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Prokaryotic Cytochromes P450 (Review)

L. E. Khmelevtsova*, I. S. Sazykin, M. A. Sazykina, and E. Yu. Seliverstova

South Federal University, Ivanovsky Academy of Biology and Biotechnology, Rostov-on-Don, 344090 Russia

**e-mail: lehmelevcova@sfedu.ru*

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Abstract—The research on the structure and role of bacterial cytochromes P450 are summarized in this review. We consider the organizational features of these enzymes, cytochrome-catalyzed reactions, the distribution of cytochromes among prokaryotes, and their functions in bacterial cells. We cite the data on cytochrome genes and the regulation of their expression in prokaryotes and classify cytochromes by components involved in the electronic transition. We consider the role of bacterial cytochromes in the biodegradation of carbohydrates and xenobiotics by microorganisms and the possible involvement of reactive oxygen species, which are generated in the catalytic cycle of these enzymes, at the initial stages of carbohydrate biodegradation.

Keywords: cytochrome P450, prokaryotes, monooxygenase, biodegradation, carbohydrates, reactive oxygen species

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Cytochromes P450 constitute a superfamily of heme-containing enzymes that are found today in animals, higher plants, algae, fungi, and bacteria. Bacterial cytochromes P450 comprise the majority of the superfamily. According to the systemic nomenclature, they represent the CYP101 and higher families, in which CYP 1050 (http://cyped.biocatnet.de) is the current limit of the described P450 bacterial families. Most bacterial types contain at least one P450, although some archaea and anaerobic bacteria do not have any P450 [1].

Bacterial cytochromes are involved in the synthesis of secondary metabolites, such as antibiotics, in the utilization of hydrophobic low molecular substrates, such as alkanes and aromatic carbohydrates. These enzymes also have monooxygenase activity towards different organic low molecular substrates. Cytochromes C450 play an important role in the degradation of environmental toxins and mutagens. The cytochrome-catalyzed reactions are extremely diverse; they include hydroxylation, N-, O-, and S-dealkylation, sulfoxidation, epoxidation, deamination, desulfurization, dehalogenation, peroxidation, and reduction of N-oxides [2].

Since cytochromes P450 regioselectively introduce one oxygen atom and nonactivated carbon atom, they can take part in the degradation of hardly decomposable natural pollutants and in the preparation of industrial products that can be hardly obtained by chemical methods, if at all [3].

Prokaryotes contain soluble P450, which is probably due to the absence of intracellular membranes to fix cytochromes P450. In eukaryotic systems, P450 is inserted into membranes, as in the case of yeasts and fungi. All cytochromes P450 of higher organisms are membrane enzymes [4].

Most likely, cytochromes P450 arose during the appearance of atmospheric oxygen, when their initial role was in the detoxification of molecular oxygen in anaerobic bacteria [1]. In the evolutionary aspect, the bacterial monooxygenase system is the most ancient system. It consists of three water-soluble components, i.e., FAD-containing flavoprotein (NADPH- or NADH-dependent P450 reductase), iron-sulfur protein (e.g., putida redoxin in the *Pseudomonas putida* system, which oxidizes camphor), and P450. A feature of this system is its absolute specificity to the substrate. The general equation of the P450-catalyzed reaction may be described by the formula:

$$A + O_2 + NADAO(P)H + H^+ + 2e^- \xrightarrow{\text{Monooxygenase P450}} AO + H_2O + NAD(P)^+ ,$$

where A and AO are the substrate and the target product of the monooxygenase reaction, respectively [5].

As seen from the equation, the monooxygenase reactions are catalyzed by cytochrome P450, which are accompanied by obtaining electrons from NADPH or NADH. Therefore, the functioning of cytochrome P450 is associated with either NAD(P)H-dependent reductase or redox cellular systems. The first self-sufficient enzyme containing domains of cytochrome P450 and P450 reductase was found in *Bacillus megaterium* in 1987 and was named P450 BM-3 (CYP102A1) [1]. Cytochromes P450 can catalyze the monooxygenase reactions in the absence of molecular

oxygen but in the presence of organic or inorganic peroxides. This type of P450 monooxygenase activity, which does not require reduction equivalents from reductase, was discovered in 1975. Some cytochromes P450 catalyze the rearrangement of the oxygen atoms in the substrate molecule; this type of the activity is also independent on reductase [7]. So-called oxygen activation occurs during the common catalytic cycle of cytochrome P450; this means that molecular oxygen is bound and successively transferred through heme iron intermediates, such that the insertion of one oxygen atom occurs in the bound substrate molecule with the formation of the target product of the monooxygenase reaction [5, 8, 9]. NAD(P)H/O_2-catalyzed monooxygenase reactions occur through a four-electron reduction of O_2, which is conjugated with the two-electron oxidation of a substrate. Sometimes, these reactions may lead to the so-called cycle separation, in which the electron intake from NAD(P)H to the P450 molecules results in the generation of a superoxide-anion radical and/or hydrogen peroxide instead of the products of the monooxygenase reaction [10, 11]. In this separated cycle, NAD(P)H is oxidized and H_2O_2 is formed as a result of the NAD(P)H-oxidase activity of cytochrome P450:

$$\mathrm{NAD(P)H + H^+ + O_2} \xrightarrow{\text{oxidase P450}} \mathrm{NAD(P)^+ + H_2O_2}.$$

The well-known peroxide shunt is the process in which H_2O_2 or organic peroxides (alkyl hydroperoxides and peroxy acids) give an oxygen atom to an oxidized substrate; in this case, the presence of O_2 or NAD(P)H as electron donors is not necessary [12]. The peroxide shunt is rarely an effective means of P450 catalysis, because peroxides oxidize both the heme and protein. Nevertheless, a small amount of cytochromes P450 can use the peroxidase shunt. In 2014, Belcher and coworkers described cytochrome P450 $OleT_{JE}$ (CYP152L1) from *Jeotgalicoccus* sp. 8456, which catalyzes oxidative decarboxylation of fat acids with alkene formation with the use of hydrogen peroxide as oxygen donors [13].

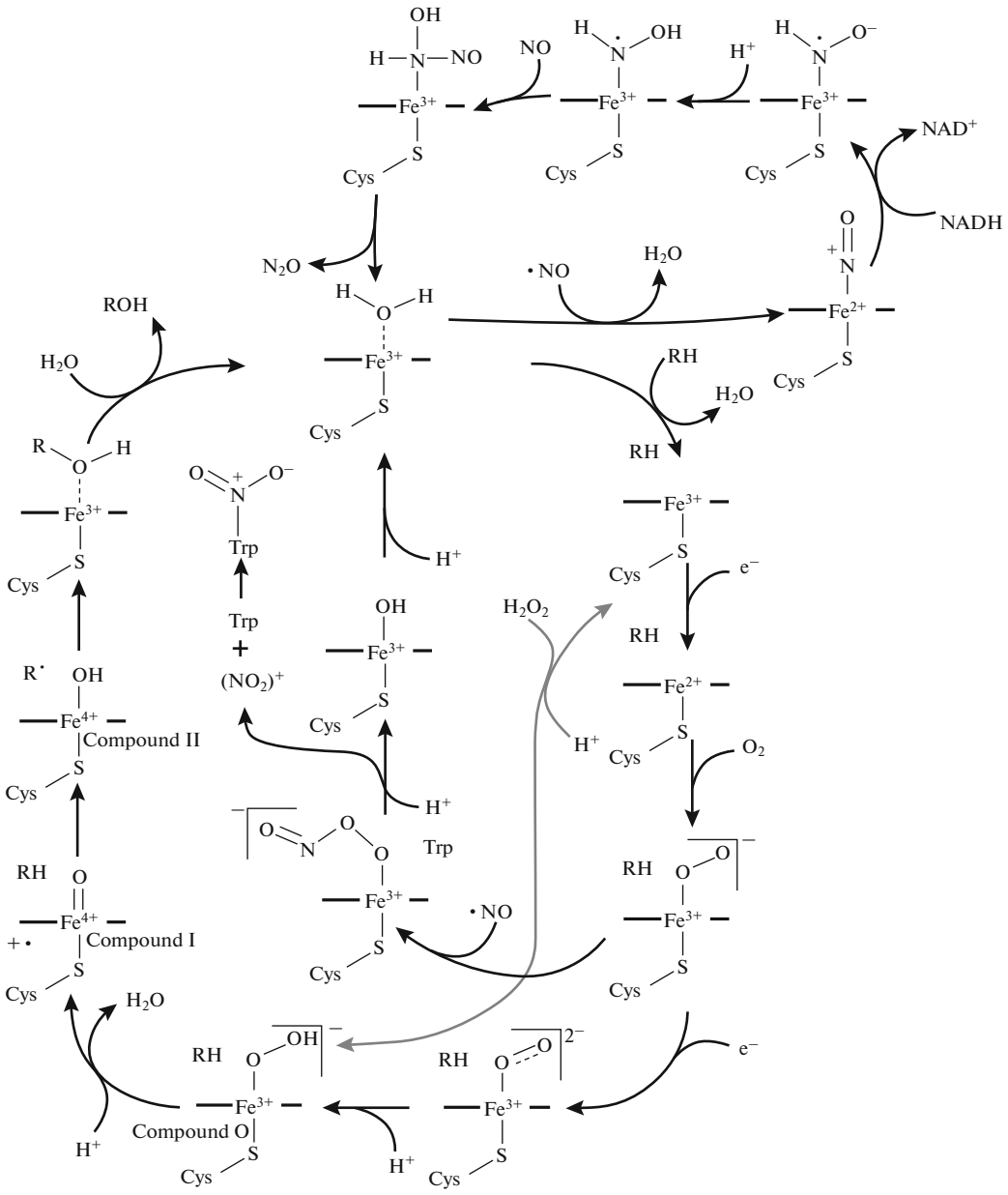
The generation of reactive oxygen species (ROS) can occur during two stages of the cycle, i.e., either during dissociation of the intermediate iron compound ($Fe^{III} - O_2^-$) with releasing trivalent iron and superoxide-anion radical, followed by dismutation of O_2^- to H_2O_2, or during dissociation of the intermediate peroxo complex ($^-Cys–Fe^{III}–O_2^-$), followed by protonation with the formation of H_2O_2 [14] (figure). This peroxide can be converted to a hydroxyl radical by the Fenton reaction in the presence of two-valent iron. Moreover, the catalytic cycle of cytochrome P450 can generate singlet oxygen.

ROS generation can lead to lipid peroxidation and the degradation of proteins, including cytochrome, thus being toxic for cells. At the same time, there are increasing opinions that ROS formation is not only an inevitable side effect but also has a certain biological relevance [15].

ROS, which are generated in the catalytic cycle of cytochromes, are known to degrade different compounds and regulate various cellular functions in eukaryotes. It was shown that singlet oxygen is involved in p-hydroxylation of aniline, which is catalyzed by cytochrome CYP2E1 in microsomes of the rat liver under the action of phenobarbital. In this case, the addition of β-carotene, i.e., a trap for $^1O_2$, inhibited the reaction of p-hydroxylation of aniline, while superoxide dismutase, catalase, and dimethylsulfoxide did not influence the reaction course. The authors suggested that the superoxide radical, hydrogen peroxide, and hydroxyl radicals are not involved in this reaction [16]. As for monooxygenase reactions in humans, there are assumptions that the formation of highly reactive radicals, such as $^1O_2$ and $OH^\bullet$, makes it possible for cells to utilize a wide range of organic compounds because these ROS can break any C–C and C–H bonds [12, 17]. Another assumption is that ROS cause some nonspecificity of monooxygenases, which makes it possible for any isoform of this enzyme to metabolize various substrates [18]. For prokaryotic organisms, there is not enough data, which would confirm that the ROS formation by cytochromes is associated with the degradation of carbohydrates and xenobiotics. This theory can be confirmed by the results of Sazykin et al. [19], who showed the inhibition of petroleum biodegradation upon the addition of antioxidants (ascorbic acid, mannitol, alpha-tocopherol acetate, and ionol). It was also shown that ROS (superoxide radical and hydrogen peroxide) are generated by two *Acinetobacter calocaoaceticus* strains during their incubation with different carbohydrates as the only source of carbon and energy. This can be indirect evidence of the role of ROS, which are generated in the active center of bacterial monooxygenases at the initial stages of carbohydrate biodegradation [20].

The cytochrome P450 system comprises more than 270 different gene families that differ in the homology of the nucleotide/amino acid sequence. In turn, the families are divided into subfamilies. Isoenzymes of cytochrome P450 with an identity of amino acid composition of more than 40% and more than 55% are combined in families and subfamilies, respectively. The cytochrome P450 (CYP from Cytochrome P) families and subfamilies are denoted by numbers and numbers along with Latin letters (e.g., CYP 1A1), respectively. The first symbol is an Arabic numeral to denote the family; the second symbol is Latin letter to denote the subfamily; the third symbol is an Arabic numeral corresponding to the polypeptide number [21].

Bacterial P450 was found for the first time in the *Rhizobium* bacteroids in 1967. Another two bacterial cytochromes were found in 1968 in *Pseudomonas*



Scheme of the catalytic cycle of cytochromes p450 [14].

putida [22] and *Corynebacterium* sp. [23]. Cytochrome P450 from *Pseudomonas,* P450cam (CYP101) catalyzes the NADPH-dependent oxidation of camphor and requires the soluble NADFH-P450 reductase system, which consists of putida-redoxin and NADPH-putida-redoxin reductase. P450 from *Corynebacterium* catalyzes the NADPH-dependent oxidation of n-octane to 1-octanol. Many soluble P450s were found in different prokaryotes over the past years. The last cytochrome P450 of the CYP1050A family was found in 2014 in *Rhodococcus erythropolis* JCM 6824 and described in the BioCatNet database (http://cyped.biocatmet.de). This cytochrome is encoded by the *rauA* gene and catalyzes hydroxylation of the nitrogen atom in the quinoline ring of auracin (an alkaloid synthesized by this microorganism). As a result, auracin acquires antibiotic properties towards a wide range of gram-positive bacteria [24].

Initially, the P450 distribution was assumed to be rather limited, because some types of bacteria, including gram-negative *Escherichia coli,* had no cytochrome P450. However, more recent studies showed that P450 is widely spread among bacteria. For example, 20 genes of cytochrome P450 were found in the chromosome of *Mycobacterium tuberculosis*, nine genes were found in *Bacillus subtilis*, and 18 genes were found in *Streptomyces coelicolor. Streptomyces avermitilis* has, presumably, 33 genes of P450 [19, 25, 26]. The

P450 genes encoding enzymes with the function of alkane hydroxylases have been discovered in many psychrophilic and mesophilic bacteria, which degrade alkanes in microorganisms from contaminated biotopes [27, 28], as well as in *Burkholderiales* and *Rhodocycalles* microorganisms, which form biofilms on the walls of aerobic reactors of water treatment facilities [29]. In addition, cytochromes P450 were found in some Archaea. These cytochromes are mostly thermostable proteins that are dissolved in the cytoplasm. The enzyme P450 from the *Sulfolobus sulfataricus* system utilizes pyruvate as an electron donor. This three-component system consists of cytochrome P450 (CYP 119), ferredoxin, and 2-oxoferredoxin oxidoreductase. This is an exceptional case because the role of electron donors in the P450 systems play, usually NADH or NADPH [7].

Lamb et al. [30] report that the mimivirus genome of *Acanthamoeba polyphaga* contains two genes that presumably encode cytochromes P450 (GenBank acc. No. YP_142886 and YP_143162, also known as MIMI_L532 and MIMI_L808, respectively). The authors believe that cytochromes P450 were probably present in the virus genome before the appearance of three domains (eukaryotes and prokaryotes consisting of bacteria and archaea). It is quite possible that the mimivirus acquired the CYP genes from an older ancestor. It is also possible, on the other hand, that different mimivirus genes, many of which are homologs of eukaryotic genes, including the CYP genes, were obtained by the horizontal transmission of genes, because *A. polyphaga* is also the host for parasitic and bacterial endosymbionts, including many mycobacteria containing a large number of CYP genes in their genomes. The mimivirus probably acquired the genes from these endosymbionts or from the ameba host. It is assumed that the mimivirus CYP gene develops into the gene encoding a protein with a completely different function not associated with its functioning as P450.

Since cytochromes P450 catalyze a huge variety of reactions, they can often be pathogenic factors in microorganisms. Chenge et al. studied the virulent strain of *M. tuberculosis* H37Rv, the genome of which contains 20 genes encoding cytochromes P450 [31]. Cytochromes of mycobacteria are involved in the metabolism of lipids and sterols, in the oxidative modification of respiratory menaquinone, and in the production of secondary metabolites. It was found that the cytochrome of the CYP144A1 family, which is encoded by the *Rv1777* gene of the above strain, binds to some azole preparations used for the tuberculosis treatment. This property makes it possible to consider CYP144A1 a promising target for the development of novel antituberculosis agents. The CYP144A1 orthologs were found in most mycobacterium species associated with human diseases; on the other hand, they are not found outside the *Mycobacterium* genus, which clearly indicates their role in the pathogenesis of these bacteria. It was shown by transcriptome analysis that different CYP144A1 forms, i.e. the complete and truncated forms with the lengths of 434 aa and 404 aa, respectively, are produced from alternative transcripts. The truncated form of CYP144A1 apparently plays an important role in mycobacterium physiology, because it represents more than 25% of all mycobacterial transcripts.

STRUCTURAL FEATURES OF BACTERIAL CYTOCHROMES P450

The structure of bacterial cytochromes P450 was characterized by X-ray crystallography. The main tertiary structure (core) is common for all studied cytochromes [32]. The amino acid sequences are variable; there are only three completely conservative residues in the CYP family. However, the common topography and structural folds of cytochromes are highly conservative. Unlike the core, the so-called flexible substrate recognition regions (SRSs) are the most variable; this makes it possible for cytochromes to be universal biological catalysts [2]. The conservative core consists of a helix (meander), four-helical beam (D, E, I, and L helices), J and K helices, and two sets of beta-sheets. They form the heme-binding region with an extremely conservative cysteine residue, which is the fifth ligand of the heme iron, the proton-transport groove, and a completely conservative EXXR motif in the K helix [http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=cl12078]. There is a highly conservative decapeptide in the beginning of the L helix, which can serve as a signature for identification of the P450 sequence in mapping of the genome. The general formula of the P450 motif is PxxGxxxCxG, where x is any amino acid residue, P is phenylalanine, G is glycine, and C is cysteine. Although the x residues can denote any amino acid, there are certain preferences that were identified when studying many P450 sequences. For example, the amino acid residue located two positions above cysteine is usually the histidine or arginine residue in most studied bacterial cytochromes P450. In addition to conservative decapeptide, there are other highly conservative regions in the P450 structure corresponding to the binding sites of redox partners or responsible for the general tertiary structure. Nevertheless, the types of the involved redox partners can vary from one P450 to another, while most bacterial cytochromes P450 utilize iron-sulfur redoxin for the electron transfer from NADH. In addition, it was revealed that the conservative threonine residue presents in most cytochromes P450 in order to attach and activate molecular oxygen, which binds to the heme iron before its involvement in catalysis of P450 substrates. This threonine residue acts along with the preceding amino acid residues in the formation of the proton transport channel inside the active site of P450, which is hydrated in the absence of a substrate [33].

The vast majority of P450 requires for the catalysis two electrons, which are eventually delivered from pyridine nucleotide coenzymes (NADPH or NADH). The electrons are transferred to the P450 heme through one or more redox proteins [34]. The first electron reduces the trivalent heme iron to the two-valent state, which can further bind molecular oxygen. The binding of a substrate facilitates the dissociation of the water ligand, which is weakly bound to iron and provides the transport of water to cysteine, which, in turn, induces electronic rearrangement in d-orbitals of the heme iron. This leads to a spin equilibrium shift of the heme iron from low spin (S = 1/2) to high spin (S = 5/2) state. In turn, this increases the reduction potential of the heme iron, which facilitates electron transfer to the heme iron from the redox partner. This mechanism can ensure that the electron transport to P450 and the subsequent ROS formation occur when a substrate is accessible for oxygenation. This phenomenon is observed for many cytochromes P450, e.g., for p450CA, (cyp101a1) from *P. putida* or P450 BM3 (CYP102A1) from *B. megaterium*, which have a strong substrate specificity. However, this is not always true for all cytochromes P450.

CLASSIFICATION OF BACTERIAL CYTOCHROMES BY COMPONENTS INVOLVED IN ELECTRON TRANSFER

The P450-dependent systems of the electron transfer can be divided into ten classes according to the involved protein components [36]. Most soluble bacterial CYPs belong to class I. Their activity is associated with ferredoxin and ferredoxin reductase. There are many genes for these proteins in bacterial genomes as, for example, for *S. coelicolor* A3 (2) CYP105D5. The functional redundancy of these genes was shown in several studies. Class I also includes archetypal CYP101 (P450 cam) from *P. putida*.

Class II includes most eukaryotic CYPs, which are localized in the endoplasmic reticulum and some other membranes via the N-terminal anchor and are attached to individual BADPH-cytochrome P450 reductase (CPR). Cytochromes of this class are responsible for a large number of reactions in eukaryotes, i.e., the oxidative metabolism of endogenous and exogenous compounds in animals, the synthesis of cutin and lignin in plants, and the synthesis of membrane sterols and mycotoxins in fungi [2]. There is only one described prokaryotic monooxygenase system in *Streptomyces carbophilus* consisting of CYP105A3 (P450sca) and NADH-dependent P450 reductase containing both FAD and FMN. This soluble monooxygenase system catalyzes the hydroxylation of mevastatin to pravastatin, which is an inhibitor of the biosynthesis of cholesterol.

Cytochromes P450 of class III from *Citrobacter braakii* were described in 2002. They resemble the classic bacterial system but have significant differences. In this system, electrons are transferred from the primary electron donors, NAD(P)H, to cytochrome P450 through NAD(P)H-dependent FAD-containing ferredoxin reductase and the second auxiliary redox protein. In the system of class I, the second mediator protein is the iron-sulfur protein, while flavodoxin (cindoxin) is presumably the immediate electron donor for cytochrome CYP176A1 ($P450_{cin}$) from *C. braakii*. In system I, in contrast to system II containing NADPH-cytochrome P450 reductase (CPR), two redox centers of FAD and FMN are two different proteins [2].

Cytochromes of class IV were found in acido-thermophilic archaea. Cytochromes CYP119 from *Sulfolobus solfataricus* can take electrons from non-NAD(P)H-dependent reductase; they are active at high temperature and pressure [36].

Class V is represented by CYP51 (sterol-14α-demethylase) from *Methylococcus capsulatus* consisting of two separate protein components, i.e., NAD(P)H-dependent reductase and cytochrome P450-ferredoxin fusion protein. This enzyme has a unique primary structure in which the heme-monooxygenase P450 domain at the C termini is fused with the 3FE–4S ferredoxin domain through the alanine-enriched linker. The linker plays a role of a flexible hinge to provide the interaction between two domains [2].

The cytochrome P450 system of class VI consists of NAD(P)H-dependent flavoprotein reductase and flavodoxin-P450 fusion protein. Thus, this system is between the $P450_{BM3}$ and P450cin systems, which utilize the same redox centers, i.e., FAD, FMN, and heme, but differ in the amount and characteristics of proteins in this system. This cytochrome was found in *Rhodococcus rhodochrous* (the 11Y strain) and was shown to be responsible for the degradation of widely used explosive hexahydro-1,3,5-trinitrotoluol-1,3,5-triazine (RDX).

The first discovered cytochrome of class VII was CYP116B2 ($P450_{RhF}$) *from Rhodococcus sp*. NCIMB 9784. This protein consists of the C-terminal domain of reductase containing FMN and NADP-binding motifs and the ferredoxin-like domain containing N-terminal heme; the latter domain is linked to the C-terminus via a linker [36]. Fusion enzymes of phthalate dioxygenase reductase of the CYP116 family and homologous to $P450_{RhF}$ were found in three pathogenic species, i.e., in *Burkholderia* and *Ralstonia eutropha JMP134, Gibberella zeae PH*-1 fungi, and in the *Ralstonia metallidurans* bacterium, which is resistant to heavy metals.

Cytochromes of class VIII include enzymes containing the N-terminal domain of the heme attached to the domain of cytochrome P450 reductase of the eukaryotic type at the C terminus. The first described cytochrome of this system was CYP102A1 ($P450_{BM3}$) from *B. megaterium*, which is involved in fatty acid metabolism. A similar membrane-binding system was

also found in *Fusarium oxysporum* (CYP505) [37] and in a number of genomes of mycelial fungi [38].

No prokaryotic cytochromes were found among classes IX and X.

CYTOCHROME GENES

The very first CYP gene probably appeared at the dawn of the evolution of living organisms. The comparison of amino acid sequences of cytochromes P450 of eukaryotes and prokaryotes leads to the conclusion that the P450 gene superfamily appeared and diverged from one ancestral prokaryotic gene. The ancestral P450 gene was developed through duplications and mutations that led to the appearance of many genes encoding different molecular types of cytochromes P450 [2]. The processes of amplification, gene conversion, gene loss, and horizontal gene transfer also occurred [39]. A sample of horizontal gene transfer was described in [40] for *Acinetobacter radioresistens* S13. The authors identified the gene of self-sufficient monooxygenase (CYP116B5) of cytochromes P450 of class VII. This is an amazing discovery, because only cytochromes of class I were identified until recently in the *Acinetobacter* species. The authors suggest that a horizontal gene transfer occurred through plasmids from the *Rhodococcus jostii* RHA1 donor to *A. radioresistens* S13, because both bacteria occupy one ecological niche and are able to destruct xenobiotics. This event was accompanied by the fusion of genes and subsequent integration of new fusion genes in the *A. radioresistens* S13 chromosome that led to the acquisition of new metabolic capabilities, such as oxidation of alkanes. The gene fusion leads to the union of proteins that operate consistently. These proteins include consecutive enzymes of metabolic pathways, the enzymes and domains involved in their regulation, or DNA-binding and ligand-binding domains in prokaryotic transcription regulators. The selective advantage of the fusion domain is improved cohesion efficiency for the corresponding stage of the biochemical reaction or signal transduction and the close interaction of the regulation of expression of the fused domains.

In most cases, cytochrome P450 is an inducible enzyme, with different organic compounds and substrates being inducers of biosynthesis. The cytochrome genes are often included in operons, the expression of which is regulated in various ways. For example, cytochrome $P450_{BM-3}$ from *B. Megaterium* with both the cytochrome P450 reductase and P450 monooxygenase activities was shown to be expressed in an operon that was under the negative regulation of transcription repressor Bm3R1. It was found that repression is inactivated and cytochrome $P450_{BM-3}$ is expressed in mutants with a single point mutation in the DNA-binding domain of Bm3R1. Thus, the inhibition of the Bm3R1 binding to its operator is closely related to the induction of the cytochrome $P450_{BM-3}$ gene [41]. Cytochrome P450 of the CYP249 family encoding by the *ethB* gene was identified in the strains of *Rhodococcus ruber* IFP 2001, *Rhodococcus zopfii* IFP 2005, and *Gordonia* sp. IFP 2009 (previously, *Mycobacterium* sp.), which were isolated due to their ability to grow on ethyl-tert-butyl ether (ETBE) and to degrade it to tert-butyl alcohol. It was shown that all *eth* clusters of these strains are under the control of the *ethR* gene, which encodes the supposed positive regulator of transcription of the AraC/XylS family. It was also shown that the specificity of the cytochrome *Eth* system is related to the regulator but not to the cytochrome. All clusters were revealed to be located on transposons, which are easily lost under the nonselective cultivation conditions. This loss led to the isolation of the *R. ruber* ETBE mutant, which was used to demonstrate the role of the *eth* cluster in the ETBE degradation. Sequence analysis of the *eth* gene clusters in *R. ruber* IFP 2001 has shown a significant similarity with the *thc* system from *R. erythropolis* NI86/21 encoding the cytochrome P450 system, which catalyzes S-dealkylation of herbicide of S-ethyl dipropyl thiocarbamate [42]. Expression of the *Alcanivorax borkumensis* genes involved in the metabolism of alkanes (two genes of AlkB hydroxylases and three genes of P450) is induced in the presence of the substrate, although the regulators of this process have been poorly studied [43].

ROLE OF BACTERIAL CYTOCHROMES P450 IN DEGRADATION OF CARBOHYDRATES

One important function of bacterial cytochromes is their involvement in the biodegradation of various carbohydrates. A large number of studies are devoted to the degradation of alkanes, because they are the bulk of crude oil, which is a common pollutant in the environment.

Alkanes are saturated carbohydrates consisting exclusively of carbon and hydrogen atoms. They may be linear (*n*-alkanes), cyclic (cyclo-alkanes), and branched alkanes (isoalkanes). Alkanes can account for up to 50% of crude oil, depending on its source. Alkanes may be formed in the process of metabolism of many living organisms, such as plants, green algae, bacteria, and animals. This is probably the reason for the presence of alkanes at low concentrations in most soil and aquatic environments. Alkanes are nonpolar chemically inert molecules; they are poorly soluble in water and tend to accumulate in cellular membranes; they need energy to be activated. All these factors complicate their metabolism in microorganisms. Nevertheless, some microorganisms, both aerobic and anaerobic, can utilize various alkanes as a source of carbon and energy [43]. There is a well-characterized alkane hydroxylase detected in many alkane-degrading strains. This enzyme is encoded by the *alkB* gene and contains nonheme iron [44]. In some cases, however, it is difficult to associate the presence of the AlkB

hydroxylase genes with the ability to degrade aliphatic carbohydrates and even to detect this gene in microorganisms in oil-polluted areas [29]. An alternative way induced by CYP153 hydroxylases was shown to be a common process for the destruction of alkanes that are not degraded by AlkB [44]. The CYP153 genes were identified in many oil-oxidizing microorganisms. These genes isolated from oil-polluted areas were shown to encode alkane hydroxylases [3, 45]. Some bacterial strains degrading medium-chain (C5-C10) alkanes contain alkane hydroxylases that belong to a separate family of soluble cytochrome P450 monooxygenases. The first member of this family was CYP153A1 from *Acinetobacter* sp. EB104. Similar enzymes were found in different strains of mycobacteria, rhodococcus, and proteobacteria [45]. Funhoff et al. [46] showed that many strains capable of metabolizing linear alkanes contain CYP153A1-related cytochromes P450. These strains include *A. borkumensis* species, which are a significant part of the biomass in oil-contaminated marine habitats, alkane degrading strains, such as *Sphingomonas* sp. HXN-200 and *Oleomonas sagaranensis* HXN-1400, and several mycobacterial species isolated from a trickling bioreactor, which ensure the removal of hexane from the air stream. These strains contain one or more alkane-degrading cytochromes P450 of the CYP153 family instead of or in addition to the well-studied enzyme systems of terminal alkane hydroxylation, e.g., AlkB. It has been shown that CYP153A6 is the first soluble cytochrome P450 that predominantly hydroxylates nonreactive aliphatic alkanes with a high regioselectivity at the terminal positions. The medium-chain and long-chain alkanes are predominant substrates, although short-chain aliphatic and cyclic alkanes also bind to the active site and can be hydroxylated. Minerdi et al. studied alkane degradation by the *A. radioresistens* S13 strain and came to similar conclusions [40]. They have found the first self-sufficient cytochrome P450 of class VII (CYP116B5), which allows *A. radioresistens* S13 to utilize the medium-chain (C14–C16) and long-chain (C24–C36) alkanes as a single source of carbon and energy.

Cytochromes can oxidize not only linear alkanes but also aromatic carbohydrates. The introduction of an oxygen atom into the molecule of an oxidized substrate in the form of the hydroxyl group increases the solubility of aromatic compounds, which is the key role of cytochrome P450 in the metabolism of these compounds. Du et al. discovered a unique means of 4-cresol degradation in *Corynebacterium glutamicum* and found a unique P450 system of class I, CreJEF, which specifically recognizes phosphorylated intermediate products and successively oxidizes the aromatic methyl groups to the functional groups of carbonic acid through the alcohol and aldehyde intermediates [47]. The *Novosphingobium aromaticivorans* genome was shown to have 15 cytochromes P450 of different families, two of which (CYP108D1 and CYP203A2) bind aromatic substrates, and the rest cytochromes bind linear alkanes (CYP153C1) and mono- and sesquiterpenoid compounds [48]. Leont'ev et al. showed the key role of cytochrome P450-dependent monooxygenase system of bacteria of *Pseudomonas* species in the biotransformation and biodegradation of aliphatic and aromatic compounds [49].

As mentioned above, the *R. ruber* IFP 2001, *R. zopfii* IFP 2005, and *Gordonia* sp. IFP 2009 strains contain P450 monooxygenase CYP249A1, which is able to decompose fuel oxygenates, such as methyl-tert-butyl ether (MTBE), ethyl-tert-butyl ether (ETBE), and tert-amyl-methyl ether (TAME) [42]. CYP249A1 in *R. ruber* IFP 2001 was active against ETBE, and growth occurred due to the C2 fragment, which was released after the cleavage of the ether bond. At the same time, tert-butyl alcohol, the intermediate product of ETBE decomposition, accumulated in the culture. It is noteworthy that cometabolic biodegradation of MEBE and TAME was shown after induction of the cytochrome P450 system in *R. ruber* IFP 2001 when grown on ETBE. Bernstein et al. showed that cytochrome of class VI from *R. rhodochrous* 11Y catalyzes the initial stage of aerobic degradation of explosive hexahydro-1,3,5-trinitro-1,3,5-triazine, which is a pollutant of soil and groundwater [50].

USE OF CYTOCHROMES IN BIOTECHNOLOGICAL PRODUCTION

The ability of cytochromes to regio- and stereospecifically oxidize substrates makes them quite promising catalysts for their use in synthetic biology and in the synthesis of valuable compounds (antibiotics, drug metabolites, steroids, and terpenes) when their chemical synthesis is unprofitable [14, 51, 52]. As a rule, the synthesis of these compounds is performed by biotransformation in the cells of bacteria, yeast, and fungi with the use of both the initial P450 forms and the enzyme forms that were changed by protein engineering to expand its substrate specificity [53]. For example, artemisinin with antimalarial activity (CYP71AV1) and analgesic morphine (CYP82Y2 and CYP719B1) can be synthesized in the *Saccharomyces cerevisiae* cells. The *Penicillium chrysogenum* strain with introduced P450Prava genes from *Amycolatopsis orientalis* is a producer of pravastatin, which can reduce the cholesterol level. CYP199A2 from *Rhodopseudomonas palustris* is involved in the two-stage fermentation of tyrosine to caffeic acid with anticancer and antioxidant activities. Cytochrome CYP107DY1 and its redox partners (BmCPR and Fdx2) were successively used to design an E. coli-based whole-cell system for the efficient biotransformation of mevastatin [54]. In addition, bacterial cytochromes P450 may be used to fight mycotoxins, which are a serious problem for agriculture because of declining yields, livestock diseases, and adverse effects on human health [55]. Ito et al.

have shown the possibility of using cytochrome P450 from the *Sphingomonas* sp. KSM1 strain for hydroxylation of deoxynivalenol, a mycotoxin produced by fungi of the *Fusarium* genus [56], which causes fusariosis in wheat and other grain crops. The final product of this enzyme system is 16-hydroxy-deoxynivalenol, which has significantly lower toxicity as compared to the initial compound. Thus, cytochromes P-450 play an important role in the synthesis of valuable compounds, and their use has broad prospects in biotechnological production.

CONCLUSIONS

Thus, bacterial cytochromes P450 play a very important role in the life of the prokaryotic cell by catalyzing a vast number of enzymatic reactions. Cytochromes are involved in the initial stages of oxidation of carbohydrates and xenobiotics by means of both the introduction of an oxygen atom in the molecule structure and, probably, the generation of the reactive oxygen species. These processes provide efficient biodegradation and biotransformation of these compounds. This allows bacteria containing the cytochrome genes to exist in conditions of contaminated biotopes and to use pollutants as the source of carbon and energy, thus taking part in the natural bioremediation of contaminated areas. Despite the large number of described cytochrome genes, not all of them have identified functions. It is therefore necessary to continue the study of this unique class of enzymes.

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