQ mechanism is based on the enzymatic degradation of quorum sensing signalling molecules to avoid the cumulation of autoinducers and finally to inhibit expression of genes. For example, the enzyme AHL-lactonase produced by *Bacillus cereus* VT96 can directly degrade AHLs molecules by cleaving the lactone ring, so it is able to control the virulence of *P. aeruginosa* and *P. carotovorum* (Rajesh and Rai 2016). Another quorum quenching enzyme, MomL, isolated from marine *Muricauda olearia* Th120, has also been investigated as a novel type of AHL-lactonase (Wang et al. 2019). AHL-acylase (Sio et al. 2006) and/or oxidoreductase (Terwagne et al. 2012) can also degrade AHL signal molecules (Fig. 14.3).

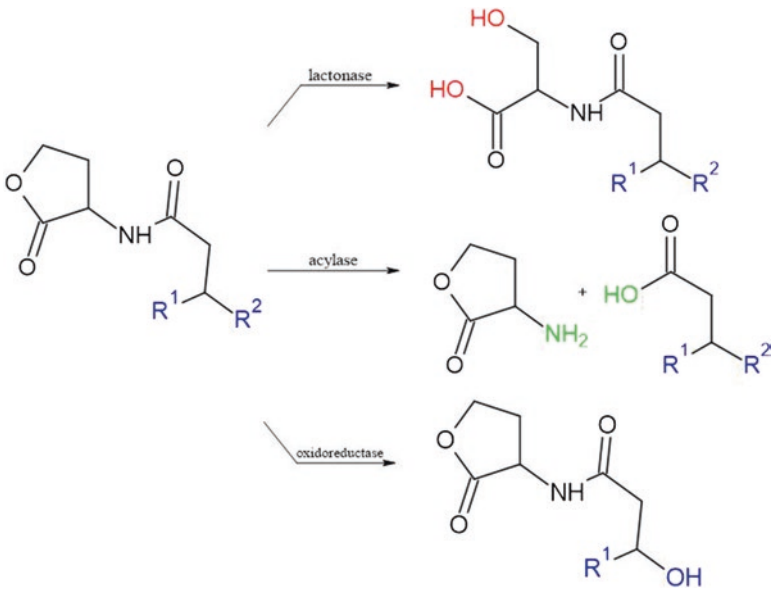

Fig. 14.3 AHL-deactivating enzymes – lactonases, acylases, and oxidoreductases – redrawn according to (Chen et al. 2013)

3.2 Methods of Determination of Quorum Quenching

As the knowledge of quorum sensing/quenching increased, the scientists are focused on finding new active quorum sensing inhibitors and investigating their properties. Many molecules have been successfully characterized and examined, but the finding of a single molecule which will inhibit all the mentioned quorum sensing mechanisms is improbable. Nevertheless, Kalia (2013) proposed some criteria for selecting an efficient QS inhibitor. The molecule should be small and chemically stable. A good QS inhibitor should be able to reduce gene expression regulated by QS. The inhibitor should also be highly specific for QS regulator, then it must not have any negative effect on the bacterial or host cells, and should be longer than native AHL (Kalia 2013).

The qualitative and quantitative measurements of QQ are proceeded using various methods, which can be classified as direct and indirect (biosensors are necessary). Most of the methods are based on the detection of autoinducers reacting with specific chemicals leading to color reaction which can be quantitatively determined (for example, by colorimetry) or have luminescence or fluorescence ability. Other analytical methods are capillary electrophoresis, TLC (thin-layer chromatography), HPLC (high performance liquid chromatography), and GC (gas chromatography) (Shaw et al. 1997; Teplitski et al. 2003; Yang et al. 2006). Liquid and gas chromatography coupled with mass spectrometry (LC-MS/MS; GC-MS) have been successfully used for the detection of AHLs (Cataldi et al. 2004; Purohit et al. 2013;

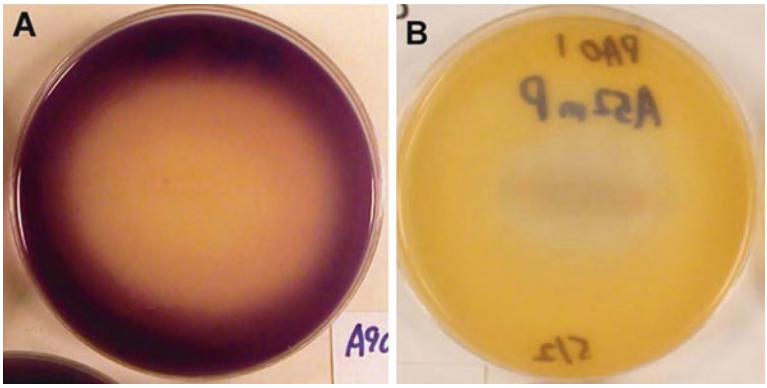

Fig. 14.4 Influence of C4 HSL and 3-oxo-C12 HSL production from *P. aeruginosa* PAO-1 on *C. violaceum* 12,472 (**a**) and *P. aureofaciens* 30–84 (**b**) overlay. (Permissions by Elsevier (McLean et al. 2004))

Patel et al. 2016; Huang et al. 2020), for example farnesol, and tyrosol produced by *Candida albicans* (Greguš et al. 2010; Pilařová et al. 2020), or peptides (Debunne et al. 2018).

Techniques based on bacterial biosensors have also been studied for AHLs detection. Bacterial biosensors represent a fast tool for detection of specific signalling molecules. Biosensors are genetically modified organisms of various species (*Pseudomonas aeruginosa, Vibrio fischeri*) which have the ability to detect quorum sensing molecules by proteins and bacterial pathways. These proteins are usually detected by optical or electrochemical methods. Most of the QS biosensors express a reporter gene from a quorum sensing response promoter. This promoter is getting activated immediately as a complex of signal molecule and quorum sensing transcriptional activator binds to the promoter (Rai et al. 2015). The detection of AHLs can also be applied by using of genetically modified bacterial strains producing bioluminescence. The most usually used assay is bioluminescence *Vibrio harveyi* BB170 method. This *V. harveyi* strain is disabled to produce AHLs and AI-2 due to deleted luxN gene, which encodes LuxN protein. The result of these mutations is that the bioluminescence is detected only if the exogenous AI-2 molecule is present in bacterial environment (O'Connor et al. 2016). The approach of using genetic modifications to create bacterial strains serving as quorum quenchers is also applicable (Oh et al. 2017).

Measurement of enzymatic activity is also the way of quantification of quorum sensing inhibition. As we mentioned, the quorum quenching inducting enzymes represent AHL-acylase, AHL-oxidoreductase, and AHL-lactonase (Chen et al. 2013). The capability of quorum quenching enzymes to decrease virulence of bacteria can also be examined by genome modification (Chen et al. 2013).

Biosensor strains, such as *Chromobacterium violaceum* CV026, *Pseudomonas aureofaciens* 30–84, or *Agrobacterium tumefaciens* A136, are quite commonly and

successfully used for the detection of QQ (Shaw et al. 1997; McLean et al. 2004; Zhu et al. 2012; Tang et al. 2013; Zaki et al. 2013; Tabbouche et al. 2017). The *C. violaceum* and *P. aureofaciens* methods are based on inhibition of the produced pigment violacein and phenazine, respectively (Fig. 14.4).

Some limitations were observed for these methods, they are time-consuming, low QQ is undetectable, and measuring of inhibition zones can be inaccurate (Liu et al. 2010; Tang et al. 2013; Lee et al. 2016).

4 Mushrooms as Quorum Sensing Inhibitors

Mushrooms are rich source of various compounds including fatty acids, amino acids, polysaccharides (in general β-glucans), minerals, secondary metabolites such as phenolics, flavonoids, β-carotenes, lycopenes, vitamins, terpenes, steroids, anthraquinones, benzoic acid derivatives, quinolines, organic acids, or high-molecular-weight molecules (peptides, proteins, nucleic acids) occurring in fruiting bodies, mycelia, and spores (Reis et al. 2012; Bedlovičová et al. 2016; Strapáč et al. 2019; Omer and Alfaig 2020). Mushroom-derived compounds possess a variety of biological activities, including antimicrobial properties (Petrović et al. 2014; Soković et al. 2014; Kostić et al. 2017; Strapáč et al. 2019). The presence of mentioned molecules is varying depending on the particular species of mushrooms, but in general, these compounds are based on phenolics, flavonoids, lactones, chitosans, quinones, coumarins, terpenoids, polysaccharides, and alkaloids (Glamočlija et al. 2015a; Bedlovičová et al. 2016).

De Carvalho et al. isolated coprinuslactone [(*3R,4S*)-2-methylene-3,4-dihydroxypentanoic acid 1,4-lactone] from edible mushroom *Coprinus comatus*, which interferes with QS and disperses biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (de Carvalho et al. 2016). Melanin from edible jelly mushroom (*Auricularia auricula*) has shown the antibiofilm activity regulated by QS (Bin et al. 2012).

Related studies showed that extracts of edible mushrooms are able to inhibit quorum sensing, but there is a problem to find out the mechanism of QSI (quorum sensing inhibition) because extracts are complex mixtures of different chemical compounds of various types. Some authors suggest that QSI is probably associated with the presence of phenolic compounds (Hossain et al. 2017; Strapáč et al. 2019; Vunduk et al. 2019), others proposed furanone-like derivatives (Zhu and Sun 2008), but in general, the exact compounds presented in extracts, which are responsible for anti-quorum sensing properties, are still unknown, so relevant studies are needed to clarify the mechanism of QS inhibition (Petrović et al. 2014; Glamočlija et al. 2015a, 2015b; Tabbouche et al. 2017; Gurgen et al. 2018; Yıldız et al. 2019).

As already mentioned, several studies related to the QSI by extracts of mushrooms were released (Table 14.1).

An interesting study was revealed by Koc et al. (2020), in which an extract of mushroom *Tricholoma terreum* was used as chitosan-based film producer.

Table 14.1 Quorum sensing inhibition by extracts of mushrooms

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
Tricholoma terreum	Water	*Chromobacterium violaceum* CV026	Inhibition of violacein pigment production	Koc et al. (2020)
Agaricus bisporus *Clitocybe nuda* *Lactarius volemus* *Macrolepiota procera* *Xerocomellus chrysenteron*	Water	*Pseudomonas aeruginosa* 119, 44	Microtiter plate method	Strapáč et al. (2019)
Pleurotus flabellatus	Water	*MRSA* *Escherichia coli* *Pseudomonas aeruginosa* *Proteus mirabilis* *Enterococcus faecalis*	Microtiter plate method	Vunduk et al. (2019)
Agaricus bisporus *Laccaria bicolor* *Bovista plumbea* *Lactarius deliciosus* *Boletus edulis*	Supercritical CO_2	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Yıldız et al. (2019)
Pleurotus ostreatus *Geastrum fornicatum* *Agaricus arvensis* *Amanita pantherina*	Methanol	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Gurgen et al. (2018)
Amanita rubescens *Lactarius* sp.	Ethanol	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Tabbouche et al. (2017)
Armillaria mellea	Methanol	*Pseudomonas aeruginosa* PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition	Kostić et al. (2017)
Polyporus squamosus	Methanol	*Pseudomonas aeruginosa* PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition	Fernandes et al. (2016)

(continued)

Table 14.1 (continued)

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
Agaricus bisporus *Agaricus bitorquis* *Agaricus campestris* *Agaricus macrosporus*	Methanol	*Pseudomonas aeruginosa* PAO1	Biofilm inhibition Twitching and flagella motility inhibition Disc-diffusion method	Glamočlija et al. (2015b)
Inonotus obliquus	Water Ethanol	*Pseudomonas aeruginosa* PAO1	Twitching and flagella motility inhibition Swarming Pyocyanin production inhibition	Glamočlija et al. (2015a)
Agrocybe aegerita	Methanol	*Pseudomonas aeruginosa* PAO1	Biofilm inhibition Twitching and flagella motility inhibition Disc-diffusion method Pyocyanin production inhibition	Petrović et al. (2014)
Agaricus blazei	Water	*Pseudomonas aeruginosa* PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition Disc-diffusion method	Soković et al. (2014)
Pleurotus florida	Methanol Chloroform	*Pseudomonas aeruginosa*	Swarming motility AHL inhibition Biofilm inhibition	Silambarasan et al. (2014)
Phellinus igniarius	Fermentation	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Zhu et al. (2012)

(continued)

Table 14.1 (continued)

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
Phellinus igniarius Auricularia auricula, Cordyceps sinensis Coriolus versicolor, Ganoderma luatdum, Inonotus obliquus Antrodia camphorata Lentinus edodes Pleurotus ostreatus Flammulina velutipe Sparassis crispa Agrocybe aegerita Agaricus bisporus Auricularia polytricha	Fermentation	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Zhu et al. (2011)
Tremella fuciformis	75% methanol	*Chromobacterium violaceum*	Inhibition of violacein pigment production	Zhu and Sun (2008)

Anti-quorum sensing activities of prepared chitosan-mushroom extract films were tested against various types of bacteria (*Escherichia coli, Salmonella typhimurium, Proteus microbilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans,* and *Bacillus thuringiensis*). The results showed that the combination of chitosan film with mushroom extracts is a good method for increasing anti-quorum sensing activity (26 ± 1 mm), due to much more inhibition capability of violacein production than gentamicin (12 ± 1 mm) or chitosan film without extract of *Tricholoma terreum* (9.1 ± 1 mm) (Koc et al. 2020).

Methanolic extracts of two different samples of *Polyporus squamosus*, a wild mushroom obtained from Serbia and Portugal, were subjected to the study of quorum sensing inhibition of *P. aeruginosa* by three methods. The first was antibiofilm activity tested at subinhibitory level (0.5 and 0.125 MIC). Inhibition of biofilm formation was observed only for extract of sample obtained from Serbia at the value of 88.3 ± 0.65%, 84.30 ± 0.55%, respectively. The inhibition of biofilm formation was better than ampicillin and streptomycin standards. The second QSI technique was study of inhibition of the twitching and flagella motility of *P. aeruginosa*. The sample from Serbia showed better activity than from Portugal, and also than the standard antibiotics. Pyocyanin production inhibition by *P. aeruginosa* PAO1 was the third method of anti-QS activity studies. *P. squamosus* extract of Portuguese sample showed higher ability to reduce pyocyanin production than Serbian sample and standard antibiotics. The strain of *P. aeruginosa* PAO1 produced a significant amount of pyocyanin (83.12%) and the methanolic extract of studied mushroom from Portugal inhibited this production to an amount of 44.5%. The QSI mechanism of action is unclear nevertheless the authors also determined chemical composition of extracts (fructose, rhamnose, mannitol, trehalose, fatty acids, organic acids, tocopherols) (Fernandes et al. 2016).

These methods for QSI study were also used for methanolic extract of *Armillaria mellea* (honey mushroom). The effect of honey mushroom on *P. aeruginosa* biofilm formation was studied using 0.5, 0.25, and 0.125 MIC. The obtained results showed that the extracts were more effective than standard antibiotics (streptomycin and ampicillin), and biofilm inhibition was in a concentration-dependent manner (for 0.5, 0.25, and 0.125 MIC, the inhibition was determined at the values of 69.8, 45.89, and 17.01%). *A. mellea* methanolic extract also reduced the twitching motility of *P. aeruginosa*. The anti-quorum sensing activity of extract was also studied against pyocyanin production. The highest ability to inhibit pyocyanin production was observed for extract of 0.5 MIC concentration (38.47%), whereas the streptomycin exhibited 10.96% and ampicillin 15.84% reduction. The chemical composition of honey mushroom was also measured. The main components were carbohydrates, sugars (mannitol, trehalose, D-xylose, D-glucose, D-galactose), fatty acids, organic acids (malic, citric, fumaric, oxalic), polyphenols, and tocopherols. The authors claimed that the role of molecules in QS mechanism is elaborate, and there are more factors affecting the mechanism, so that it is important to study different mechanisms of action and specifically with the biomolecules present in the species of *A. mellea* (Kostić et al. 2017).

Ethanolic extracts of *Agaricus* species (*A. bisporus, A. bitorquis, A. campestris, A. macrosporus*) were also tested against quorum sensing. All the samples showed anti-biofilm effects (reduction was observed in the range of 53–87%), the best results were obtained for *A. macrosporus*. The reduction of biofilm formation by standard antibiotics was detected for streptomycin in 51% and for ampicillin in 31%. The QS inhibition zones obtained by disc diffusion method showed comparable results as ampicillin standard. On the other side, the streptomycin standard possessed the best anti-QS activity.

All the extracts also showed a promising inhibition of twitching of *P. aeruginosa* and flagella motility (Glamočlija et al. 2015b).

The methanolic extract of *Agrocybe aegerita* also possessed antibiofilm activity of *P. aeruginosa*. The tested extract at subMIC concentrations (0.5, 0.25, and 0.125 MIC) showed better ability to reduce biofilm formation than standard streptomycin and ampicillin antibiotics. The best results were observed for 0.5 MIC extract which reduced formation of biofilm in 82.24%, whereas ampicillin and streptomycin reduced biofilm generation by 30.84% and 50.60%, respectively. The QS zones of inhibition were designated by disc diffusion technique. The extracts of all concentrations showed a better anti-QS effect between 7.70–10.30 mm of inhibition zone, while ampicillin standard possessed lower activity, but at higher concentration (7.60 mm). On the other side, the streptomycin activity was much higher (15.50–22.06 mm). Pyocyanin pigment reduction was observed for all the *Agrocybe aegerita* extracts in concentration-depending manner. The best results were noticed for 2 MIC concentration of extract, and all the extracts showed better reduction of pigment than standard antibiotics used for determination. In addition, authors were also focused on the twitching and flagella motility inhibition, which are responsible for initializing the formation of biofilm by *P. aeruginosa*. They observe reduction of

twitching and flagella motility by the extract, streptomycin reduced flagellas absolutely, ampicillin did not affect the flagella formation (Petrović et al. 2014).

Another study demonstrated that hot water extracts of *Agaricus blazei* reduced *P. aeruginosa* biofilm formation more effectively than commercial antibiotics (streptomycin and ampicillin). The QS-inhibiting zones were also observed in the range of 7.0–17.7 mm. Water extracts of *A. blazei* also much more efficiently reduced pyocyanin pigment formation at subMIC concentrations and are able to reduce motility of flagella and twitching (Soković et al. 2014).

Chaga mushroom (*Inonotus obliquus*) is a known medicinal mushroom. Glamočlija et al. studied its chemical composition and anti-quorum sensing properties (Glamočlija et al. 2015a). The organic acids presented in the extracts were oxalic acid, phenolic acids, such as gallic acid, protocatechuic, and *p*-hydroxybenzoic acid. All the extracts exhibited unequivocal activity against *P. aeruginosa* PAO1 biofilm formation, pyocyanin productions, and twitching and flagella motility (Glamočlija et al. 2015a).

Methanolic extracts of three cultivated mushrooms of *Pleurotus ostreatus* and three wild mushrooms (*Geastrum fornicatum, Agaricus arvensis, Amanita pantherine*), ethanolic extracts of *Amanita rubescens,* and *Lactarius* sp. collected in Turkey were subjected to anti-quorum sensing activity study by the method of inhibition of violacein pigment production by *Chromobacterium violaceum*. The authors found out that all the extracts of studied mushrooms demonstrated anti-QS activity due to inhibition of pigment formation without change of the bacterial count (Tabbouche et al. 2017; Gurgen et al. 2018).

Five edible mushrooms (*Agaricus bisporus, Clitocybe nuda, Lactarius volemus, Macrolepiota procera,* and *Xerocomellus chrysenteron*) were studied regarding their anti-quorum sensing properties using *E. coli* JM109 with pSB1142 plasmid reporter strain against *P. aeruginosa.* All the extracts showed significant anti-quorum sensing activity without affecting the growth of *P. aeruginosa* (Strapáč et al. 2019).

Zhu et al. (2011) tested 14 mushrooms against inhibition of violacein produced by *C. violaceum*. All the tested supernatants obtained by fermentation inhibited violacein production without affecting bacterial growth (Zhu et al. 2011, 2012).

Tremella fuciformis dimethyl sulfoxide extract was successfully subjected to violacein inhibition study. The studied mushroom extract inhibited violacein production without affecting the growth of *C. violaceum* (Zhu and Sun 2008).

In the study of Yıldız et al. (2019), four wild mushroom extract (*Lactarius deliciosus, Laccaria bicolor, Bolista plumbea,* and *Boletus edulis*) and one cultivated mushroom extract (*Agaricus bisporus*) prepared by extraction using supercritical CO_2 were tested. Three of four wild mushroom extracts possessed anti-quorum sensing activity using violacein pigment inhibition method. *Lactarius deliciosus, Boletus edulis,* and *Laccaria bicolor* remarkably reduced production of pigment produced by *C. violaceum*. The growth of bacteria was unvaried or only slightly affected. QSI was not noticed for cultivated *A. bisporus* (Yıldız et al. 2019).

Pleurotus florida methanolic and chloroform extracts were studied as anti-QS agents. Authors demonstrated that *P. florida* has the potential to inhibit signalling

molecules produced by *P. aeruginosa* and obstruct its virulence factors. A study of swarming motility indicated that extracts are able to reduce motility. Authors also determined inhibition of AHL (acyl-homoserine lactone) and biofilm formation in concentration-dependent manner. Inhibition of AHL for methanolic and chloroform extracts was in the range of 37.89–58.94% and 50.05–70.05%, respectively. These results are in correlation with biofilm formation inhibition study, when the methanolic extracts decreased the formation of biofilm in the range of 33.9–83.9%, while using chloroform extracts, it was between 60.7 and 82.1%. The authors declared that both types of the extracts showed considerable ability to inhibit QS, and chloroform extracts exhibited a higher percentage of inhibition of AHL and biofilm production (Silambarasan et al. 2014).

These findings propose that mushrooms have the ability to produce compounds serving as a source of anti-quorum sensing agents, but the key molecule and mechanism of action have not been clarified yet.

5 Conclusions

The problem of microbial resistance is the reality of the current world. This fact forced the research communities around the world to exploit new and alternative strategies to fight against harmful resistant, or lethal microbes. The good and promising approach is quorum sensing inhibition.

A broad spectrum of compounds inhibiting QS have been reported, and various mechanisms of inhibition quorum sensing were reported. Mushrooms as quorum sensing inhibitors are also studied, due to broad spectrum of pharmacological activities (antimicrobial, antiviral, immunomodulatory, or antioxidant). Mushrooms represent rich source of bioactive compounds, namely polysaccharides, proteins, peptides, or secondary metabolites, such as phenolic compounds, flavonoids, vitamins, terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolines, organic acids, which are perspective antimicrobial substances. The various mushroom extracts underwent the study of anti-quorum sensing activity by various methods, but with perspective and promising results. But, on the other hand, there is a quite difficult challenge to find a single molecule responsible for quorum sensing inhibition of mushroom extract, and finally to clarify the mechanism of action.

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