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Emerging chemicals and the evolution of biodegradation capacities and pathways in bacteria

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The number of new chemicals produced is increasing daily by the thousands, and it is inevitable that many of these chemicals will reach the environment. Current research provides an understanding of how the evolution of promiscuous enzymes and the recruitment of enzymes available from the metagenome allows for the assembly of these pathways. Nevertheless, physicochemical constraints including bioavailability, bioaccessibility, and the structural variations of similar chemicals limit the evolution of biodegradation pathways. Similarly, physiological constraints related to kinetics and substrate utilization at low concentrations likewise limit chemical-enzyme interactions and consequently evolution. Considering these new data, the biodegradation decalogue still proves valid while at the same time the underlying mechanisms are better understood.

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Introduction

New chemicals are in constant development and production to satisfy the increasing demand for new applications and products. The abstract 'chemical space' has been estimated to host a population of at least 10^{60} possible small organic molecules [1]. The CAS registry currently references more than 72 million chemicals and notably reports on adding about 15 000 compounds daily. Inevitably, a large number of these chemicals will be released into the environment during product life cycle, legitimizing concerns about their respective occurrence in the environment and their potential effects on living organisms.

Nowadays, it is broadly accepted that anthropogenic or naturally occurring chemicals can only be degraded under favorable conditions. This idea has been discussed and perpetuated in the form of a biodegradation decalogue by Dr Martin Alexander nearly 40 years ago and rules have been formulated that may limit degradation ([2], see also Box 1). Bacteria are constantly developing new catabolic pathways directed against moving targets in order to either access sources of carbon, energy and nutrients or simply to detoxify them. However, unravelling these degradation processes in nature is made nearly impossible due to the sheer number of chemicals, their occurrence at mostly low concentrations, and the number of unknown chemicals resulting from bacterial transformation and biodegradation. In this review, we account for our growing understanding of the bacterial biotransformation and biodegradation of emerging contaminants, the evolution of catabolic enzymes, and the assembly of novel biodegradation pathways. We conclude with a discussion on the physicochemical and physiological limitations to biodegradation and consequently to the evolution of biodegradation capacities.

Biotransformation and biodegradation of emerging chemicals

The term biotransformation typically refers to an enzyme-catalyzed reaction that results in the formation of intermediate products with a slightly modified structure. Biodegradation is the recommended term for a biotransformation leading to either the loss of certain chemical properties (primary biodegradation) or fully reduced or oxidized products such as carbon dioxide and water (ultimate biodegradation) [3].

There is no general rule to the biodegradation of emerging chemical contaminants; a variety of mechanisms may enable efficient use of a compound as a carbon or energy source. Examples of rather simple biotransformation reactions include additions, such as the acetylation of amino groups [4] or the methylation of hydroxyl groups [5], along with a variety of substitution and cleavage-type reactions [6]. Often, relatively weak bonds, such as esters (e.g. in carbamates [7], organophosphates [8] or pyrethroids [9], amides (in numerous compounds [10,11]), are prone to these first biotransformation steps. However, these

Box 1 The biodegradation decalogue by Alexander [2]

1. Thou shalt not degrade a compound for which there exists no enzyme (or enzymes) catalyzing an initial reaction (or reaction sequence) in the catabolic pathway.
2. Thou shalt not readily degrade a substrate which provides insufficient energy or no carbon for growth.
3. Thou shalt not degrade a compound in environments where at least one essential nutrient is missing.
4. Thou shalt not destroy a substrate in environments containing factors inimical to microbial proliferation.
5. Thou shalt not metabolize a large molecule that fails to penetrate thy cells.
6. Thou mayest have difficulty in degrading a compound present in aqueous solution in exceedingly low concentrations.
7. Thou shalt not degrade a chemical that fails to induce the formation of enzymes needed for its breakdown.
8. Thou mayest not be able to decompose rapidly a molecule whose degradation requires several extracellular enzymes produced by different populations.
9. Thou shalt have trouble dealing with inaccessible substrates.
10. Thou shalt similarly find difficulty in cleaving a molecule when the sites to be cleaved are not readily accessible.

simple reactions are not necessarily catalyzed by organisms able to further degrade these substances [12•]. Rather, these reactions are more often catalyzed by enzymes with broad substrate specificity [13] and oxidized products are either further transformed through step-wise transformation reactions or enter into central metabolic pathways. Sometimes, single oxidation steps can even lead to efficient breakdown of molecules, when a destabilization of the whole compound is initiated (e.g. in sulfonamide antibiotics [14•]). Other functional groups of anthropogenic origin such as nitro (e.g. in explosives [15,16]), cyano (e.g. in herbicides [17]), or halogen groups (e.g. in flame retardants [18]) are likewise either cleaved or transformed [19] to obtain intermediates which can be further processed in central metabolism. Lightly substituted aromatic rings are often targets for ring-cleavage reactions which can be catalyzed by a variety of ubiquitous enzymes [20].

Bacteria can utilize the full space of catabolic biochemical reaction types to initiate biodegradation and for some compounds several different biodegradation strategies seem to have evolved concurrently. For example, at least three distinct biodegradation mechanisms have been described for bisphenol A (BPA), an industrial chemical found in a variety of plastics and epoxies and a putative endocrine disrupting compound (Figure 1). In the first pathway discovered, hydroxylation of the isopropyl moiety led to intramolecular rearrangement, the product of which was dehydrated to a stilbene and was eventually cleaved at the double bond [21,22]. Later, a substantially different mechanism responsible for the degradation of alkylphenols (nonylphenols, octylphenol, and BPA) was identified in strains of the genus *Sphingomonas* sensu latu [23,24]. Here, alkylphenols including BPA are hydroxylated at the *ipso*-position of the aromatic ring carbon atom bearing the alkyl group. The respective unstable intermediates spontaneously fragment into hydroquinone and transient carbocationic species, which ultimately hydrolyze and yield hydroxylated forms of the alkyl moieties. Most recently, a third pathway has been elucidated in which one of the two aromatic rings is hydroxylated and is subsequently the target of a meta-cleavage reaction [25]. These examples demonstrate the wide range of mechanisms bacteria may exploit to acquire energy and/or carbon or other nutrients from emerging chemical substrates.

Enzyme promiscuity and the evolution of 'novel' enzymes

The idea that enzymes can also catalyze unspecific reactions gained acceptance over the past decades [26••]. In fact, many enzymes are actually promiscuous biocatalysts capable of transforming a variety of substrates that share structural similarity with their primary substrate. However, these promiscuous reactions are often catalyzed at drastically lower rates [27].

It is generally accepted that gene duplication is one of the driving forces in the evolutionary process of generating novel genes by subsequent mutation [28]. It has more recently been proposed that duplication would most likely occur for genes with dual function and under circumstances in which both of these functions were needed [29]. Lab results have shown that evolution of genes encoding for enzymes with promiscuous activity could lead to drastically increased activities for their alternate substrates in few or even single steps [30], while their respective physiological functions were only slightly changed [27]. In a case where genes had already been duplicated, and conditions required the activity of both functions, divergence of the former duplicates could be observed within a few thousand generations [31••]. A field example for this can be found in the hexachlorocyclohexane (HCH) degradation pathway, where new genes appear to be obtained from copies of genes already responsible for xenobiotic degradation. *Sphingobium indicum* B90A hosts two isoenzymes of a lindane dehydrochlorinase, which are 88% identical to each other based on the amino acid sequence and show preferences for different HCH isomers [32]. In many cases, however, similarities of genes involved in the transformation of xenobiotic substrates to other known genes are low. This is the case for genes encoding for linA [33] and opdA, a monooxygenase degrading BPA and nonylphenol [34]. Therefore it often remains unclear what the physiological functions of enzymes responsible for xenobiotic transformations are, and how novel functions evolve so quickly.

Assembly of pathways

If genes enabling steps in the degradation of xenobiotics are already present in the metagenome, they can be disseminated by horizontal gene transfer (HGT). Based