Biodegradation of emerging pollutants: focus on pharmaceuticals

Irina Ivshina[A,B,D], *Elena Tyumina*[B] *and Elena Vikhareva*[C]

[A]Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, Perm, Russia

[B]Perm State University, Perm, Russia

[C]Perm State Pharmaceutical Academy, Perm, Russia

[D]Tel: + 7 342 280 81 14, Email: ivshina@iegm.ru

A priority environmental problem is pollution and disturbance of natural environments by emerging pollutants – substances of various origins and structures and with known and/or potential ecotoxic effects. One of the most dangerous groups of emerging pollutants is pharmaceutical substances due to their highly stable chemical structure and pronounced biological activity. They are found in soil, bottom sediments, surface, sewage, groundwater and drinking water. Uncontrolled release of pharmaceuticals in open ecosystems is potentially dangerous, entailing environmental consequences. Their negative impacts on living organisms are evident. This has driven the search for effective ways to neutralise persistent pollutants. In Russia, pharmaceutical pollution of the environment has commenced recently and is still presented as research with a local focus. In particular, the dynamics and metabolic mechanisms of pharma pollutants by *Rhodococcus* actinobacteria, outstanding among other microorganisms for their capacity to degrade a great diversity of degradable pollutants, are most intensively investigated. These studies are implemented at the junction of organic chemistry, molecular biology, biotechnology, and pharmacology. They include a set of interrelated fundamental tasks, such as developing drug detection methods in the cultivation media of microorganisms, elucidating the relationships between the systematic affiliation of microorganisms and their ability to degrade chemically different drug substances, as well as studying the degree of biodegradability and toxic effects of new compounds on the degrading microorganisms, and also the features of their decomposition and co-metabolism. Solving these tasks is important to enable understanding of the environmental fate of pharmaceuticals and to create prerequisites for innovative technical solutions in the advanced treatment of pharmaceutical wastewater. It is also essential for the development of environmentally safe approaches to hazardous pharmaceutical waste management.

Emerging pollutants are a new global environmental concern. The term emerging pollutants refers to natural or synthetic substances found in ecosystems. Their ecotoxic impacts on the environment and humans are already known, whereas the occurrence and environmental fate are uncontrolled or unregulated[1,2]. Emerging contaminants are not necessarily new substances. Such ecotoxicants comprise compounds long present in the environment, but now detected due to improved analytical methods. In 2016, the Norman network listed more than 1,000 most frequently reported emerging pollutants[3]. This list includes pharmaceuticals, personal

care products, pesticides, industrial and household chemicals, metals, surfactants, industrial additives and solvents.

Of particular concern are the release and accumulation of highly persistent and bioactive pharmaceutical pollutants in the environment. According to aus der Beek *et al.*[4], about 700 pharmaceuticals were found in the aquatic ecosystems of 71 countries. Pharmaceutical substances and their metabolites have been detected in soil, sediments, surface, sewage, ground and even drinking water[4–8]. Trace amounts of drugs found in bottled water is an unprecedented case[9]. Stumm-Zollinger and Fair[10], and Hignite and Azarmoff[11] first reported on pharmaceuticals in wastewater. The toxicity and biodegradability of these compounds was first discussed in the 1980s by Richardson and Bowron[12].

Since the late 1990s, pharmaceutical pollutants present in natural ecosystems have been seen as an emerging environmental problem[13]. The limited knowledge about their negative impacts on animals and humans remains the weak link. Despite the relatively low concentrations (ng/L to µg/L) of pharmaceutical pollutants in nature, their constant replenishment can lead to high permanent concentrations and stimulate negative effects on humans and the environment. The best-known case is the decline in vulture populations (*Gyps bengalensis*, *G. indicus, and G. tenuirostris*) in the Indian subcontinent. Toxic exposure was caused by veterinary-used diclofenac in South Asia. Birds fed on cattle carcasses medicated with the anti-inflammatory drug diclofenac died of intoxication and kidney failure[14]. Pharmaceutical pollutants can move across food chains. British scientists have found diclofenac in otter wool, indicating diclofenac contamination of aquatic ecosystems, fish and fauna, the habitat and food for these animals[15]. Several studies reported on feminisation of male fish caused by a synthetic hormone 17-α-ethinyl-estradiol in their habitat[16,17].

For most drugs detected environmentally, potential acute and chronic effects on ecosystem components have not yet been studied. Though drug influence on animals and humans is intensively studied, and reports describing impacts of widely used antipyretics and analgesics on plants have appeared[18,19], they are not sufficiently studied in ecologically relevant microorganisms. Upon contact with these xenobiotics, microorganisms detoxify them, and as principal biosphere constituents, they are sensitive to changes in the habitat. The relevant studies have recently commenced.

Pharma pollutants in ecosystems of Russia

According to aus der Beek *et al.*[4], the prevalence of antibiotics and analgesics in the environment, including non-steroidal anti-inflammatory drugs, is a typical situation for the eastern European countries and Russia.

Investigations on pharmaceuticals and their metabolites in wastewater and surface waters in Russia are few and concentrated mainly in the Central and North-Western regions. Table 1 summarises data on pharmaceutical occurrences and concentrations detected in aqueous samples. The average concentrations of pharmaceuticals found in surface water, untreated wastewater, and treated wastewater were 136, 360 and 181ng/L, respectively.

Pharmaceuticals-related studies of aquatic ecosystems and bottom sediments in the Northwest region detected a number of over-the-counter medications, including the psychostimulant caffeine, anti-inflammatories ketoprofen and diclofenac, and antispasmodic drotaverine hydrochloride[20].

Recent studies under the project 'Implementation of the Baltic Sea Action Plan in Russia' (BASE) detected 20 pharmaceuticals in the wastewater of St. Petersburg, though the initial targets were only diclofenac and ethinylestradiol as the most cosmopolitan pharma pollutants[6]. The effluent contained diclofenac ranging from 355 ng/L in summer to 550 ng/L in winter. The researchers calculated the predicted environmental concentration (PEC_{river}) in the Neva River to be *circa* 5 ng/L. At the same time, a significantly increased diclofenac concentration in the wastewater effluent was found compared to that in the influent. This phenomenon is apparently explained by the release of conjugated diclofenac metabolites during secondary wastewater treatment. The ethinylestradiol concentration was 0.4 ng/L.

Sampling performed at water intakes and reservoirs (Moscow region) revealed 105 pharmaceuticals and their residues[21]. Diclofenac (0.025–0.35 ng/L), caffeine (26 ng/L) and tetracycline (0.662 ng/L) were most frequently detected. Considering the potential risks of pharmaceutical pollutants for living organisms, the authors used the PASS program (Prediction of Activity Spectra for Substances) tailored to simulate the drug toxicity. According to the structural formula of an organic compound the PASS program estimates its probable biological activity[21]. They predicted the possible toxic effects (embryotoxicity, carcinogenicity, mutagenicity, etc.) on living organisms and developed an ecotoxicological map of some Moscow aquatic ecosystems.

Because of the limited knowledge on the topic of this review, the priority research in Russia is still focused on environmental detection and identification of pharmaceuticals, their effective analyses in wastewater, and clinical trials of low drug concentrations against

Table 1. Pharmaceuticals detected in the environment (in ng/L) in Russia.

Therapeutic group	Pharmaceuticals	Source	Concentration	Reference
Antibiotic	Amoxicillin	Wastewater (influent)	525	6
	Ampicillin	Wastewater (influent)	32	6
		Sediments	0.005[A]	21
	Azithromycin	Wastewater (influent)	332	6
	Ciprofloxacin	Surface water (lake)	271	20
		Wastewater (influent)	871	6
	Clarithromycin	Wastewater (influent)	230	6
	Norfloxacin	Wastewater (influent)	502	6
	Tetracycline	Wastewater (influent)	124	6
		Surface water (storage lake)	6.62	21
	Triclosan	Sediments	500−23 600[A]	20
	Trimethoprim	Wastewater (influent)	457	6
	Erythromycin	Wastewater (influent)	216	6
Analgesic	Codeine	Wastewater (influent)	191	6
Antihistamine	Ranitidine	Wastewater (influent)	252	6
Lipid-lowering	Bezafibrate	Wastewater (influent)	48	6
Anticancer	12-Methyl-tridecanoic acid	Sediments	38	21
Antiepileptic	Carbamazepine	Wastewater (influent)	76	6
Psychoactive	Caffeine	Surface water (river)	3.8−446	20
		Surface water (river)	26	21
		Sediments	27[A]	21
NSAIDs	Ketoprofen	Surface water (river)	260	20
		Wastewater (influent)	756	6
	Diclofenac	Surface water (river)	270	20
		Wastewater (effluent)	355−550	6
		Wastewater (effluent)	0.025−0.35	21
		Surface water (storage lake)	0.025	21
Antihypertensive	Enalapril	Wastewater (influent)	611	6
	Enalaprilat	Wastewater (influent)	461	6
Antiplasmodic	Drotaverine	Surface water (lake)	36.1−41.1	20
		Wastewater (influent)	452	6
Hormone	Ethinylestradiol	Wastewater (effluent)	0.4	6

[A]Concentrations are indicated in ng/kg.

humans and other living organisms, including environmentally relevant microorganisms. The latter are capable of pharmaceutical pollutant detoxification in natural ecosystems.

Biodegradation of pharma pollutants

The role of microorganisms role in the environmental degradation of xenobiotics is pivotal. Of those involved in water and soil ecosystem self-cleaning processes, *Rhodococcus* actinobacteria exhibit nonspecific enzymatic actions. They are first to attack compounds novel for microbial cells. In recent years, rhodococci are often considered as promising biodegraders and biotransformers of various xenobiotics. *Rhodococcus*' ecological versatility and exceptional polyfunctionality, high catalytic activity in extreme environments, and biodegradation of organic compounds from many known classes clearly indicate the suitability of rhodococci for degradation of pharmaceutical pollutants[22,23].

Biocatalysis activity studies revealed *Rhodococcus*' ability to decompose chemically diverse pharmaceuticals. Gauthier *et al*.[24] used *R. rhodochrous* ATCC 13808 to biodegrade heterocyclic nitrogen-containing pharmaceuticals, such as sulfamethisole (43.4 mg/L), sulfamethoxazole (32 mg/L), and carbamazepine (9.5 mg/L). These compounds were biodegraded only in the presence of glucose. In co-metabolic conditions, biodegradations of sulfamethisole, sulfamethoxazole and carbamazepine were 14, 20, and 15%, respectively. Each drug biodegradation process did not exceed 36 days and metabolites formed were unstable.

Yoshimoto *et al*.[25] studied rhodococcal interactions with steroid compounds (specifically17β-estradiol). *R. zopfii* Y 50158 degraded this pharmaceutical in the presence of glucose within 24 hours.

R. erythropolis, *R. equi* and *R. rhodochrous* were examined as potential 17α-ethinylestradiol (1.4 mg/L) biodegraders, with *R. erythropolis* being the most active. 17α-ethinylestradiol depletion as the sole carbon and energy source was 10% after 75 hours, and 47% in the presence of glucose within 13 hours[26]. According to Larcher and Yargeau[27], *R. rhodochrous* ATCC 13808 completely utilised 17α-ethinylestradiol (5 mg/L) after 48 hours. Complete testosterone (1 mg/ml) decomposition was achieved with resting cells of *R. equi* ATCC 1488728 within 2 days[28]. Biological testosterone degradation proceeded in two stages: formation of 9α,17β-dihydroxyandrost-4-en-3-one and androst-4-ene-3,17-dione further converted into 9α-hydroxyandrost-4-ene-3,17-dione and 9α-hydroxy-1,4-adrostadien-3,17-dione, respectively. The final products contained 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (3-HSA) further degraded to CO_2 and H_2O[28]. There are also studies published on *Rhodococcus* biodegradation of

ester-based drugs. Possible clofibric acid biodegradation to clofibrate using *R. rhodochrous* was shown[29].

The authors of this review employed resources of the Regional Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM 768, http://www.iegmcol.ru)[30] to investigate biodegradation of pharmaceutical pollutants typically found in ecosystems and widely used in Russia, such as non-steroidal anti-inflammatory drugs (phenylacetic acid derivatives diclofenac and ibuprofen), spasmolytic (an isoquinoline derivative drotaverine or No-Spa)[31,32], analgesic (a phenol group-containing *n*-acetaminophen or paracetamol)[33]. To elucidate biodegradation mechanisms, the genomes of environmentally significant *R. erythropolis* IEGM 267 and *R. ruber* IEGM 231, which actively biodegrade a variety of complex organic compounds, were sequenced[34]. Comparative bioinformatic analysis applied to sequencing data allowed analysis of functional genes, which control the pharma pollutant biodegradation. Kinetics and decomposition patterns of pharma pollutants were studied depending on physiological state of biodegraders (growing, washed, immobilised, and dormant bacterial cells) and their culture conditions (mineral composition, aeration rate and acidity, temperature, initial ecotoxicant concentrations, and selection of effective co-substrates). Regulation mechanisms (induction, inhibition) of *Rhodococcus* catalytic activity towards pharmaceutical pollutants were elucidated. Less hazardous metabolites were identified. The main biotransformation pathways of the parent ecotoxicants were determined. Features of rhodococcal interaction with pharmaceutical pollutants were studied[32]. The pharmaceutical biodegradation process was most significantly enhanced with immobilised rhodococci[31]. The wooden waste available in the Perm region was used as carrier material. To enhance the adsorbent surface affinity to bacterial cells, the carrier was treated with selected hydrophobisers[35]. Stable polyfunctional biocatalytic systems based on immobilised *Rhodococcus* cells were developed. They increased the rate of the biodegradation processes of pharmaceutical pollutants and their metabolites. Additionally, they are characterised by high functional stability over 3–8-month storage and are reusable.

The research results indicate that *Rhodococcus* actinobacteria may act as important bio-oxidants of chemically diverse pharma pollutants typically detected in the environment. The experimental data on mechanisms and tentative detoxification and bioconversion pathways of pharma pollutants together with biochemically and catalytically characterised biodegrader strains may offer new environmentally safe methods for hazardous pharmaceutical waste management. Another practical application is to use actinobacteria-based biodegraders as a suitable model to study the novel pathways

of drug metabolism that allows prediction of metabolites expected from degradation of closely related pharmaceutical pollutants.

Conclusion

There is a rapid growth in pharmaceuticals and novel pharmaceutical agents on the market. This, combined with their exponentially increasing annual consumption, only partial removal of pharmaceuticals and their metabolites in wastewater treatment, and a lack of highly effective disposal methods for hazardous pharmaceutical wastes contribute to the dramatically growing pharmaceutical pollution of the biosphere and an imbalance in natural ecosystems. Society only recently realised the real scope of the imminent danger and the urgent need to work out ways to deal with the pharmaceutical pollution challenge. Environmental disamenities stimulate novel technical solutions on how to mitigate the environmental burden and to assess environmental risks due to possible pharmaceutical pollutant exposure. They force us to seek detoxification and removal methods of these anthropogenic toxicants from aquatic and terrestrial ecosystems to reduce and even exclude the problem completely in future.

In Russia, such studies are still at the stage of intensive accumulation of factual data on both the expansion and analysis of pharmaceutical pollution of the environment (principally water bodies) and drug conversion by microbes. Attempts are being made to propose a set of measures reducing environmental risks associated with drug pollution. However, it will take years of research to evolve fundamentally new, experimentally based solutions that require large investment, and more deliberate strategies to prevent drug release into the environment.

Acknowledgements

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Biographies

Professor Irina Ivshina carries out research in environmental microbiology and industrial biotechnology. She leads the Laboratory of Alkanotrophic Microorganisms at the Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, and performs teaching activities at the Microbiology and Immunology Department, Perm State University. She is an academician of the Russian Academy of Sciences, awarded Laureate of the Russian Federation Government for the development and implementation of a complex of remediation biotechnologies for hydrocarbon-polluted biogeocenoses, and Vice-President of the Russian Interregional Microbiological Society.

Elena Tyumina, MS (Biotechnology), graduated with honors from Perm State University, and is now a post-graduate student of the Microbiology and Immunology Department, Perm State University. Her research interests are general microbiology, ecology, microbial diversity. She is awarded a Laureate of the V.I. Vernadsky Non-governmental Ecological Foundation in the field of science.

Professor Elena Vikhareva, Dr Sci. (Pharmacology), is Head of the Department of Analytical Chemistry, Perm State Pharmaceutical Academy. Her research interests are ecology, biotechnology and analysis of pharmaceuticals.

New thermophilic prokaryotes with hydrolytic activities

Elizaveta Bonch-Osmolovskaya[A,B], *Alexander Elcheninov*[A], *Ksenia Zayulina*[A] *and Ilya Kublanov*[A]

[A]Federal Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

[B]Tel: +7 499 135 44 58, Email: elizaveta.bo@fbras.ru

Thermophilic microorganisms are capable of growing on polymeric substrates and have been intensively studied for their enzymes, thermostable hydrolases (glycosidases, proteinases, lipases), which have important applications in many fields of bioindustry: production of detergents, food processing, paper and textile industry, biofuel formation