Chapter 15
Bioremediation Strategies for Removing Antibiotics from the Environment

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Abstract Antibiotics are used to treat/prevent infections in humans and animals, but their overuse causes soil, water, and environmental pollution. Additionally, this will lead to the rise of antibiotic-resistant bacteria and antibiotics resistance genes in environment causing serious health problems in human and animals. Bioremediation is an inexpensive and eco-friendly process that utilizes the living organisms (bacteria, fungi, algae, plants, and animals) to remove or detoxify pollutants within a given environment. This chapter summarizes recent scientific reports on the use of bioremediation strategies to remove antibiotics from the environment.

Keywords Bioremediation · Strategies · Antibiotics · Environment

15.1 Introduction

Antibiotics are major pharmaceutical compounds extensively used to treat/prevent human infections and increase feed conversion in animal pharming (Bunce and Hellyer 2018; Kirchhelle 2018). This results in world over increase in demand and production of antibiotics (Van Boeckel et al. 2014, 2015). The antibiotics were released into the environment through various anthropogenic activities like manuring and overthrown antibiotics ensuing soil, water, and environmental pollution. The antibiotics are persistent in the environment (Ezzariai et al. 2018; Tasho and Cho 2016) (Fig. 15.1).The selective pressures that are imposed by antibiotic compounds on bacterial population in the environment promote emergence of antibiotic-resistant bacteria (ARB) carrying antibiotic resistance genes (ARGs). The spread and acquisition of ARGs by clinically relevant bacteria led to develop serious problem for health of human and animals (Berendonk et al. 2015; Hinchliffe et al. 2018). The

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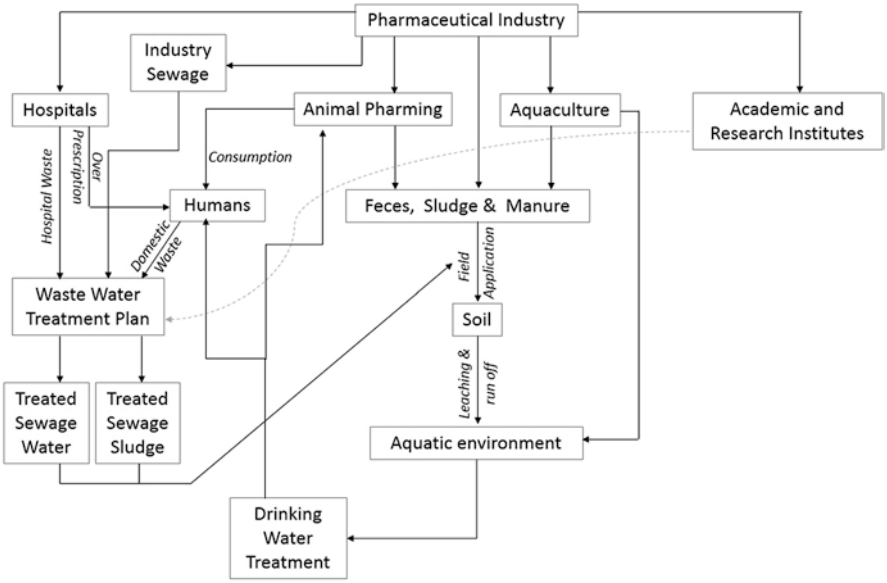

Fig. 15.1 Potential sources, pathways, and different entry routes of antibiotics in environment. (Du and Liu 2012; Li 2014; Tasho and Cho 2017)

large number of deaths are caused by antimicrobial resistance and that number is likely to rise to 300 million by 2050 if the problem is left unaddressed (Bunce and Hellyer 2018; O'Neill 2016). This chapter summarizes recent scientific reports on the use of bioremediation strategies to remove antibiotics from the environment.

15.2 Bioremediation

Bioremediation is a process that utilizes the living organisms (bacteria, fungi, algae, plants, and animals) to remove or detoxify pollutants within a given environment. Bioremediation approaches are generally classified as in situ or ex situ. In situ bioremediation involves treating the polluted material at the site while ex situ involves the removal of the polluted material to be treated elsewhere (Azubuike et al. 2016). The different types and strategies of bioremediation are shown in Fig. 15.2.

15.2.1 Bacterial Remediation: Removal of Antibiotics by Bacteria

The bacteria are most common group of organisms used for the bioremediation. Bacterial remediation can play an important role and offers inexpensive and eco-friendly option for removal of antibiotics from environment. The bacteria which is

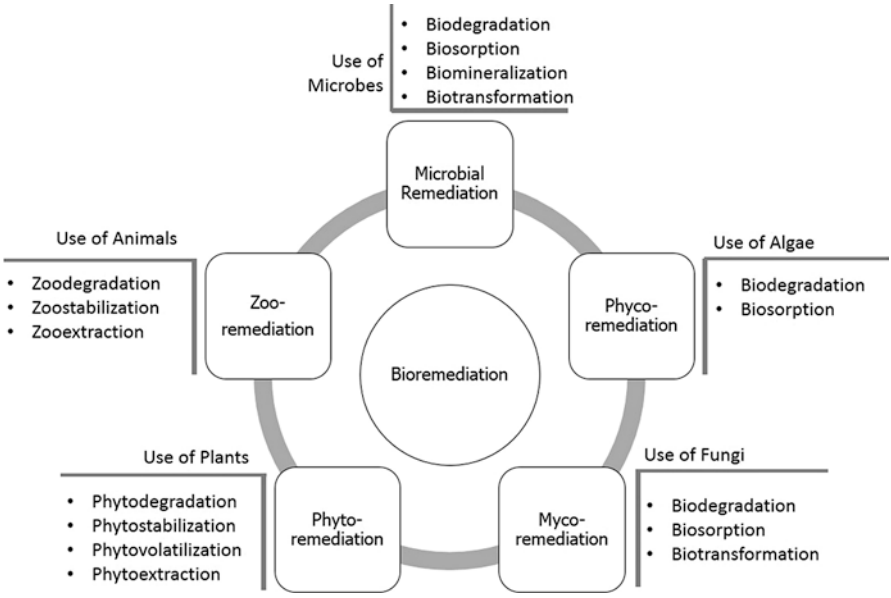

Fig. 15.2 Bioremediation techniques and strategies

used for bioremediation process should possess certain characteristics such as the ability to survive under extreme conditions (e.g., redox, moisture, nutrient, osmotic factor, and pH) and compete with indigenous microbial populations (Morikawa 2006). The exploitation of the bacterial methods such as biosorption and enzymatic biodegradation processes for the removal of heavy metals and antibiotics has been reviewed by Al-Gheethi et al. (2015).

The increased loads and persistent presence of antibiotics in the environment can cause selective pressure for bacteria that leads to the development of antibiotic resistance/or tolerance in bacteria. Antibiotic resistance and antibiotic tolerance do not seem to be equal. "Resistance" is used to describe the inherited ability of microorganisms to grow at high concentrations of an antibiotic, irrespective of the duration of treatment, and is quantified by the minimum inhibitory concentration (MIC) of the particular antibiotic, whereas "tolerance" is more generally used to describe the ability, whether inherited or not, of microorganisms to survive transient exposure to high concentrations of an antibiotic without a change in the MIC, which is often achieved by slowing down an essential bacterial process (Brauner et al. 2016).

A microbial bioremediation approach may involve both biosorption and biodegradation processes. Diverse strains of algae have been effectively employed for the removal of antibiotics (Table 15.1). Al-Gheethi et al. (2014) studied the biosorption of heavy metal ions and the antibiotic cephalexin from secondary effluents by the cell biomass of tolerant bacterial strains isolated from a secondary effluents generated by sewage treatment plants at Penang, Malaysia. The maximum biosorptive capacity of cephalexin was observed in mixed living cell biomass (60 mg g^{-1}). For

Table 15.1 Some antibiotic degrading bacterial species

Organism	Strain	Source	Biodegradation condition	Biomass	Removal	Time	References
Shewanella oneidensis	MR-1	Soil Microbiology Laboratory of Northwest Agriculture and Forestry University (Shanxi, China).	LB + 10 mg/L SPY or SMX, 30 °C	10^{-7} CFU/mL	SPY (23.91%); SMX (59.88%)	5 days	Mao et al. (2018)
Shewanella sp.	MR-4	Soil Microbiology Laboratory of Northwest Agriculture and Forestry University (Shanxi, China).	LB + 10 mg/L SPY or SMX, 30 °C	10^{-7} CFU/mL	SPY (23.43%); SMX (63.89%)	5 days	Mao et al. (2018)
Alcaligenes faecalis	CGMCC 1.767	Pure culture	MMSM + 1 g/L acetate + 1 g/L glucose + 50 mg/L SMX + 100 ppm vitamin C; 30 °C	OD540 = 0.20	80–90% SMX	16 h	Zhang et al. (2016)
Rhodococcus rhodochrous	ATCC 13808	Pure culture	MMSM + 3 g/L glucose + 31.6 mg/L SMX or 43.4 mg/L SMZ, 26 °C	–	SMX (20%); SMZ (14%)	SMX 36 days; SMZ (12 days)	Gauthier et al. (2010)

Rhodococcus equi	ATCC 13557	Pure culture	MMSM + 6 mg/L SMX + 0.5 g/L glucose,26 °C	_	SMX (15–29%)	120 h	Larcher and Yargeau (2011)
Microbacterium sp.	BR1	Activated sludge (AS)	MSM + 127 mg/L SMX	OD600 = 1.0	SMX (24–44%) mineralization	16 days	Bouju et al. (2012)
			MMSM + 0.5 mg/L yeast extract + 0.5 mg/L vitamins + 127 mg/L SMX, 28 °C	OD600 = 1.0	SMX (100%)	56 h	Birkigt et al. (2015)
Bacillus subtilis	1556WTNC	Sewage treatment plants (STPs) at Penang, Malaysia	Sterilized treated sewage effluents + 0.2 to 5 mg/L amoxicillin or ampicillin or cephalexin orcefuroxime or ciprofloxacin, 35 °C	5.98 log10 CFU mL^{-1}/50 mL sewage effluent	Amoxicillin (25.03% at 1 mg/mL); ampicillin (15.59% at 0.8 mg/mL); cephalexin (22.59% at 1 mg /mL); cefuroxime (10.62% at 1 mg/mL); ciprofloxacin (2.45% at 0.6 mg/mL)	12 days	Al-Gheethi and Norli (2014)
Achromobacter denitrificans	PR1	AS	MSM + 152 ppm SMX	OD600 = 1.00	100%	14 days	Reis et al. (2014)
			MSM + 152 ppm SMX + 590 ppm succinate	OD600 = 1.00	100%	1.8 days	
			MSM + 25 ppm SDZ	OD600 = 1.00	98%	2.3 days	
			MSM + 31 ppm SDM	OD600 = 1.00	48%	2.3 days	
			MSM + 28 ppm SMT	OD600 = 1.00	98%	2.3 days	
			MSM + 25 ppm SPY	OD600 = 1.00	100%	2.3 days	
Methylobacterium sp.	SDZ-W2-SJ40	AS	AMS + 0.04% yeast extract + 5 mg/L SDZ	OD600 = ~1.00	SDZ (55.2%)	12 days	Mulla et al. (2016)

(continued)

Table 15.1 (continued)

Organism	Strain	Source	Biodegradation condition	Biomass	Removal	Time	References
Arthrobacter sp.	D2	AS	MSM + 50 mg/L SDZ + 2 g/L glucose, 37 °C	OD595 = 0.30	SDZ (99%) degradation; SDZ (82.5%) mineralization	53 h	Deng et al. (2016)
Arthrobacter sp.	D4	AS	MSM + 50 mg/L SDZ + 2 g/L glucose, 37 °C	OD595 = 0.30	SDZ (99.8%) degradation; SDZ (34.4%)mineralization	11 days	Deng et al. (2016)
Geobacillus thermoleovorans	S-07	AS	MMSM + 0.5 g/L glucose + 10 mg/L sulfonamides; 70 °C	OD600 = 2.00	95% SM2 degradation; 72.96–85.96% other sulfonamide degradation	24 h	Pan et al. (2017)
Acinetobacter sp.	W1	AS	MSM + 5–240 mg/L SMX + 5 mg/L DO, 25 °C	OD600 = 0.2	100% SMX degradation; 95–100% SMX mineralization	24 h	Wang and Wang (2018)
Paracoccus sp.	SDZ-PM2-BSH30	Pig manure	AMS + 0.04% yeast extract + 5 mg/L SDZ; 30 °C	OD600 = ~0.8	50.0% SDZ degradation	12 days	Mulla et al. (2016)
Kribbella sp.	SDZ-3S-SCL47	Sediment	AMS + 0.04% yeast extract + 5 mg/L SDZ; 30 °C	OD600 = ~1.1	60.6% SDZ degradation	12 days	Mulla et al. (2016)

SDZ sulfadiazine; *SDM* sulfadimethoxine, *SMZ* sulfamethazine; *SMT* sulfamethizole; *SPY* sulfapyridine; *SMX* sulfamethoxazole; *MMSM* minimum mineral salt medium, *MSM* mineral salts medium, *LB* Luria-Bertani medium, *AMS* ammonium mineral salts

living cells, Gram-positive bacteria had a higher biosorptive capacity than Gram-negative ones (50.91 vs. 40.44 mg g^{-1}). For dead cells, Gram-negative bacteria had a higher biosorptive capacity (25.11 vs. 15.99 mg g^{-1}). Among all individual bacterial strains, the highest biosorptive capacities were observed in living cell biomass of *B. subtilis* 1612WTNC (35.02 mg g^{-1}) and dead cell biomass of *B. cepacia* 103WTNC (40.74 mg g^{-1}). Furthermore, the authors evaluated the biosorption of cephalexin by bacterial biomass (living and dead cells) in aqueous solutions contaminated with the heavy metals Ni^{2+} (1 mg L^{-1}), Cu^{2+} (1 mg L^{-1}), Zn^{2+} (2 mg L^{-1}), Pb^{2+} (0.5 mg L^{-1}), and Cd^{2+} (0.1 mg L^{-1}). The efficiency of cephalexin biosorption was reduced by more than 40.83 and 82.88% (living and dead cells, respectively) in the presence of 1 mg L^{-1} Ni^{2+} ions compared with the control, whereas no biosorption by dead cell biomass was recorded in aqueous solutions contaminated with cadmium, zinc, copper, and lead ions.

In another report, Al-Gheethi and Norli (2014) have investigated the biodegradation of antibiotics (cephalexin, cefuroxime, ampicillin, and amoxicillin) in sewage-treated effluents by b-lactamase produced from *B. subtilis* 1556WTNC. The biodegradation process was performed at the optimal conditions for b-lactams production (5.9 log10 CFU mL^{-1}; pH 6.5; temperature 35 °C for 12 days). They revealed that the maximum biodegradation was 25.03% at 1 mg mL^{-1} for amoxicillin, 15.59% at 0.8 mg mL^{-1} of ampicillin, 22.59% at 1 mg mL^{-1} of cephalexin, 10.62% at 1 mg mL^{-1} of cefuroxime, while it was 2.45% at 0.6 mg mL^{-1} of ciprofloxacin.

15.2.2 Phytoremediation: Removal of Antibiotics by Algae

Phytoremediation is a part of bioremediation where algae are being used for the removal or biotransformation of pollutants, including nutrients, xenobiotic, and CO_2. Algae are aquatic and photoautotrophic organisms offering cost-effective, nonintrusive, and safe cleanup technology for removal of antibiotics from environment. The algae offer several advantages as follows: (1) The blue-green alga (cyanobacteria) uses light energy source and CO_2 for its growth and survival. This way it helps in carbon sequestration and mitigation of global warming. (2) They are capable of not only photosynthesis but also fixing up atmospheric nitrogen, and they can survive better under the nutrient-limited conditions. (3) Microalgae have the greatest abundance of plant biomass in aquatic environments. Microalgae cultures can be cultivated in open ponds or in large-scale water reservoirs. At the same time, the algal growth under laboratory conditions provides reliable and consistent supply of biomass. (4) As the nontarget organism, green algae have higher tolerance to antibiotics than bacteria. (5) They have the potential to treat sites polluted with more than one type of pollutant. (6) They are economically more viable and an eco-friendly tool. (7) They generate lesser volume of chemical and/or biological sludge to be disposed of (Dixit and Singh 2015).

Guo and Chen (2015) have applied alga-activated sludge combined system as a novel treatment to remove cephalosporins. The green alga *C. pyrenoidosa* performed excellent removal capacity for the four target antibiotics (cefradine, cefalexin, ceftazidime, and cefixime). In addition, the green alga has high tolerance to the impact of the antibiotics. A satisfactory growth ability of *C. pyrenoidosa* was observed during the treatment and the algal cell size increased with the removal process. Cefradine could be partly removed by the acclimated activated sludge after a long time adaptation, while an excellent removal efficiency was obtained based on the un-acclimated green alga and un-acclimated activated sludge directly in the combined system.

However, most of the studies focused on the removal capability of algae, which grown in an unpolluted environment before the treatment and ignored whether the feedback of alga to the toxic stress influenced the removal capability in a subsequent treatment batch. Algal tolerance of contaminants plays a decisive role in continuous pollution treatment processes. It is possible that the sensitivity or tolerance of algae changes after the first treatment and therefore causes feedbacks during continuous treatment that influences the final removal efficiency. Therefore, in another study, Chen et al. (2015) investigated and compared algal feedback and removal efficiency of *C. pyrenoidosa* in a sequencing batch reactor algae process (SBAR) to remove cefradine. The results revealed that during the first treatment batch, the antibiotic cefradine influenced the biomass of the green algae *C. pyrenoidosa*. Meanwhile, the "toxic background" of the algae also produced a physiological response and degraded the antibiotic in the subsequent treatment batch. However the maximum population inhibition rate was observed 96 h after the second treatment batch for all tested concentrations. The result indicated that the green algae were also able to adapt to varied pollution loads in different treatment batches.

The use of lipid-accumulating microalgae to remove antibiotics from wastewater has offered additional benefit of biofuel production. Guo et al. (2016) observed that the use of microalgal strains (namely, *Chlorella* sp. Cha-01, *Chlamydomonas* sp. Tai-03, and *Mychonastes* sp. YL-02) improves removal of cephalosporin antibiotics 7-amino cephalosporanic acid (7-ACA) by hydrolysis and photolysis reactions without affecting microalgal lipid accumulation ability. However, 7-ACA had slight inhibition effects on the microalgal growth (9.6–12%). Thus, the current approach of the use of microalgal strains is to establish the best conditions for simultaneous removal of 7-ACA in real wastewater and production of lipid-rich microalgal biomass for subsequent biofuels generation seems to be a cost-effective and bio-safe technology.

C. pyrenoidosa algae was evaluated in the elimination of antibiotic ceftazidime and its basic parent structure 7-ACA with removal rates of 92.70% and 96.07%, respectively (Yu et al. 2017). The algal removal mainly involved a rapid adsorption, a slow cell wall transmission, and the final biodegradation. The LC-MS analysis revealed that Δ-3 ceftazidime and trans-ceftazidime were regarded as the metabolites of ceftazidime and the metabolite of 7-ACA was regarded as a compound which shared the similar structure with 4-chlorocinnamic acid. This study

demonstrates that using green algae to treat antibiotic is promising for the application due to the potential of high removal efficiency and low environmental impact (Yu et al. 2017).

Numerous microalgae species in a single, natural habitat interact with each other synergistically or antagonistically and may compete for nutrients and/or light. Therefore, multispecies tests are expected to provide a more realistic appraisal of the response of microalgae to the exposure of toxic compounds. This fact represents a motivation for the study of ecotoxicity and removal of a fluoroquinolone antibiotic enrofloxacin (ENR) by five individual microalgae species (*Scenedesmus obliquus*, *Chlamydomonas mexicana*, *Chlorella vulgaris*, *Ourococcus multisporus*, *Micractinium resseri*) and their consortium (Xiong et al. 2017). The authors have found that the microalgae consortium showed a higher sensitivity toward ENR than the individual microalgae species. However, ENR removal efficiency of the constructed microalgae consortium was comparable to that of the most effective microalgal species.

A phycoremediation approach may also involve biosorption, where the biomolecule binds to the algal wall (i.e., biosorbent). The high surface area to volume ratio (*S*/*V* ratio) of the algae and functional groups (amino, carboxyl, hydroxyl, and carbonyl groups) on the surface of algal biomass makes algae an attractive choice for biosorption. Santaeufemia et al. (2016) showed that, the living biomass of the microalga *Phaeodactylum tricornutum* is a useful tool for oxytetracycline (OTC) phycoremediation. The use of living biomass was much more effective and efficient than the same amount of dead biomass. A culture of *Phaeodactylum tricornutum* microalga (equivalent to 0.4 g of dry biomass L^{-1}) eliminated 97% of 2.5 mg L^{-1} of OTC in 11 h. The highest sorption capacity was 29.18 mg g^{-1}. The culture conditions of this microalga allowed to combine bioremediation with photodegradation. Thus, the results obtained in this study demonstrated that living biomass of this microalga was a promising low-cost and an eco-friendly alternative to be used in the OTC removal from seawater solutions. Similarly, algae have been investigated as a biosorbent for the removal of antibiotics such as tetracycline (de Godos et al. 2012), norfloxacin (Zhang et al. 2012), and spiramycin (Liu et al. 2012).

15.2.3 Mycoremediation: Removal of Antibiotics by Fungi

Mycoremediation is a part of bioremediation where fungi are being used for the removal or biotransformation of pollutants. Recently, excellent reviews have been published describing the role of fungi in biodegradation of pharmaceutical compounds (Olicón-Hernández et al. 2017) and pesticides (Spina et al. 2018). The unique characteristics of fungi such as the ability to form extended mycelial networks, the low specificity of their catabolic enzymes, and their independence from using pollutants as a growth substrate make these fungi well suited for bioremediation processes (Harms et al. 2011).

Currently, fungi have been proven to be effective in degrading and mineralizing recalcitrant antibiotics due to their powerful enzymatic machinery (extracellular ligninolytic enzyme system), robust morphology, and diverse metabolic capacity (Čvančarová et al. 2015). A number of fungi that are antibiotic degraders belong to the phyla Ascomycota and Basidiomycota followed by the sub-phylum Mucoromycotina (Table 15.2).

Fungi have a variety of strategies to counteract with a myriad of toxic compounds such as recalcitrant polycyclic aromatic hydrocarbons (PAHs), pesticides, and antibiotics. These strategies include nonenzymatic process such bioadsorption, biomineralization (bio-precipitation) as well as biotransformation and biodegradation mediated by enzymatic systems (Olicón-Hernández et al. 2017). The role of various fungal species in remediation of antibiotics were summarized in Table 15.2

15.2.4 Phytoremediation: Removal of Antibiotics by Plants

The usage of natural or genetically modified plants and their associated rhizospheric microbes to remediate contaminated soil, sediments, and water is known as phytoremediation. The fate and effect of pharmaceutical compounds in the environment and their uptake and remediation by plants have been well reviewed in previous publications/review articles (Carvalho et al. 2014; Jagtap 2017; Tasho and Cho 2016).

Phytoremediation has recently been receiving attention as a promising, cost-effective, and eco-friendly method to remove active pharmaceutical ingredients from contaminated soil and water as compared to conventional methods. However, the phytoremediation technologies are less utilized for the removal of antibiotics from soil (Jagtap 2017).

Pteris vittata (L.) was evaluated for the removal of tetracycline (TC) antibiotics from water. The results showed that more than half of the TCs could be removed from the water solution (with the starting concentration of TCs about 1.0 mg kg^{-1}) after 1 day of treatment. No TCs (less than 0.01 mg kg^{-1}) were detected in the solution after 5 days of treatment. Accumulation of TCs was very low in both the roots and the pinnae of *Pteris vittata*, which indicates that accumulation in the fronds is not the main removal mechanism. The main removal mechanism was plant uptake and/or degradation in the fronds (Li et al. 2015). Present results provide a feasible method for removal of TCs from livestock-polluted wastewater. However, more research work should be done before any real-world application is made.

Preliminary results by Gahlawat and Gauba (2016) demonstrated that *Brassica juncea* could remove 71% tetracycline after 24 days in in vitro conditions. However, as initial tetracycline concentrations were increased in the media, the remediation rate also improved. However, at higher concentrations, the plants showed phytotoxicity as depicted by the decrease in shoot length of the germinated seeds (Gahlawat and Gauba 2016).

Table 15.2 Some antibiotic degrading fungi

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
Basidiomycota	*Pleurotus ostreatus*	Ciprofloxacin	–	(1) maximum enzyme production at the highest concentration of CIP (500 ppm). (2) degraded products exhibited a decreased antimicrobial activity	Titrimetric and spectrophotometric assays, HPLC	Biodegradation	Singh et al. (2017)
Basidiomycota	*Irpex lacteus*[b], *Trametes versicolor*[a]	Ciprofloxacin	Desethylene-N-ciprofloxacin; 7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid; N-acetylciprofloxacin[a]; Desethylene-N-acetylciprofloxacin; N-formylciprofloxacin; desethylene-N-formylciprofloxacin; hydroxymethyl-N-ciprofloxacin	(1) *Irpex lacteus* and *Trametes versicolor* degraded fluoroquinolones most efficiently. (2) the compounds are attacked at the piperazinyl moiety: Substitution or decomposition.	HPLC-MS/MS	Biodegradation	Čvančarová et al. (2015)

(continued)

Table 15.2 (continued)

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
				(3) Only *I. lacteus* removed the antibiotic activity during the degradation. (4)manganese peroxidase might participate in the degradation			
		Ofloxacin	9-Fluoro-10-[(2-formamidoethyl) amino]-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid[b]; 9-fluoro-10-(4-formylpiperazin-1-yl)-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid; desethylene-Norfloxacin; 10-[(2-acetamidoethyl) amino]-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij] quinoline-6-carboxylic				

			Acid; 10-amino-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid; 10-(4-acetylpiperazin-1-yl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4] oxazino[2,3,4-Ij]quinoline-6-carboxylic acid[a]; desmethyl-N-ofloxacin[a]				
		Norfloxacin	Desethylene-N-norfloxacin[a]; 7-amino-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid; desethylene-N-acetylnorfloxacin; N-formylnorfloxacin;				
Basidiomycota	*Trametes versicolor*	Ofloxacin	–	–	–	–	Gros et al. (2014)
Ascomycota	*Aspergillus terreus FZC3*	Gentamicin	–	(1) the gentamicin removal efficiency exceeded 95% by day 7 under optimized culture Conditions	–	Biosorption and biodegradation	Liu et al. (2016)

(continued)

Table 15.2 (continued)

Division (fungal taxon)	Name of organisms (fungal organism)	Antibiotic	Metabolites	Comments	Detection method	Focus of study	References
Basidiomycota	*Gloeophyllum striatum*	Ciprofloxacin, Enrofloxacin	–	–	–	Biodegradation and metabolite identification	Wetzstein et al. (1997, 1999)
Zygomycota	*Mucor ramannianus*	Enrofloxacin	–	–	–	Biotransformation	Parshikov et al. (2000)
Ascomycota	*Pestalotiopsis guepini*	Ciprofloxacin and norfloxacin	–	–	–	Biotransformation	Parshikov et al. (2001)
Basidiomycota	*Trametes versicolor*	Norfloxacin and ciprofloxacin	–	–	–	Biodegradation	Prieto et al. (2011)
Ascomycota	*Trichoderma viride*	Ciprofloxacin and norfloxacin	4-Hydroxy-3-oxo-4-vinylcyclopent-1-enyl ciprofloxacin; 4-hydroxy-3-oxo-4-vinylcyclopent-1-enyl norfloxacin.	Metabolites found to be optically active	HPLC; LC/ESI MS; NMR	Identification of degraded products	Parshikov et al. (2002)
Mucoromycotina	*Cunninghamella elegans*	Flumequine	–	–	–	Biotransformation	Williams et al. (2007)

[a,b]Indicates metabolites formed by respective fungal organism after antibiotic degradation

HPLC high performance liquid chromatography, *MS* mass spectrometry, *NMR* nuclear magnetic resonance, *LC/ESI-MS* liquid chromatography electrospray ionization mass spectrometry

Sulfamethazine (SMN) was taken up and translocated by alfalfa (*Medicago sativa*) grass grown hydroponically in a commercially available nutrient solution supplemented with 10 mg L^{-1} of SMN antibiotic. Analysis of alfalfa sap, root zone, middle one-third, and top portion of the foliage showed varying uptake rate and translocation of SMN. The highest average amount of SMN (8.58 μg kg^{-1}) was detected in the root zone, followed by the top portion (1.89 μg kg^{-1}), middle one-third (1.30 μg kg^{-1}), and sap (0.38 μg kg^{-1}) samples, indicating a clear distribution of SMN within the sampled regions. The ultraviolet spectra of parent SMN and translocated SMN identified in different parts of the plant present the possibility of metabolization during the uptake process. Uptake of SMN using alfalfa grown under hydroponic conditions has potential as a promising remediation technology for removal of similar antibiotics from wastewater lagoons (Kurwadkar et al. 2017).

In another study Singh et al. (2018) studied the phytotoxicity pertaining to growth, oxidative stress, and biochemical traits as well as degradation of amoxicillin antibiotics in the duckweed *Spirodela polyrhiza*. The results showed that the high dose (1 mg L^{-1}) of amoxicillin caused a significant ($p < 0.05$) decrease in photopigments, protein, starch, and lipid content and an increase in carotenoids/total Chlorophyll and Chlorophyll a/Chlorophyll b ratios in fronds of *Spirodela polyrhiza*. The results showed a shift in biomarkers: a decrease in frond growth and relative growth rate (16.2–53.8%) and an increase in the activities (mmol mg $protein^{-1}$) of catalase (0.021–0.041), ascorbate peroxidases (0.84–2.49), and superoxide dismutase (0.12–0.23) in fronds. The significantly ($p < 0.05$) greater reduction in amoxicillin content in duckweed setups (84.6–100%) than in the control (62.1–73%) suggested that phytodegradation is an important mechanism in removing antibiotics from water, apart from hydrolysis and photodegradation, which occur in control setups. Overall, the results suggested a toxic effect of amoxicillin on *Spirodela polyrhiza*, even at low concentrations, and nonetheless, the duckweed contributed directly to the degradation of antibiotics in the water and throughout the phytoremediation process.

15.3 Conclusion and Future Perspectives

The use of antibiotics also plays a vital role in medical treatment, veterinary, and agriculture farms to cure or prevent bacterial infections in humans, to increase feed efficiency as well as growth performance in animals and plants, respectively. However, due to its intensive use, antibiotic pollution has emerged as an urgent issue. Furthermore, widespread antimicrobial resistance poses a threat to public and animal health. The inveterate undesirable effects of antibiotics on the environment should attract considerable attention to remove antibiotics from the contaminated environments.

Bioremediation in the broad sense offers a powerful technology for the removal of various contaminants from environment. Hitherto, varieties of organisms have been characterized to degrade/remove antibiotics from the environment. However, genetics and biochemistry of the highly efficient/desired organism have yet not been properly explored. This will lead to the identification of the functional genes and enzymes and their role in the antibiotic degradation. Moreover, the advances in genetic engineering and synthetic biology tool box will also be helpful in order to develop large-scale applications of antibiotic degrading organisms for bioremediation.

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