Front. Environ. Sci. Eng. 2020, 14(1): 9
https://doi.org/10.1007/s11783-019-1188-2

REVIEW ARTICLE

Transmembrane transport of polycyclic aromatic hydrocarbons by bacteria and functional regulation of membrane proteins

Hongqi Wang (✉)[1], Ruhan Jiang[1], Dekang Kong[1], Zili Liu[1], Xiaoxiong Wu[1], Jie Xu[1], Yi Li (✉)[2,3]

1 College of Water Sciences, Beijing Normal University, Beijing 100875, China
2 Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Ministry of Education), Guangxi Normal University, Guilin 541004, China
3 College of Environment and Resource, Guangxi Normal University, Guilin 541004, China

HIGHLIGHTS

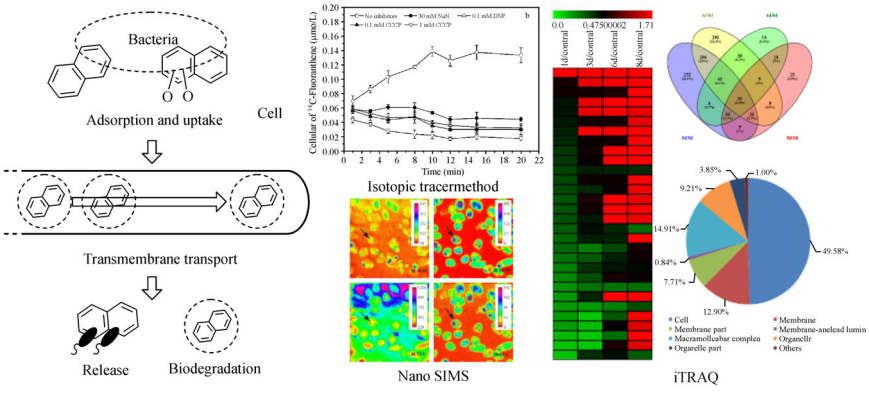
- Explainteadsorption, uptake and transmembrane transport of PAHs by bacteria.
- Analyze functional regulation of membrane proteins inthe transmembrane transport.
- Proteomics technology such as iTRAQ labeling was used to access expressed proteins.
- Single cell analysis technology wereused to study the morphological structure.

ARTICLE INFO

Article history:
Received 10 June 2019
Revised 27 August 2019
Accepted 16 September 2019
Available online 28 October 2019

Keywords:
Polycyclic aromatic hydrocarbons
Transmembrane transport
Adsorption and uptake of hydrocarbons
Proteomics
Functional regulation of membrane protein
Single cell analysis technology

GRAPHIC ABSTRACT



ABSTRACT

In recent years, increasing research has been conducted on transmembrane transport processes and the mechanisms behind the microbial breakdown of polycyclic aromatic hydrocarbons (PAHs), including the role of membrane proteins in transmembrane transport and the mode of transmission. This article explains the adsorption, uptake and transmembrane transport of PAHs by bacteria, the regulation of membrane protein function during the transmembrane transport. There are three different regulation mechanisms for uptake, depending on the state and size of the oil droplets relative to the size of the microbial cells, which are (i) direct adhesion, (ii) emulsification and pseudosolubilization, and (iii) interfacial uptake. Furthermore, two main transmembrane transport modes are introduced, which are (i) active transport and (ii) passive uptake and active efflux mechanism. Meanwhile, introduce the proteomics and single cell analysis technology used to address these areas of research, such as Isobaric tags for relative and absolute quantitation (iTRAQ) technology and Nano Secondary ion mass spectrometry (Nano-SIMS). Additionally, analyze the changes in morphology and structure and the characteristics of microbial cell membranes in the process of transmembrane transport. Finally, recognize the microscopic mechanism of PAHs biodegradation in terms of cell and membrane proteins are of great theoretical and practical significance for understanding the factors that influence the efficient degradation of PAHs contaminants in soil and for remediating the PAHs contamination in this area with biotechnology.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more benzene rings arranged according to certain rules; they usually contain only

✉ Corresponding authors
E-mail: amba@bnu.edu.cn (H. Wang); liyi412@mailbox.gxnu.edu.cn (Y. Li)

carbon (C) and hydrogen (H), but heterocyclic aromatic hydrocarbons have oxygen (O), nitrogen (N) and/or sulfur (S) atoms (Gan et al., 2009). PAHs constitute a range of potential environmental pollutants, which are often carcinogenic, teratogenic, mutagenic, and highly toxic. In addition, Fluoranthene are considered as the typical persistent organic pollutants because they are difficult to degrade in the environment (Bautista et al., 2009; Hua and Wang, 2012). PAHs in the environment are mainly derived from incomplete combustion of coal, oil, gasoline, wood and other fuels used by humans in industrial and agricultural production, transportation and daily life. Because of their high hydrophobicity, PAHs are stable in soil, water, sediment and the atmosphere, and the magnitude of their toxic effects increases with increases in their molecular weight. PAHs are harmful to the human body, which they can enter through inhalation, ingestion and skin contact, and long-term exposure to PAHs contamination can cause respiratory diseases and cancer (Gao et al., 2016). At present, there are more than 100 known PAHs, and of these, 28 PAHs were among the contaminants that the US Environmental Protection Agency listed in its 2008 call for "priority monitoring of pollutants" (Bansal and Kim, 2015).

Microbial degradation is the main mechanism of PAHs degradation in nature. Because it is economical, safe, and capable of treating various forms of pollutants without creating secondary pollution, microbial remediation technology has been rapidly developed in recent years. For decades, most studies have focused on the physical or chemical factors affecting the biodegradation of PAHs in the aerated zone of soil (Stapleton et al., 1994; Volkering et al., 1995). However, in recent years, increasing research has been conducted on the transmembrane transport process and the mechanism of microbial breakdown of PAHs, specifically, the role of membrane proteins and the transmission mode in transmembrane transport. Analyzing changes in morphology, structure and characteristics of microbial cell membranes during transmembrane transport and recognizing the microscopic mechanism of PAHs biodegradation in terms of cell and membrane proteins are of great theoretical and practical significance for understanding the factors that improve the degradation efficiency of PAHs contaminants in the aerated soil zone and for remediating PAHs contamination in this area with biotechnology. Degradation of petroleum pollutants by microorganisms can be divided into two ways: intracellular degradation and extracellular degradation. The whole process of degradation can be divided into three steps. The first is the adsorption and uptake of compounds by microorganisms, which is a dynamic equilibrium process. The second is the process by which compounds adsorbed on the surface of the cell membrane enter the cell membrane, which is called transmembrane transport. Finally, the compounds enter the cell membrane of microorganisms and combine with degradation enzymes for enzymatic reactions (Hua and Wang, 2014). This article explains the adsorption, uptake and transmembrane transport of hydrocarbons by bacteria; the functional regulation of membrane proteins during the transmembrane transport process; and the proteomics and single cell analysis technology used to address these areas of research.

2 Adsorption and uptake of hydrocarbons by bacteria

Adsorption and degradation are two important processes in microbial repair of damage caused by PAHs (Chen et al., 2010; Chen and Ding, 2012). However, at present, more research has been conducted on the microbial degradation of PAHs, and less attention has been paid to the adsorption of PAHs by microorganisms. Some studies have shown that the adsorption of PAHs by microorganisms greatly affects the contamination levels of PAHs in the environment. Microorganisms have abundant adsorption sites, and the mechanism by which pollutants are adsorbed differs. At present, the mechanism of by which microorganisms adsorb water-soluble particles such as heavy metals, dyes and pesticides has been studied in depth for some time. However, the adsorption and mechanism of microorganisms for nonpolar organic pollutants, such as PAHs, have been developed in only recent years (Stringfellow and Alvarez-Cohen, 1999; Xiao et al., 2007; Ke et al., 2010).

Research on the adsorption of PAHs by different bacteria was conducted for the first time in 1999 and revealed that the ability of different species and different strains of bacteria to adsorb phenanthrene differed; for example, the adsorption ability of *Nocardia* bacteria was the strongest, as it was related to cell hydrophobicity, but other parameters (such as the specific surface area, gram characteristics, polymerization characteristics, surface protein, and carbohydrate content) were not related. In addition, naphthalene could be added to reduce the adsorption of phenanthrene, indicating that phenanthrene undergoes surface-specific adsorption. Some special effects also play an important role in the adsorption process of PAHs onto bacteria (Stringfellow and Alvarez-Cohen, 1999). Researchers studied the adsorption of phenanthrene to gram-negative *Escherichia coli* and found that complex cations could neutralize the negative charges on the surface of bacteria and thus eliminate hydrophilic groups such as organic acids, proteins and polysaccharides, thus reducing the hydrophilicity and increasing the hydrophobicity on the surface of bacteria and thereby increasing the capacity of bacteria to adsorb phenanthrene by 1.5−4 times (Xiao et al., 2007).

The adsorption of microorganisms can promote the degradation of pollutants. Although the adsorption of PAHs by microorganisms decreases the rate of microbial degradation in the short-term, the PAHs adsorbed by microorganisms are eventually degraded with the prolon-

gation of incubation time. In addition, some microorganisms secrete extracellular polymers or surfactants, which contain a large number of water molecules, and through the combination of hydrophobic forces and hydrophobic organic pollutants, thus promote the release of pollutants into soil and other substances where the microorganism can readily adsorb them, thereby shortening the distance between pollutants and microorganisms and ultimately increasing the degradation and utilization of pollutants by microorganisms (Lai et al., 2009; Jia et al., 2011). Furuno et al. (2010) proved by agar block simulation experiments that the mycelia of growing fungi adsorbed PAHs and stored them in lipid vesicles, which transported them by protoplasmic flow in mycelia, from which they were released; this finding can be used to explain bacterial degradation at release sites. Scheme of the proposed steps involved in mycelial PAHs transport. The hypha (light dotted) contains lipid-rich vesicles (circles) which are transported inside the hypha via cytoplasmic streaming (indicated by the arrow inside the hypha). The steps are (i) PAHs uptake from the source, (ii) transport inside the hyphae, and (iii) release from the hyphae in the presence of a contaminant sink (e.g., bacteria) (Fig. 1). This process is important for the remediation of contaminants in soil environments where bacterial mobility is usually poor (Furuno et al., 2010; Schamfuss et al., 2013; Yin et al., 2015).

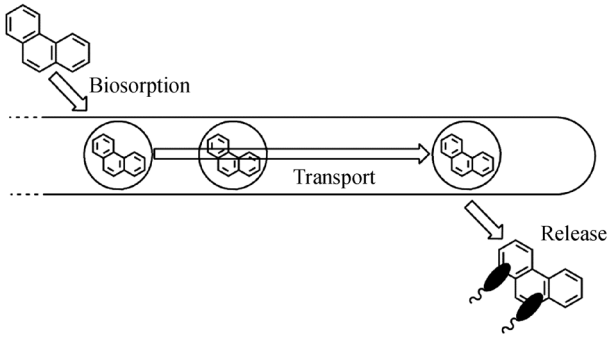


Fig. 1 Scheme of mycelia fluoranthene biosorption, transport and release (Schamfuss et al., 2013).

Despite the high hydrophobicity and the low bioavailability of PAHs, the hydrophobic nature of the bacterial surface has been cited as a factor in the growth of cells on water-insoluble hydrophobic substrates (Rosenberg, 1993). Typically, there are three different regulation mechanisms, depending on the state and size of the oil droplets relative to the size of the microbial cells (**Table 1**): (i) direct adhesion, where which microorganisms attach to the soluble hydrocarbon drops, which are much smaller than cells, and substrate uptake presumably takes place through diffusion or active transport at the point of contact (Miyata et al., 2004); (ii) emulsification and pseudosolubilization, which are mediated by cell biosurfactant contact with emulsified small hydrocarbon droplets (Bouchez-Naitali et al., 1999; García-Junco et al., 2001); and (iii) interfacial uptake, in which highly specific uptake systems have affinity for pollutants and the pollution-attached microorganisms grow as a confluent biofilm (Westgate et al., 1995).

2.1 Direct adhesion

Bacterial uptake of dissolved PAHs in the aqueous phase is generally only applicable to water-soluble PAHs and is also known as the "single diffusion-dissolution uptake" model. When microorganisms directly absorb hydrocarbons dissolved in water, they use dissolved PAHs as carbon and energy sources to grow. At this time, the efficiency of microorganism adsorption and uptake of hydrocarbons is closely related to the solubility of hydrocarbons in water. If the growth rate of microorganisms is slow and the solubility of hydrocarbons is high, microorganisms will have enough carbon and energy to use (Rosenberg et al., 1980). When the bacteria continue to grow, the PAHs dissolved in the water phase can no longer supply sufficient carbon and energy needed for the growth of bacteria. When the solubility of PAHs in water droplets become undetectable, the growth rate of microorganisms due to adsorbing and absorbing hydrocarbons also drops sharply (Thomas et al., 1986). Stucki et al. indicated that the solubility of hydrocarbons in water limits the degradation efficiency of hydrocarbons by microorganisms (Stucki and Alexander, 1987). In addition, the extracellular and organic substances (EPS) play an important role in direct adsorption. EPS produced by bacteria is a key factor mediating the formation of biofilm of bacteria attached to other surfaces (Pan et al. 2012). So as to improve the bioavailability of insoluble PAHs and promote biodegradation. EPS isolated from *Zoogloea* sp. and *Aspergillus Niger (a. Niger)* were used to study the degradation of pyrene in contaminated soil. The results showed that any kind of EPS could degrade pyrene, and the reduction amount of pyrene increased with the increase of the initial concentration of EPS, indicating that EPS plays a very important role in the direct adsorption of PAHs by bacteria (Chunyun et al., 2011). In addition, Compared to inoculation of EPS-extracted bacteria, the inoculation of bacteria without EPS-extraction improved biodegradation of both PHE and PYR. EPS forms a microenvironment on the surface of bacterial cells, helping them avoid direct contact between PHE and PYR molecules on the surface of bacterial cells, thus protecting the cells from hydrophobic damage. Meanwhile, EPS is conducive to the formation of bacteria attaching to the surface of humus and biofilm, which may allow higher bacterial growth rate (Zhang et al., 2015).

Table 1 Three different regulation mechanisms in the adsorption and uptake of hydrocarbons by bacteria

Adsorption type	Microbial species	Hydrocarbons species	Parameters and results	References
Direct adhesion	*Pseudomonas* sp. M1	*n*-Hexadecane	The degradation was not significantly affected by 4 mM EDTA.	Goswami and Singh (1991)
	Acinetobacter sp. CR	*n*-Heneicosane	Higher impeller speed resulted in both lower microbial growth and lower *n*-alkane degradation rate of the bacterium, although it increased the specific surface area of the oil, which was measured by a previously developed device. This result was due to the decreased number of cells adhering to the oil surface, i.e., intense agitation inhibited the adhesion of cells to the oil surface.	Hori et al. (2002)
	Rhodococcus equi OU2	Hexadecane	The surfactant is unnecessary for hexadecane to be degraded by OU2	Bouchez-Naitali and Vandecasteele (2008)
	Rhodococcus baikonurensis EN3	Diesel oil	Tergitol 15 S, APG, LAE 9 and Tween 80 were effective only to a small extent in the enhancement of the biodegradation of diesel oil, and SDS inhibited rather than stimulated the degradation of diesel oil.	Lee et al. (2006)
Emulsification and pseudosolubilization	*Pseudomonas* sp. N1	*n*-Hexadecane	The degradation was strongly inhibited by the 4 mM EDTA.	Goswami and Singh (1991)
	Pseudomonas aeruginosa GL1	Hexadecane	The surfactant is necessary for hexadecane to be degraded by GL1.	Bouchez-Naitali and Vandecasteele (2008)
	Pseudomonas aeruginosa AT10	Total petroleum hydrocarbons, the group of isoprenoids from the aliphatic fraction and the alkylated PAHs from the aromatic fraction	The rhamnolipid MAT10 reduced the surface tension (cST) up to 26.8 mN/m and the interfacial tension (cIT) against hexadecane to 1 mN/m. The CMC of MAT10 was 150 mg/L.	Geddes et al. (2008)
	Mixed consortia of *Pseudomonas aeruginosa strain* PG201, *Rhodococcus* sp. H131A, and a *Pseudomonas* strain which produced the rhamnolipid Dyna270	Hexadecane, dodecane, benzene, toluene, *iso*-octane, pristane (2,6,10,14-tetramethyl pentadecane), naphthalene, and phenanthrene	The rhamnolipid biosurfactants enhanced the rate of linear alkane biodegradation more than the biodegradation rate of the monoaromatics.	Inakollu et al. (2004)

(*Continued*)

Adsorption type	Microbial species	Hydrocarbons species	Parameters and results	References
	Rhodococcus baikonurensis EN3	Diesel oil	These hydrocarbon solubilization studies for pure hydrocarbons and mixed hydrocarbon systems have indicated that the biosurfactant shows hydrocarbon specificity, with hydrocarbon substrate packed within the micelle core and is influenced by the size and shape of the hydrocarbon substrate in the mixed waste systems.	Lee et al. (2006)
	Rhodococcus sp.	Pyrene	The experimental kinetic data were fitted well with a mathematical model showing PAHs uptake both from the interface and from the aqueous medium by a population consisting of adsorbed cells in dynamic equilibrium with cells in the aqueous medium; interfacial uptake was predominant in these experiments.	Bouchez et al. (1997)
	Mycobacterium PYR-1	PAHs	Results showed that the agitation rate affected cell growth and PAH degradation rates, while the substrate concentration did not; these are two characteristics of systems that exhibit an interfacial uptake mechanism. Moreover, detailed examination using fluorescence microscopy revealed that, in addition to associating with the aqueous-organic interface, this bacterium exists exclusively on the organic side of the interface.	MacLeod and Daugulis (2005)
	Candida lipolytica	Hexadecane	Interfacial tension between oil and water and Sauter mean drop size decreased as cultivation proceeded.	Nakahara et al. (1977)
	Candida lipolytica NRRL Y-6795 *Candida intermedia* IF0 0761 *Candida tropicalis* ATCC 20336 *Saccharomyces cerevisiae* HANSEN IF0 0305	*n*-Decane	*C. intermedia* and *C. tropicalis*, which can utilize hydrocarbon, adhere well to hydrocarbon, but *S. cerevisiae*, which cannot utilize hydrocarbon, did not adhere.	Miura et al. (1977)

2.2 Emulsification and pseudosolubilization

In the process of bacterial degradation of PAHs, surfactants often improve the bioavailability of PAHs and accelerate the mass transfer rate of microorganisms such that PAHs can be degraded by microorganisms more quickly. These surfactants, including fatty acids, proteins, lipids and extracellular polymers, are secreted by the microorganisms themselves. For some reaction processes, surfactants can also be artificially added to improve the degradation rate. The surface activity of the surfactant is due to the polarity and nonpolarity of the molecule itself. Hydrophobic groups prevent the surfactant from dissolving in water and migrate away from the water, while hydrophilic groups force the molecule to enter the water (Bramwell and Laha, 2000).

PAHs in soils mostly exist in the form of Non-Aqueous Phase Liquids (NAPLs), such that they degrade like hydrocarbons in the aqueous phase. PAHs in unsaturated soils are trapped and degraded through the reduction of interfacial tension during contact with bacteria. Goswami et al. indicated that bacteria can ingest hydrocarbons in soil by direct contact. However, bacteria cannot directly adhere to solid substrates. Therefore, hydrocarbons in soil need to be dissolved before contact with bacteria so that they can adsorbed and degraded (Goswami et al., 1983). The kinetic relationship between the dissolution rate of solid substrates in soil and the growth rate of microorganisms was studied by Mulder et al. The results showed that the degradation rate of naphthalene by *Pseudomonas* sp. was limited by the dissolution rate of naphthalene. However,, the products secreted by bacteria did not play a substantial role in improving the solubility (Mulder et al., 1998). Adsorption of surfactant molecules to soil depends on the properties of the soil and the surfactant (Edwards et al., 1992, Edwards et al., 1994). Although surfactant adsorption is an adverse process for micellar solubilization (West and Harwell, 1992), it may play an important role in promoting transport of adsorbed substrates. A schematic overview of the interactions between bacteria, soil, pollutants, and surfactants by Volkering et al. (1997). is presented in Fig. 2: I. sorption of pollutant, II. sorption of surfactant molecules by soil, III. solubilization of pollutant, IV. uptake of pollutant from the water phase by bacteria, V. partitioning of pollutant between the water phase and the micelles; VI. sorption of micelles by bacteria; VII. direct uptake of pollutant from the solid phase by bacteria; and VIII. sorption of bacteria by soil. However, due to the specificity of the soil itself, the interaction between soil, microorganisms, PAHs and biological surfactants is more complex in the actual remediation process.

2.3 Interfacial uptake

Bacteria are hydrophilic, while PAHs are highly hydrophobic. Therefore, bacteria overcome their own hydrophilic characteristics to absorb hydrophobic PAHs for degradation in a process shown feasible in current studies. Westgate et al. indicated that microorganisms contact hydrocarbon substrates at the liquid-liquid interface for

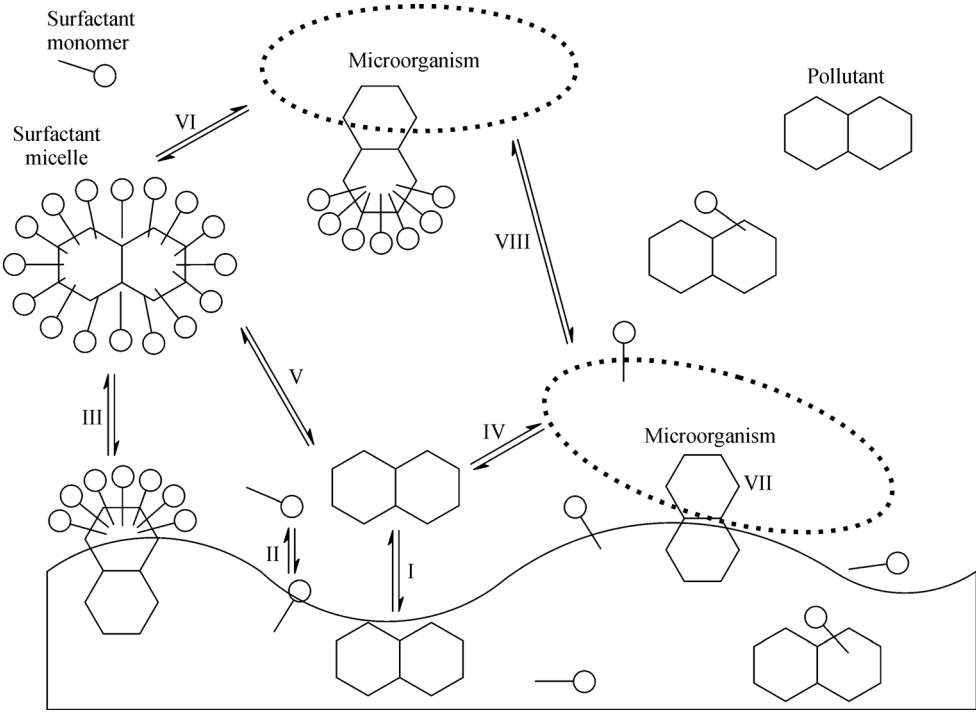


Fig. 2 Schematic overview of the interactions between bacteria, soil, pollutants, and surfactants (Volkering et al., 1997).

adsorption and uptake. During interfacial contact, the degradation rate depends on the concentration of the solvent phase. Therefore, a high affinity between the microorganisms and solvent phase (substrate) is required (Westgate et al., 1995). A series of kinetic studies on pyrene degradation by *Rhodococcus* sp. showed that the solubility of microorganisms in the aqueous phase could be neglected when the cell density at the interface was high and the transfer rate of substrates to the aqueous phase was low. In this case, the uptake mode of PAHs by microorganisms was mainly demonstrated by the interface uptake mode (Bouchez et al., 1997). The degradation of PAHs by *Mycobacterium* PYR-1 was studied by MacLeod et al. As shown by fluorescence microscopy, bacteria are mainly located at the organic phase interface when they take up PAHs (Fig. 3) (MacLeod and Daugulis, 2005).

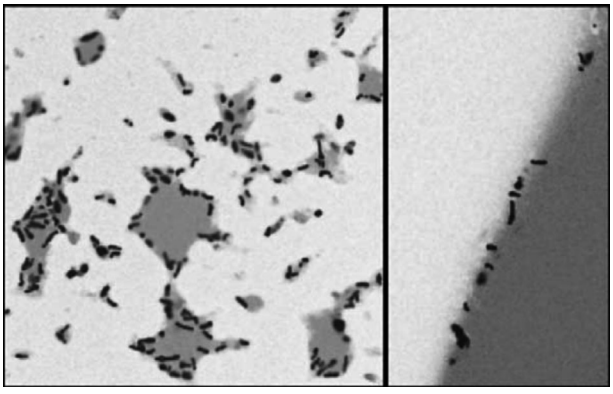


Fig. 3 Uptake of PAHs by *Mycobacterium* PYR-1 exanimated using fluorescence microscopy (MacLeod and Daugulis, 2005).

3 Transmembrane transport and biodegradation of hydrocarbons by bacteria

3.1 Transmembrane transport of hydrocarbons by bacteria

Passive transport and active are two kinds of transmembrane transport by which hydrocarbons are taken up by bacteria (Table 2). In passive transport, matter is transferred from areas of high concentration to areas of low concentration by the concentration gradient and includes the processes of simple diffusion and alienation diffusion. Simple diffusion, also known as free diffusion, is characterized by pollutants that are spread along the concentration gradient, no requirement for added during transport and no assistance from membrane proteins (Borbás et al., 2016; Foster and Miklavcic, 2016). The differentiating characteristic of alienation diffusion is that the rate of transfer is high such that, within a certain range, it is proportional to the concentration of the substance being transported. In contrast, active transport has the following four characteristics: it can be transported against the concentration gradient, it requires energy or is coupled with a process for releasing energy, it relies on transport proteins in the membrane, and it is selective and specific (Vedovato and Gadsby, 2014).

Hearn et al. (2008) proposed that bacterial biodegradation of hydrocarbons requires the passage of hydrophobic substrate across the cell membrane, which means transmembrane transport of hydrocarbons is the first step in biodegradation. Batman et al. found that naphthalene crosses the membrane of *Pseudomonas putida* PpG1064 by simple diffusion because ATP was not essential for association with or movement of the compound into the cell (Bateman et al., 1986). However, Bugg et al. (2000) found two conflicting transport mechanisms for PAHs uptake by *Pseudomonas fluorescens* LP6a: passive diffusion and excretion by an active efflux mechanism. The isotopic tracer method was used by Hua and Wang (2014) and Li et al. (2014) to analyze the transmembrane transport process of hydrocarbons. Hua and Wang (2014) investigated the transmembrane transport process of ^{14}C-octadecane by *Pseudomonas* sp. DG17 and found that addition of the energy inhibitor NaN_3 inhibited the uptake of ^{14}C-octadecane by *Pseudomonas* sp. DG17, suggesting that the transmembrane transport of ^{14}C-octadecane into *Pseudomonas* sp. DG17 is based on an active transport process that requires energy. Li et al. (2014) investigated the transmembrane transport process of ^{14}C-fluoranthene by *Rhodococcus* sp. BAP-1 and demonstrated that the mechanism for fluoranthene travel across the cell membrane of *Rhodococcus* sp. BAP-1 requires energy. In this study, Figure 4 showed that when the ATP inhibitors were absence, after incubation for 10 min, the cellular ^{14}C-fluoranthene level was 0.1384 ± 0.016 mol/L, and then at 12 min declined to 0.1255 ± 0.076 mol/L, after that the cellular ^{14}C-fluoranthene level was maintained at 0.1330 ± 0.010 mol/L at 20 min. This finding suggests that the fluoranthene concentration between cells is kept below the equilibrium level by the efflux pump. But in the presence of inhibitors, the uptake and efflux processes were strongly prevented. These finding demonstrated that the mechanism for fluoranthene travel across the cell membrane in *Rhodococcus* sp. BAP-1 requires energy, which was an energy-dependent active transport process (Fig. 4).

3.2 Biodegradation of hydrocarbons by bacteria

Changes in the environment include changes in temperature, salinity, nutrient elements, etc., which are the basic factors affecting the biodegradation process of PAHs by microorganisms.

As the temperature has a direct impact on microbial enzymatic reactions, Suszek-Łopatka et al. (2016) investigated the degradation of microorganisms at different temperatures, specifically, as the temperature increased from 15°C to 30°C, and found that, when the soil organic matter content was high, the degradation rate of micro-

Table 2 Passive and active transmembrane transport of hydrocarbons by bacteria

Type of transport	Microbial species	Hydrocarbon species	Features and conclusions	References
Active transport	Pseudomonas putida	Toluene	The results show that the efflux system in *P. putida* S12 is specific for organic solvents and does not export antibiotics or other known substrates of multidrug-resistant pumps.	Isken and De Bont (2000)
	Pseudomonas putida KT2442	Toluene	Solute molecules are transported across the cell membrane by consuming energy of an inverse concentration gradient with the participation of pump proteins.	Fukumori et al. (1998)
	Pseudomonas putida DOT-T1E	1,2,4-[C-14] Trichlorobenzene, toluene, xylenes, benzene	The concentration of organic molecules at the cell membrane increased with the addition of energy inhibitors.	Ramos et al. (1998)
	Pseudomonas sp. DG17	Octadecane	Addition of the energy inhibitor NaN_3 inhibited the uptake of ^{14}C-octadecane by *Pseudomonas* sp. DG17.	Hua and Wang (2014)
	Rhodococcus sp. BAP-1	Fluoranthene	Demonstrated that the mechanism for fluoranthene travel across the cell membrane of *Rhodococcus* sp. BAP-1 requires energy	Li et al. (2014)
Passive uptake and active efflux mechanism	Mixed microbial consortium *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Serratia liquefaciens*, *Pseudomonas fluorescens*	Phenol	This study was carried out to understand the effect of varying sublethal concentrations of phenol on isolated individual bacterial cultures.	Sharma et al. (2002)
Assisted diffusion of ingestible material and an active efflux mechanism	*Pseudomonas fluorescens* LP6a	Phenanthrene	The mechanism of transport of PAHs by *Pseudomonas fluorescens* LP6a, a PAM-degrading bacterium, was studied by inhibiting membrane transport and measuring the resulting change in cellular uptake. The data were consistent with the presence of two conflicting transport mechanisms: uptake by passive diffusion and an energy-driven efflux system to transport PAHs out of the cell.	Bugg et al. (2000)

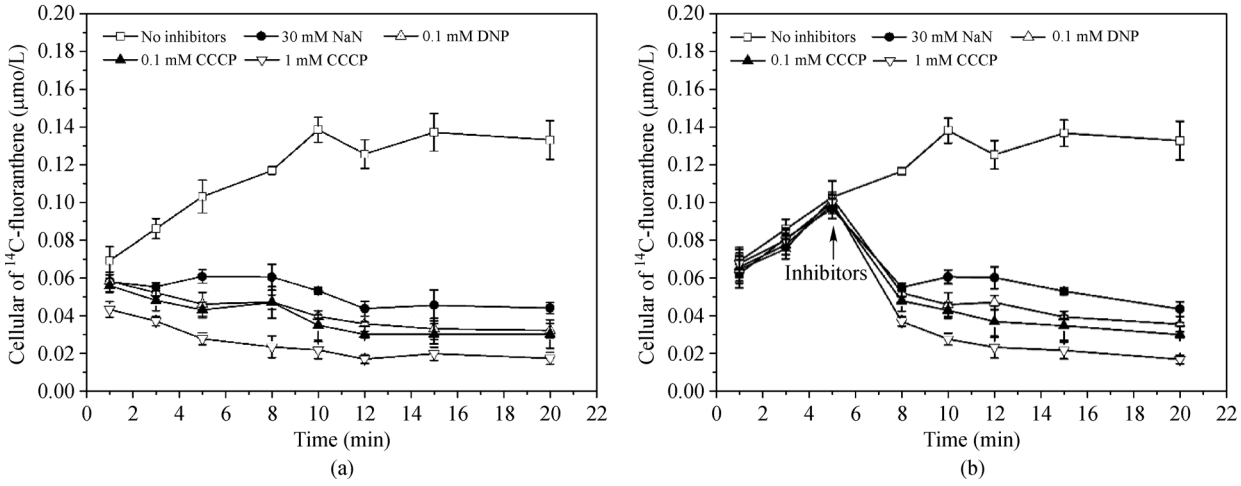


Fig. 4 Effect of different inhibitors on the trans-membrane transport of ^{14}C-fluoranthene by *Rhodococcus* sp. BAP-1 was added as an inhibitors at 0 min (a) and 5 min (b) (Li et al., 2014).

organisms for phenanthrene is significantly improved. Salt is an indispensable factor in the process of microbial growth and metabolism, and the effects of salinity change on the emulsification and the solubility of naphthalene were studied by Abouseoud et al. (2010); the results showed that the solubility rate of naphthalene decreased significantly under high-salinity conditions.

The biodegradation of PAHs requires suitable redox conditions, and the content of oxygen in soil is affected to a large extent by moisture, ventilation and other conditions. Militon et al. (2015) found that under permanent hypoxia, very little PAHs is degraded within 60 days, and in permanently oxygen-rich soil, bacteria degraded most of the PAHs within 15 days. Nutritional elements are important limiting factors for microbial growth and metabolism and thus the maintenance of microbial populations, and the types of nutrients required by different microorganisms differ. Appropriate addition of carbon sources or nitrogen sources can improve the activity of microorganisms in the soil, thus improving the degradation efficiency of the target pollutants. Studies by Jonsson and Östberg (2011) have shown that adding additional carbon sources, especially lactic acid and vitamins, during the degradation of phenanthrene can effectively promote the degradation of this compound. The content of organic matter is the determining factor for microbial type and quantity in the aerated soil zone, especially organic matter that contributes to a high concentration of carbon, which will have a great influence on the bioavailability of PAHs. Tian et al. (2017) found that a certain amount of amino acids in the soil can promote the growth of microorganisms, thus improving the removal efficiency of pyrene in the soil.

Changes in the enzyme environment of the bacterial envelope will also affect the biodegradation process of PAHs as they can to developed plant roots in the aerated zone of soil, which provide enough growth surface area for microorganisms but also create a good degradation environment. A large number of root secretions and cells from shedding root crowns promote biochemical reactions in the soil, and plant root secretion of some special chemicals, such as low molecular weight organic acids, can regulate the pH of the soil (Polak et al., 2019). For different species of plants, the root surface area, root secretions, enzymes and other types and quantities of biomaterial will be significantly different, resulting in rhizosphere soil for which PAHs degradation differences are very large (Xiao et al., 2015). Soil enzymes are an important in soil metabolism, and the enzymes mainly involved in PAHs degradation in soil are oxygenase, dehydrogenase, phosphatase and lignin enzymes. Lu and Lu (2015) found that the common interaction between plants, fungi and indigenous microorganisms stimulated microbial growth, increased the activity of polyphenol oxidase in soil, and increased the removal rate of PAHs.

The activity of catalase, as an index of soil fertility and ecological toxicity, plays an important role in converting aromatic compounds into quinone compounds (Xu et al., 2014). Wang et al. (2014) detected 6 low molecular weight organic acids, noting the activity of succinic acid and lactic acid in rhizosphere sediments, in an experiment in which mangrove forests were planted to remediate PAHs-contaminated soil, and the removal rate of mixed PAHs in sediments was improved. Changes in the microbial community structure of the aerated soil zone also have an impact on the bioavailability of PAHs. Ling et al. (2015) studied the response of endogenous bacteria and rhizosphere bacteria to PAHs pollution in salt marsh plants and found that endogenous bacterial communities played an important role in the remediation of contaminated soil by plants. Tejeda-Agredano et al. (2013) found that with the increase in the number of degradable bacteria, the soil

bacterial community structure undergoes great changes and PAHs degradation capacity enhances the direction of development.

4 Functional regulation of membrane proteins in the transmembrane transport process

Membrane protein is an important part of the cell membrane, and it is also the main component of cell membrane function (Wang et al. 2016). According to the different binding mechanisms used by membrane proteins and lipid molecules, membrane proteins can be divided into two kinds of membrane integration: peripheral and lipid anchor proteins (Miyata et al., 2004). Because of the difficulty of separating proteins and the their localized distribution in cell membranes, membrane proteins can be further categorized as external membrane and intrinsic membrane proteins (Bouchez-Naïtali et al., 1999).

External membrane proteins are mainly distributed on the inner surface of the cell membrane, with noncovalent bonds either formed at the distal end of the intrinsic protein or in combination with the hydrophilic end of the phospholipid molecule; for instance, water-soluble proteins, which constitute 20%~30% of total membrane protein content, bind through hydrogen bonds or ionic bonds and are associated with membrane lipid molecules, but these proteins can also bind indirectly to cell membranes through the interaction with intrinsic proteins (Westgate et al., 1995). Intrinsic proteins, also known as integrated proteins, account for 70%~80% of the total amount of membrane protein, are embedded in the phospholipid double molecular layer to varying degrees and are both parent and molecule bearing. The binding of the inner membrane protein to the cell membrane is very tight; the inner membrane protein exposed outside the cell membrane is hydrophilic, contains more polar amino acids, and is close to the phospholipid molecules, while the membrane protein embedded in the phospholipid double layer is composed of nonpolar amino acids that interact with the hydrophobic ends of the lipid molecules (Volkering et al., 1997).

Transmembrane proteins are intrinsic and span the double-layer lipid-protein membrane, with one end internally positioned in cytoplasm and the other end exposed to the external surface of the cell membrane; in theory, transmembrane proteins can float horizontally and can exchange cellular and environmental matter, energy and information (Rosenberg et al., 1980). The two main types of transporter proteins present on cell membranes are carrier and channel proteins (Goswami et al., 1983). Carrier proteins transfer solutes to the opposite side of the cell membrane by changing their own structure, for which some, such as ATP-driven carriers, require energy, while others do not need energy because they directly assist in diffusion (Thomas et al., 1986). Channel proteins form a hydrophilic channel through which specific solutes pass, and all channel proteins transport the solute in a way that assists diffusion (Stucki and Alexander, 1987).

There are two basic types of transmembrane proteins: (i). The alpha-helix protein is found in the endometrium of bacterial cells, in the membranes in eukaryotic cells, and in the outer membranes of some eukaryotic cells (Almén et al., 2009), and (ii) the beta-cylindrical protein is found only in the outer membranes of gram-negative bacteria, the cell walls of gram-positive bacteria, the outer membrane of mitochondria and the membrane around chromatin. Studies such as that of Lepore et al. (2011) show that most microorganisms take up nutrients with the help of a membrane pore protein, which has an outer membrane protein of multiple beta-cylindrical orifice structures, and the beta-cylindrical structure has universally conserved extracellular membrane proteins. At present, two main transmembrane transporter proteins in microorganisms are *Escherichia coli* (*E. coli*) FadL and OmpW proteins, which feature two distinct actions.

4.1 FadL family protein

The *E. coli* FadL protein is a kind of extracellular membrane protein that can play a role in the ingestion of hydrophobic components and cross-membrane transport. The FadL transmembrane protein is an integral part of cell ingestion of long-chain fatty acids and has a cell cavity region associated with the Tonb transporter protein, which can close its beta-cylindrical structure (van den Berg et al., 2004). Call et al. (2016) found that the absence of FadL family membrane protein can lead to the inability of *E. coli* to ingest octane, and after the introduction of a plasmid that encodes FADL into *E. coli*, the ability to ingest octane was restored; Zhou et al. (2015), when studying the tolerance of *E. coli* BL21 to benzene and its ability to transport membrane proteins associated with benzene, found 16 types of membrane protein, including the outer membrane protein FadL.

Current studies have found four species of transmembrane proteins associated with BTEX transport, such as the TodX membrane protein in *Pseudomonas putida* F1 (Wang et al., 1995), Tbux membrane protein in *Ralstonia pickettii* PKO1 (Kahng et al., 2000), Xyln protein in *P. putida* (Kasai et al., 2001) and Tmox membrane protein in *Pseudomonas mendocina* (Ramos-González et al., 2002), as shown in Fig. 5. These four kinds of outer membrane protein have approximately 60% structural similarity and approximately 20% gene similarity. An important feature of the FADL crystal structure is the torsion of the inward side of the third beta chain (S3), which forms the beta-cylindrical orifice structure that may be related to its function (Hearn et al., 2008).

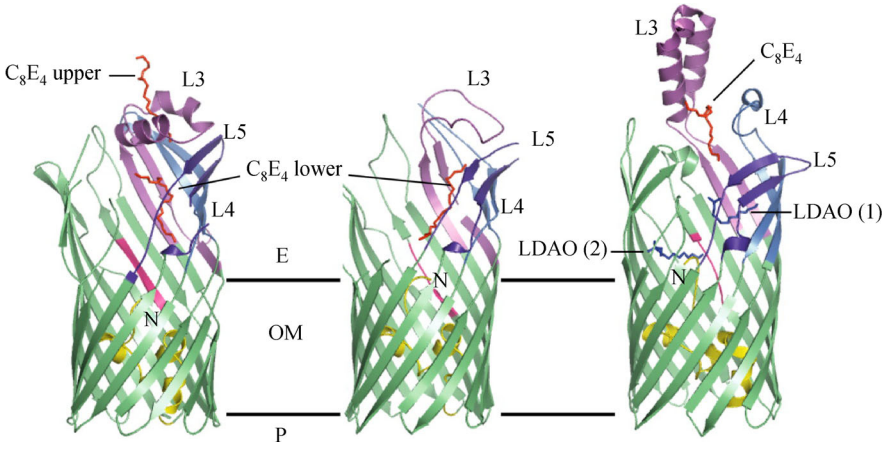


Fig. 5 Structural features of TodX, TbuX, and FadL [TodX (left), TbuX (center), FadL (right)] (Ramos-González et al., 2002).

4.2 OmpW family protein

The *E. coli* OmpW protein is a common transmembrane protein in gram-negative bacteria. A study shows that the OmpW protein is composed of 200−230 amino acids that forms 8 beta chain strands of the beta-cylindrical orifice (2.7 å resolution), forming a channel that has a certain hydrophobicity, as indicated by a multitude of *n*-dodecyl-N,N-dimethylamine-N-oxide hydrophobic molecules in the hydrophobic channel (Hong et al., 2006), as shown in Fig. 6.

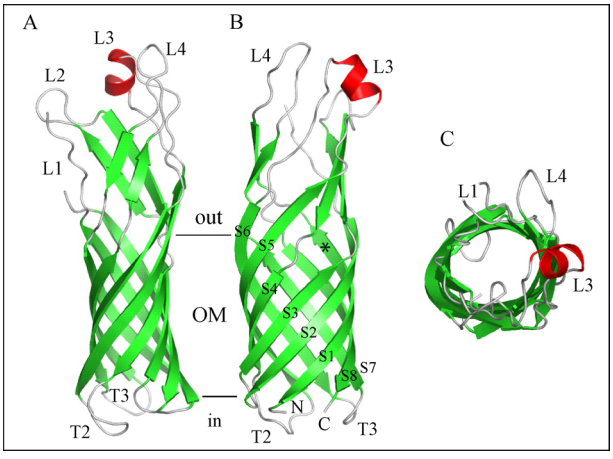


Fig. 6 Structural features of OmpW (Hong et al., 2006).

Studies have shown that OmpW protein makes bacteria resistant to complement systems mediated by bypass pathways, as explained by Li et al. (2016): The study found binding of OmpW to a complement regulating protein, H factor, which is a major bypass pathway inhibitor; the results showed that the combination of OmpW and H factor is an important means for bacteria to avoid the attack of the complement system. In addition, OmpW family proteins can assume the role of carriers in the transmembrane transport of hydrophobic molecules, such as medium-chain alkanes and naphthalene (Vanbeilen et al., 1992; Eaton, 1994), and participate in the regulation of osmotic pressure in a high-salinity environment (10%, w/v) such that microorganisms maintain the ability to exert a good biodegradation effect (Wu et al., 2006).

In addition, by adding the surfactant Tween 80, the expression of some ion transporter proteins in cell membranes (such as those for H^+) increases (Liu et al., 2017). A plasmid has been used to show that H^+-ATPase is a universal electrical H^+ pump that uses energy from ATP to pump H^+ into the extracellular membrane. Its key function is to produce an H^+ electrochemical gradient at the plasma membrane, which provides the driving force for the active transport of ions and metabolites. The transmembrane transport of PAHs cannot be separated from the driving force of the H^+ pump. Similarly, the H^+-ATPase in cytoplasmic membrane of soybean has shown consistency in its ability to take in phenanthrene (Yin et al., 2015).

5 Proteomics and single cell analysis technology

5.1 Proteomics technology

In recent years, proteomics technology and the recently emerging single cell analysis technology have been used to analyze the localization, catalytic properties and structural changes of proteins in cells from the perspective of molecular biology and are used for studying the morphology of cells at the single cell and subcellular levels (Martorana et al., 2016, Taha et al., 2015). Knowledge of the structural and functional characteristics help to better understand the function of microbial cell membrane proteins from a microscopic perspective and advances

comprehensive understanding about the changes in the ability of microbial cell membrane proteins to regulate the functions of transmembrane transport and the metabolism of PAHs (Wang et al., 2019). Proteomics technology is based on the proteome and is used to study the composition and regulation of proteins based on the overall activity level, and it includes two-dimensional electrophoresis (2-DE), biological mass spectrometry, bioinformatics and differential proteomics technologies (Murthy et al., 2015) (Table 3).

Isobaric tags for relative and absolute quantitation (iTRAQ) is a differential protein quantification technique that uses a variety of isotopic reagents to label N-terminal or lysine side chains of protein peptides. This peptide group was compared with the results from high-throughput mass spectrometry for simultaneous analysis of protein expression among eight samples. The high-throughput screening technique has commonly been used in quantitative proteomics in recent years (Ross et al., 2004). Clusters of orthologous groups of proteins (COGs), gene ontology (GO) (Table 4) and Kyoto Encyclopedia of Genes and Genomes (KEGG) have been utilized to represent major phylogenetic lineages and unify the representations of gene and gene product attributes across all species.

Wang et al. (2019) studied the regulation mechanism of proteins in bacteria *Rhodococcus* sp. BAP-1 that are the highly efficient in degrading fluoranthene and used iTRAQ technology combined with LC-MS/MS, used to cluster proteins based on differential spectral patterns, and bioinformatics analysis. Through COG (Fig. 7), GO and KEGG (Fig. 8) pathway enrichment analysis, it was found that fluoranthene significantly changed protein expression during metabolism and biosynthesis and most of the differentially expressed proteins involved in metabolism and energy production. In addition, *Rhodococcus* sp. BAP-1 upregulated aldehyde dehydrogenase, cytochrome c oxidase and oligopeptide transport ATP binding proteins, and downregulated several proteins that regulate fluorescein exposure.

Zhao et al. (2015) identified 79 differentially expressed proteins, including some surface proteins and some proteins involved in quorum sensing, using iTRAQ technology during their study of the minimally inhibitory concentration of *S. suis* to induce erythromycin inhibition of biofilm formation.

5.2 Single cell analysis technology

In the field of cell biology, various novel imaging analysis techniques are used to study the morphological structure and functional properties of cells at the single cell,

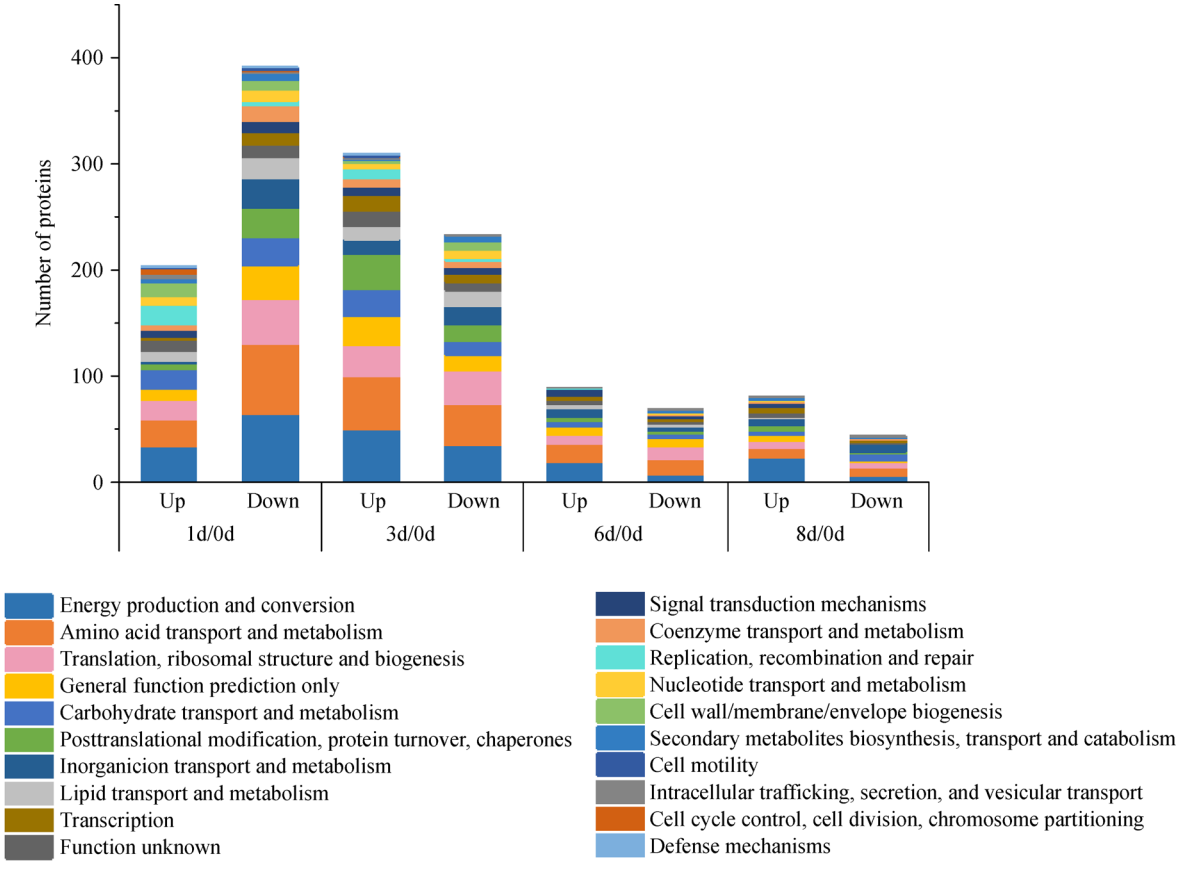


Fig. 7 Functional analysis of differentially expressed proteins in every cluster (Wang et al., 2019).

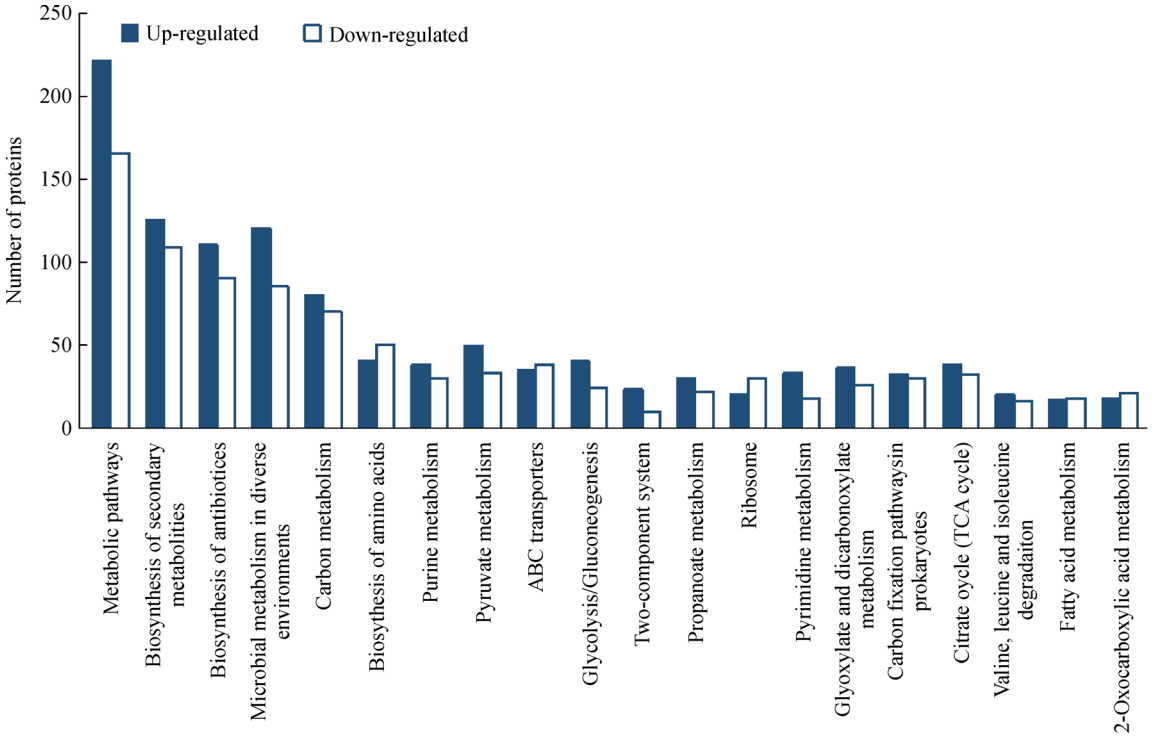


Fig. 8 The number of upregulated and downregulated proteins in different KEGG-identified pathways (Wang et al., 2019).

subcellular, single molecule or even the single atom level. Single cell imaging analysis techniques include infrared and Raman microscopy, magnetic resonance imaging, scanning probe microscopy, fluorescence microscopy, and nanosecond ion mass spectrometry (Azevedo et al., 2015; Kanbayashi and Miyafuji, 2016; Kumar et al., 2016; Peng et al., 2016) (Table 5).

Fluorescence microscopy imaging is by far the most mature single cell imaging technique. It is mainly used to study biological components in cells that have fluorescence activity or can be bound by fluorescent probes. Fluorescence in situ hybridization (FISH) is used to label microorganisms, and then, laser scanning is used. Confocal microscopy can be used to observe the morphological structure of microorganisms (Azevedo et al., 2015). Aspridou et al. (2019) stained cells to view with a confocal scanning laser microscope for direct assessment of *Salmonella Agona* individual cell inactivation in small two-dimensional colonies exposed to osmotic stress. The effect of colony size on microbial inactivation was confirmed by experiments, and the population consisting of single cells or small colonies had a higher rate of inactivation than cells in larger colonies.

Nano Secondary ion mass spectrometry (Nano-SIMS) has high sensitivity and ion transport efficiency, high mass resolution and spatial resolution, representing the highest level of ion probe imaging technology today (Peng et al., 2016). Through Nano-SIMS imaging analysis, the physiologic and ecological characteristics of microorganisms be observed at the single cell level as can the metabolically active microbial cells, and system classification information can be accurately identified from the sample (Gao et al., 2015). In 2001, Orphan first used secondary ion mass spectrometry in combination with fluorescence in situ hybridization to link the phylogenetic properties of microbial cells with metabolic activity (Orphan et al., 2001). Su et al. (2016) used stable isotope labeling combined with Nano-SIMS technology and transmission electron microscopy to study bacterial morphology and individual bacteria from samples of pure microorganisms. The utilization of ^{13}C-glucose by the degrading bacteria BDG-3 was analyzed (Fig. 9).

6 Summary and outlook

At present, most of the research focuses on the effects of physical or chemical factors on the microbial degradation of PAHs in the aerated zone of soil, the core metabolic links in the process of microbial transmembrane transport and degradation of PAHs in the aerated zone, and the key rapid control steps carried out by microorganisms. The functional regulation of cell membrane proteins during transmembrane transport is unclear. To scientifically guide polycyclic aromatic hydrocarbon-microbial system remediation and treatment efforts and effectively improve the degradation efficiency of PAHs in the aerated zone, it is necessary to fully study the microscopic mechanisms and

Table 3 Proteomics technology

Specific technology	Microbial species	Substances that interact with cells	Features and conclusions	References
iTRAQ	*Streptococcus suis*	Erythromycin	Seventy-nine differentially expressed proteins were identified in sub-MIC erythromycin inhibiting planktonic cell. Several cell surface proteins, as well as those involved in quorum-sensing, were found to be implicated in biofilm formation.	Zhao et al. (2015)
	Rice suspension cells	Pathogenic bacteria of bacterial blight (*Xanthomonas oryzae* pv. oryzae).	Proteomics analysis of rice PM can be applied to identify potential PM components involved in the rice defense response to microbes.	Chen et al. (2007)
	Resistance to fusarium	Pathogenic bacteria of head blight (FHB)	The first report of the application of proteomic techniques in studying the interaction between a series of barley genotypes representing various levels of resistance to FHB and the *F. graminearum* pathogen. The induced plant defense responses following fungal infection were diverse among resistant, intermediate and susceptible barley genotypes.	Geddes et al. (2008)
	Madam Vinous' sweet orange plants	Pathogenic bacteria of citrus Huanglongbing (HLB)	iTRAQ technology has been used to in identified 20 proteins and 10 differentially expressed proteins. These proteins may be good candidates for biomarkers to identify HLB-diseased plants prior to the expression of typical symptoms. However, similar changes in protein expression may be caused by other abiotic stresses, such as wounding and pathogen infections other than those induced by CLas.	Fan et al. (2011)
	Abomasal mucosa	Nematode, Hemonchus contorts	The study identified and quantified more than 4400 unique proteins, of which 158 proteins showed>1.5-fold difference between resistant and susceptible sheep. Trefoil factor 2, a member of RAS oncogene family (RAP1A) and ring finger protein 126 were among the proteins found to be highly abundant in the abomasal surface of resistant sheep, whereas adenosine deaminase and the gastrokine-3-like precursor were found at higher levels in susceptible sheep.	Nagaraj et al. (2012)
	E. chaffeensis	Tick-borne rickettsial pathogen, *Ehrlichia chaffeensis*	The study documents the impact of mutations on the global expression of pathogen protein and the influence of protein abundance on attenuation of mutations and protection of vertebrate host against infection.	Kondethimmanahalli et al. (2019)
	Rubrivivax benzoatilyticus JA2	Glucose	The present study deciphered the molecular/metabolic events associated with glucose-grown cells of strain JA2 and unraveled how a carbon source modulates metabolic phenotypes. Proteomic profiling revealed extensive metabolic remodeling in the glucose-grown cells wherein signal transduction, selective transcription, DNA repair, protein transport and quality control processes were upregulated to cope with the changing milieu. Proteins involved in DNA replication, translation, electron transport, photosynthetic machinery were downregulated, possibly to conserve energy.	Gupta et al. (2019)
	Clostridium *difficile*	Metronidazole	This study provided an in-depth proteomic analysis of a stable, metronidazole-resistant *C. difficile* isolate. The results suggested that a multifactorial response may be associated with high level metronidazole resistance in *C. difficile*, including the possible roles in altered iron metabolism and/or DNA repair.	Chong et al. (2014)
1-DE/LC-MS/MS	*Novosphingobium pentaromativorans* US6-1	PAHs	1-DE/LC-MS/MS analysis of *N. pentaromativorans* US6-1 cultured in the presence of different PAHs and monocyclic aromatic hydrocarbons (MAHs) identified approximately 1,000 and 1,400 proteins, respectively.	Yun et al. (2014)
2-DE	*Cyanobacterium Anabaena* PD-1	Polychlorobiphenyl	Twenty-five upregulated proteins were identified using 2-DE coupled with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). These proteins were involved in (i) PCB degradation, (ii) transport processes, (iii) energetic metabolism, (iv) electron transport, (v) general stress response, (vi) carbon metabolism, and (vii) nitrogen reductase.	Zhang et al. (2014)

Table 4 Functional classification of the combination of differentially expressed proteins based on GO

GO	Protein sources	Interference factors	Protein functions	References
Cellular components	*Synechocystis* sp. PCC 6803	Butanol	Cytoplasm, cytoplasmic part, protein transport, establishment of protein localization and protein localization.	Tian et al., (2013)
	Fillets treated by microwave thawing (MT)	Magnetic nanoparticles plus microwave exposure	Membrane, membrane par, cell, cell part, organelle, organelle part.	Cao et al. (2019)
	Vibrio parahemolyticus ATCC 17802	Food preservative and low temperature	Cell outer membrane.	Zhong et al. (2018)
	Cotton Roots and Leaves	Salt stress	Cell, cell part, intracellular part, intracellular organelle, organelle-related components.	Chen et al. (2016)
	Aspergillus flavus	Water activity	Cell, cell part, extracellular region, extracellular region part.	Zhang et al. (2015)
	Rhodococcus sp. BAP-1	Fluoranthene	Cell, macromolecular complex, membrane.	Wang et al. (2019)
Molecular functions	*Synechocystis* sp. PCC 6803	Butanol	Ribonucleoprotein complex, macromolecular complex, structural molecule activity, RNA binding, nucleic acid binding, gene expression and macromolecule metabolic processes.	Tian et al. 2013)
	Fillets treated by microwave thawing (MT)	Magnetic nanoparticles plus microwave	Binding, catalytic activity, transporter activity.	Cao et al. 2019)
	Vibrio parahemolyticus ATCC 17802	Food preservative and low temperature	Siderophore transmembrane transporter activity, receptor activity, iron ion binding, receptor activity.	Zhong et al. (2018)
	Rice (*Oryza sativa*) Embryogenesis	–	Metabolic enzymes, binding (protein binding and nucleotide binding), transporters.	Zi et al. (2013)
	Aspergillus flavus	Water activity	Catalytic activity, binding, molecular transducer activity, nucleic acid binding transcription factor activity, nutrient reservoir activity, receptor activity, structural molecule activity.	Zhang et al. (2015)
	Rhodococcus sp. BAP-1	Fluoranthene	Catalytic activity, binding followed by transporter activity.	Wang et al. (2019)
Biological processes	*Synechocystis* sp. PCC 6803	Butanol	Inorganic cation transmembrane transporter activity, hydrogen ion transmembrane transporter activity, monovalent inorganic cation transmembrane transporter activity, intracellular protein transport.	Tian et al. (2013)
	Fillets treated by microwave thawing (MT)	Magnetic nanoparticles plus microwave	Biological regulation, cellular processes, regulation biological processes, response to stimuli and signaling, metabolic processes, developmental processes.	Cao et al. (2019)
	Vibrio parahemolyticus ATCC 17802	Food preservative and low temperature	Choline transport, iron ion transport and homeostasis.	Zhong et al. (2018)
	Rice (*Oryza sativa*) Embryogenesis	–	Proteins that participate in metabolism and respond to stimuli.	Zi et al. (2013)
	The resurrection plant *Xerophyta viscosa*	Dehydration stress	Cellular processes, binding.	Abdalla and Rafudeen (2012)
	Cotton Roots and Leaves	Salt stress	Metabolic processes, organic substance metabolic processes, primary metabolic processes, cellular metabolic processes.	Chen et al. (2016)
	Aspergillus flavus	Water activity	Metabolic processes, cellular processes, single-organism processes.	Zhang et al. (2015)
	Rhodococcus sp. BAP-1	Fluoranthene	Metabolic processes, cellular processes, single-organism processes.	Wang et al. (2019)

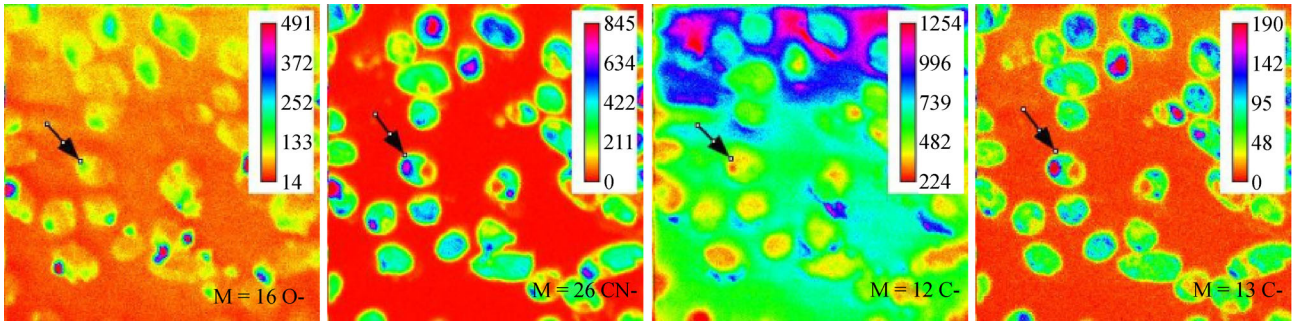


Fig. 9 High resolution Nano-SIMS images of cultured BDG-3 (Su et al., 2016).

Table 5 Single cell analysis technology—FISH and Nano-SIMS

Specific technology	Microbial species	Features and conclusions	References
FISH	–	FISH not only enables the detection of culturable microorganisms but also yet-to-be cultured (so-called unculturable) organisms and can therefore help in understanding complex microbial communities. In this review, methodological aspects, as well as problems and pitfalls of FISH, are discussed in an examination of past, present and future applications.	Moter and Göbel (2000)
	Marine bacteria	FISH with horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide signal amplification, also known as catalyzed reporter deposition (CARD), is currently not generally applicable to heterotrophic bacteria in marine samples. The enhanced fluorescence intensities and signal-to-background ratios make CARD-FISH superior to FISH with directly labeled oligonucleotides for the staining of bacteria with low rRNA content in the marine environment."	(Pernthaler et al. (2002)
	Bacterial populations in human feces	FISH with group-specific 16S rRNA-targeted oligonucleotide probes. The combination of the two Bacteroides-specific probes detected a mean of 5.4×10^{10} cells·g^{-1} (dry weight) in feces; the *Clostridium coccoides-Eubacterium rectale* group-specific probe detected a mean of 7.2×10^{10} cells·g^{-1} (dry weight) in feces. The *Clostridium histolyicum*, *Clostridium lituseburense*, and *Streptococcus-Lactococcus* group-specific probes detected only numbers ranging from 1×10^{7} to 7×10^{8} cells·g^{-1} (dry weight) in feces.	Franks et al. (1998)
Nano-SIMS	*E. coli*	*E. coli* exposed to food-grade TiO_2 showed some internalization of TiO_2 (7% of cells), as observed with high-resolution nano secondary ion mass spectrometry (nano-SIMS) chemical imaging.	Radziwill-Bienkowska et al. (2018)
	Spleen cells	Nano-SIMS makes it possible to both capture images of and quantify molecules labeled with stable or radioactive isotopes within subcellular compartments.	Lechene et al. (2006)
	Individual bacteria within eukaryotic host cells	In this study, with multi-isotope imaging mass spectrometry we directly captured images and measured nitrogen fixation by individual bacteria within eukaryotic host cells and demonstrated that fixed nitrogen is used for host metabolism.	Lechene et al. (2007)
	E. coli	*E. coli* in water were treated with OD radicals, and D atom incorporation into cells was visualized using time-of-flight SIMS and nano-SIMS. The results show that D atoms from NTPJ reach the cytoplasm of *E. coli* in H_2O, indicating the usefulness of this OD-tracking method for the study of radical interactions within living cells.	Lee et al. (2014)
	Paracoccus sp. strain HPD-2	Nano-SIMS results provided a direct evidence for the contribution of hematite to the formation of iron ions inside of HPD-2 cell, which resulted in the prominent generation of ROS (including EPFRs) that has been demonstrated to be able to affect cells.	Gan et al. (2018)

regulatory functions of microorganisms in the process of degrading PAHs. Therefore, it is necessary to study the dynamic processes of the microbial transmembrane transport of PAHs and to analyze the changes in the functional regulation ability of microbial cell membrane proteins to transport and degrade PAHs, such that the bioavailability of PAHs can be improved by regulating the microbial living environment. This article re-identifies the core links and key points in controlling the speed of microbial transport and metabolic processes of PAHs from the level of cells and membrane proteins and provides an important theoretical basis and practical guidance for the establishment of an efficient microbial-PAHs remediation system.

Acknowledgements This work is sponsored by the National Natural Science Foundation of China (Grant No. 41772234) and the Natural Science Foundation of Guangxi (Grant No. 2017GXNSFBA198168).

References

Abdalla K O, Rafudeen M S (2012). Analysis of the nuclear proteome of the resurrection plant *Xerophyta viscosa* in response to dehydration stress using iTRAQ with 2DLC and tandem mass spectrometry. Journal of Proteomics, 75(8): 2361–2374

Abouseoud M, Yataghene A, Amrane A, Maachi R (2010). Effect of pH and salinity on the emulsifying capacity and naphthalene solubility of a biosurfactant produced by *Pseudomonas fluorescens.* Journal of Hazardous Materials, 180(1-3): 131–136

Almén M S, Nordström K J V, Fredriksson R, Schiöth H B (2009). Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biology, 7(1): 1–14

Aspridou Z, Balomenos A, Tsakanikas P, Manolakos E, Koutsoumanis K (2019). Heterogeneity of single cell inactivation: Assessment of the individual cell time to death and implications in population behavior. Food Microbiology, 80: 85–92

Azevedo A S, Almeida C, Pereira B, Madureira P, Wengel J, Azevedo N F (2015). Detection and discrimination of biofilm populations using locked nucleic acid/2'-O-methyl-RNA fluorescence in situ hybridization (LNA/2'OMe-FISH). Biochemical Engineering Journal, 104: 64–73

Bansal V, Kim K H (2015). Review of PAH contamination in food products and their health hazards. Environment International, 84: 26–38

Bateman J N, Speer B, Feduik L, Hartline R A (1986). Naphthalene association and uptake in *Pseudomonas putida.* Journal of Bacteriology, 166(1): 155–161

Bautista L F, Sanz R, Molina M C, Gonzalez N, Sanchez D (2009). Effect of different non-ionic surfactants on the biodegradation of PAHs by diverse aerobic bacteria. International Biodeterioration & Biodegradation, 63(7): 913–922

Borbás E, Sinkó B, Tsinman O, Tsinman K, Kiserdei É, Démuth B, Balogh A, Bodák B, Domokos A, Dargó G, Balogh G T, Nagy Z K (2016). Investigation and Mathematical Description of the Real Driving Force of Passive Transport of Drug Molecules from Supersaturated Solutions. Molecular Pharmaceutics, 13(11): 3816–3826

Bouchez M, Blanchet D, Vandecasteele J P (1997). An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain. Microbiology, 143(4): 1087–1093

Bouchez Naïtali M, Rakatozafy H, Marchal R, Leveau J Y, Vandecasteele J P (1999). Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake. Journal of Applied Microbiology, 86(3): 421–428

Bouchez-Naïtali M, Vandecasteele J P (2008). Biosurfactants, an help in the biodegradation of hexadecane? The case of *Rhodococcus* and *Pseudomonas* strains. World Journal of Microbiology & Biotechnology, 24(9): 1901–1907

Bramwell D A P, Laha S (2000). Effects of surfactant addition on the biomineralization and microbial toxicity of phenanthrene. Biodegradation, 11(4): 263–277

Bugg T, Foght J M, Pickard M A, Gray M R (2000). Uptake and active efflux of polycyclic aromatic hydrocarbons by *Pseudomonas fluorescens* LP6a. Applied and Environmental Microbiology, 66 (12): 5387–5392

Call T P, Akhtar M K, Baganz F, Grant C (2016). Modulating the import of medium-chain alkanes in *E. coli* through tuned expression of FadL. Journal of Biological Engineering, 10(1): 5

Cao M, Cao A, Li Y, Wang W, Wang Y, Cai L (2019). Effects of magnetic nanoparticles plus microwave on the thawing of large-mouth bass (*Micropterus salmoides*) fillets based on iTRAQ quantitative proteomics. Food Chemistry, 286: 506–514

Chen B, Ding J (2012). Biosorption and biodegradation of phenanthrene and pyrene in sterilized and unsterilized soil slurry systems stimulated by *Phanerochaete chrysosporium.* Journal of Hazardous Materials, 229-230: 159–169

Chen B, Wang Y, Hu D (2010). Biosorption and biodegradation of polycyclic aromatic hydrocarbons in aqueous solutions by a consortium of white-rot fungi. Journal of Hazardous Materials, 179 (1-3): 845–851

Chen F, Yuan Y, Li Q, He Z (2007). Proteomic analysis of rice plasma membrane reveals proteins involved in early defense response to bacterial blight. Proteomics, 7(9): 1529–1539

Chen T, Zhang L, Shang H, Liu S, Peng J, Gong W, Shi Y, Zhang S, Li J, Gong J, Ge Q, Liu A, Ma H, Zhao X, Yuan Y (2016). iTRAQ-based quantitative proteomic analysis of cotton roots and leaves reveals pathways associated with salt stress. PLoS One, 11(2): 1–15

Chong P M, Lynch T, McCorrister S, Kibsey P, Miller M, Gravel D, Westmacott G R, Mulvey M R, Canadian Nosocomial Infection Surveillance Program (CNISP) (2014). Proteomic analysis of a NAP1 *Clostridium difficile* clinical isolate resistant to metronidazole. PLoS One, 9(1): 1–10

Chunyun J, Peijun L, Xiaojun L, Peidong T, Wan L, Zongqiang G (2011). Degradation of pyrene in soils by extracellular polymeric substances (EPS) extracted from liquid cultures. Process Biochemistry, 46(8): 1627–1631

Eaton R W (1994). Organization and evolution of naphthalene catabolic pathways: Sequence of the DNA encoding 2-hydroxychromene-2-carboxylate isomerase and trans-o-hydroxybenzylidenepyruvate hydratase-aldolase from the NAH7 plasmid. Journal of Bacteriology, 176(24): 7757–7762

Edwards D A, Liu Z, Luthy R G (1992). Interactions between nonionic surfactant monomers, hydrophobic organic-compounds and soil. Water Science and Technology, 26(1-2): 147–158

Edwards D A, Liu Z B, Luthy R G (1994). Experimental-data and modeling for surfactant micelles, hocs, and soil. Journal of Environmental Engineering, 120(1): 23–41

Fan J, Chen C, Yu Q, Brlansky R H, Li Z G, Gmitter F G Jr (2011). Comparative iTRAQ proteome and transcriptome analyses of sweet orange infected by "*Candidatus Liberibacter asiaticus*". Physiologia Plantarum, 143(3): 235–245

Foster K J, Miklavcic S J (2016). Modeling Root Zone Effects on Preferred Pathways for the Passive Transport of Ions and Water in Plant Roots. Frontiers in plant science, 7: 914

Franks A H, Harmsen H J M, Raangs G C, Jansen G J, Schut F, Welling

G W (1998). Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Applied and Environmental Microbiology, 64(9): 3336–3345

Fukumori F, Hirayama H, Takami H, Inoue A, Horikoshi K (1998). Isolation and transposon mutagenesis of a *Pseudomonas putida* KT2442 toluene-resistant variant: involvement of an efflux system in solvent resistance. Extremophiles, 2(4): 395–400

Furuno S, Päzolt K, Rabe C, Neu T R, Harms H, Wick L Y (2010). Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems. Environmental Microbiology, 12(6): 1391–1398

Gan S, Lau E V, Ng H K (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). Journal of Hazardous Materials, 172(2-3): 532–549

Gan X, Teng Y, Zhao L, Ren W, Chen W, Hao J, Christie P, Luo Y (2018). Influencing mechanisms of hematite on benzo(a)pyrene degradation by the PAH-degrading bacterium *Paracoccus* sp. Strain HPD-2: insight from benzo(a)pyrene bioaccessibility and bacteria activity. Journal of Hazardous Materials, 359: 348–355

Gao D, Huang X, Tao Y J C R I B (2015). A critical review of NanoSIMS in analysis of microbial metabolic activities at single-cell level. Critical Reviews in Biotechnology, 36(5): 1

Gao F, Nan F, Feng J, Lv J, Liu Q, Xie S J R B (2016). Identification and characterization of microRNAs in Eucheuma denticulatum by high-throughput sequencing and bioinformatics analysis. RNA biology, 13 (3): 343–352

García-Junco M, De Olmedo E, Ortega-Calvo J J (2001). Bioavailability of solid and non-aqueous phase liquid (NAPL)-dissolved phenanthrene to the biosurfactant-producing bacterium *Pseudomonas aeruginosa* 19SJ. Environmental Microbiology, 3(9): 561–569

Geddes J, Eudes F, Laroche A, Selinger L B (2008). Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, Hordeum vulgare. Proteomics, 8(3): 545–554

Goswami P, Singh H D (1991). Different modes of hydrocarbon uptake by two *Pseudomonas* species. Biotechnology and Bioengineering, 37 (1): 1–11

Goswami P C, Singh H D, Bhagat S D, Baruah J N (1983). Mode of uptake of insoluble solid substrates by microorganisms. I: Sterol uptake by an arthrobacter species. Biotechnology and Bioengineering, 25(12): 2929–2943

Gupta D, Mohammed M, Mekala L P, Chintalapati S, Chintalapati V R (2019). iTRAQ-based quantitative proteomics reveals insights into metabolic and molecular responses of glucose-grown cells of *Rubrivivax benzoatilyticus* JA2. Journal of Proteomics, 194: 49–59

Hearn E M, Patel D R, van den Berg B (2008). Outer-membrane transport of aromatic hydrocarbons as a first step in biodegradation. Proceedings of the National Academy of Sciences of the United States of America, 105(25): 8601–8606

Hong H, Patel D R, Tamm L K, van den Berg B (2006). The outer membrane protein OmpW forms an eight-stranded -barrel with a hydrophobic channel. The Journal of biological chemistry, 281(11): 7568–7577

Hori K, Matsuzaki Y, Tanji Y, Unno H (2002). Effect of dispersing oil phase on the biodegradability of a solid alkane dissolved in non-biodegradable oil. Applied Microbiology and Biotechnology, 59(4-5): 574–579

Hua F, Wang H (2012). Uptake modes of octadecane by *Pseudomonas* sp. DG17 and synthesis of biosurfactant. Journal of Applied Microbiology, 112(1): 25–37

Hua F, Wang H Q (2014). Uptake and trans-membrane transport of petroleum hydrocarbons by microorganisms. Biotechnology, Biotechnological Equipment, 28(2): 165–175

Inakollu S, Hung H C, Shreve G S (2004). Biosurfactant enhancement of microbial degradation of various structural classes of hydrocarbon in mixed waste systems. Environmental Engineering Science, 21(4): 463–469

Isken S, De Bont J A (2000). The solvent efflux system of *Pseudomonas putida* S12 is not involved in antibiotic resistance. Applied Microbiology and Biotechnology, 54(5): 711–714

Jia C Y, Li P J, Li X J, Tai P D, Liu W, Gong Z Q (2011). Degradation of pyrene in soils by extracellular polymeric substances (EPS) extracted from liquid cultures. Process Biochemistry, 46(8): 1627–1631

Jonsson A P, Östberg T L (2011). The effects of carbon sources and micronutrients in whey and fermented whey on the kinetics of phenanthrene biodegradation in diesel contaminated soil. Journal of Hazardous Materials, 192(3): 1171–1177

Kahng H Y, Byrne A M, Olsen R H, Kukor J J (2000). Characterization and role of tbuX in utilization of toluene by *Ralstonia pickettii* PKO1. Journal of Bacteriology, 182(5): 1232–1242

Kanbayashi T, Miyafuji H (2016). Microscopic characterization of tension wood cell walls of Japanese beech (*Fagus crenata*) treated with ionic liquids. Micron (Oxford, England: 1993), 88: 24–29

Kasai Y, Inoue J, Harayama S (2001). The TOL plasmid pWW0 xylN gene product from *Pseudomonas putida* is involved in m-xylene uptake. Journal of Bacteriology, 183(22): 6662–6666

Ke L, Luo L, Wang P, Luan T, Tam N F Y (2010). Effects of metals on biosorption and biodegradation of mixed polycyclic aromatic hydrocarbons by a freshwater green alga *Selenastrum capricornutum.* Bioresource Technology, 101(18): 6950–6972

Kondethimmanahalli C, Liu H, Ganta R R (2019). Proteome Analysis Revealed Changes in Protein Expression Patterns Caused by Mutations in *Ehrlichia chaffeensis.* Frontiers in Cellular and Infection Microbiology, 9: 58

Kumar S, Verma T, Mukherjee R, Ariese F, Somasundaram K, Umapathy S (2016). Raman and infra-red microspectroscopy: towards quantitative evaluation for clinical research by ratiometric analysis. Chemical Society Reviews, 45(7): 1879–1900

Lai C C, Huang Y C, Wei Y H, Chang J S (2009). Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. Journal of Hazardous Materials, 167(1/3): 609–614

Lechene C, Hillion F, McMahon G, Benson D, Kleinfeld A M, Kampf J P, Distel D, Luyten Y, Bonventre J, Hentschel D, Park K M, Ito S, Schwartz M, Benichou G, Slodzian G (2006). High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. Journal of Biology, 5(6): 20

Lechene C P, Luyten Y, McMahon G, Distel D L (2007). Quantitative imaging of nitrogen fixation by individual bacteria within animal cells. Science, 317(5844): 1563–1566

Lee C B, Na Y H, Hong T E, Choi E H, Uhm H S, Baik K Y, Kwon G (2014). Evidence of radicals created by plasma in bacteria in water.

Applied Physics Letters, 105(7): 073702

Lee M, Kim M K, Singleton I, Goodfellow M, Lee S T (2006). Enhanced biodegradation of diesel oil by a newly identified *Rhodococcus baikonurensis* EN3 in the presence of mycolic acid. Journal of Applied Microbiology, 100(2): 325–333

Lepore B W, Indic M, Pham H, Hearn E M, Patel D R, van den Berg B (2011). Ligand-gated diffusion across the bacterial outer membrane. Proceedings of the National Academy of Sciences of the United States of America, 108(25): 10121–10126

Li W, Wen L, Li C, Chen R, Ye Z, Zhao J, Pan J (2016). Contribution of the outer membrane protein OmpW in *Escherichia coli* to complement resistance from binding to factor H. Microbial Pathogenesis, 98: 57–62

Li Y, Wang H, Hua F, Su M, Zhao Y (2014). Trans-membrane transport of fluoranthene by *Rhodococcus* sp. BAP-1 and optimization of uptake process. Bioresource Technology, 155(155C): 213–219

Ling J, Jiang Y F, Wang Y S, Dong J D, Zhang Y Y, Zhang Y Z (2015). Responses of bacterial communities in seagrass sediments to polycyclic aromatic hydrocarbon-induced stress. Ecotoxicology (London, England), 24(7-8): 1517–1528

Liu S, Guo C, Dang Z, Liang X (2017). Comparative proteomics reveal the mechanism of Tween80 enhanced phenanthrene biodegradation by *Sphingomonas* sp. GY2B. Ecotoxicology and Environmental Safety, 137: 256–264

Lu Y F, Lu M (2015). Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms. Journal of Hazardous Materials, 285: 535–541

MacLeod C T, Daugulis A J (2005). Interfacial effects in a two-phase partitioning bioreactor: Degradation of polycyclic aromatic hydrocarbons (PAHs) by a hydrophobic *Mycobacterium.* Process Biochemistry, 40(5): 1799–1805

Martorana A M, Motta S, Sperandeo P, Mauri P, Polissi A (2016). Bacterial Cell Wall Homeostasis: Methods and Protocols. Hong H J, ed. New York: Springer, 57–70

Militon C, Jézéquel R, Gilbert F, Corsellis Y, Sylvi L, Cravo-Laureau C, Duran R, Cuny P (2015). Dynamics of bacterial assemblages and removal of polycyclic aromatic hydrocarbons in oil-contaminated coastal marine sediments subjected to contrasted oxygen regimes. Environmental Science and Pollution Research International, 22(20): 15260–15272

Miura Y, Okazaki M, Hamada S I, Murakawa S I, Yugen R (1977). Assimilation of liquid hydrocarbon by microorganisms. I. Mechanism of hydrocarbon uptake. Biotechnology and Bioengineering, 19 (5): 701–714

Miyata N, Iwahori K, Foght J M, Gray M R (2004). Saturable, energy-dependent uptake of phenanthrene in aqueous phase by *Mycobacterium* sp. strain RJGII-135. Applied and Environmental Microbiology, 70(1): 363–369

Moter A, Göbel U B (2000). Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. Journal of Microbiological Methods, 41(2): 85–112

Mulder H, Breure A M, Van Andel J G, Grotenhuis J T C, Rulkens W H (1998). Influence of hydrodynamic conditions on naphthalene dissolution and subsequent biodegradation. Biotechnology and Bioengineering, 57(2): 145–154

Murthy R V V S N, Arora J S, Kumar B V S J I J O a R (2015). Comparative proteome analysis of *Brucella abortus* under different growth conditions by two dimensional electrophoresis. International Journal of Advanced Research, 3(2): 795–800

Nagaraj S H, Harsha H C, Reverter A, Colgrave M L, Sharma R, Andronicos N, Hunt P, Menzies M, Lees M S, Sekhar N R, Pandey A, Ingham A (2012). Proteomic analysis of the abomasal mucosal response following infection by the nematode, *Haemonchus contortus*, in genetically resistant and susceptible sheep. Journal of Proteomics, 75(7): 2141–2152

Nakahara T, Erickson L E, Gutierrez J R (1977). Characteristics of hydrocarbon uptake in cultures with two liquid phases. Biotechnology and Bioengineering, 19(1): 9–25

Orphan V J, House C H, Hinrichs K U, McKeegan K D, DeLong E F (2001). Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science, 293(5529): 484–487

Pan X L, Liu J, Song W J, Zhang D Y (2012). Biosorption of Cu(II) to extracellular polymeric substances (EPS) from *Synechoeystis* sp.: A fluorescence quenching study. Frontiers of Environmental Science & Engineering, 6(4): 493–497

Peng X T, Guo Z X, House C H, Chen S, Ta K W (2016). SIMS and NanoSIMS analyses of well-preserved microfossils imply oxygen-producing photosynthesis in the *Mesoproterozoic* anoxic ocean. Chemical Geology, 441: 24–34

Pernthaler A, Pernthaler J, Amann R (2002). Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Environmental Microbiology, 68(6): 3094–3101

Polak F, Urik M, Matus P (2019). Low molecular weight organic acids in soil environment. Chemické Listy, 113: 307–314

Radziwill-Bienkowska J M, Talbot P, Kamphuis J B J, Robert V, Cartier C, Fourquaux I, Lentzen E, Audinot J N, Jamme F, Réfrégiers M, Bardowski J K, Langella P, Kowalczyk M, Houdeau E, Thomas M, Mercier-Bonin M (2018). Toxicity of Food-Grade TiO_2 to commensal intestinal and transient food-borne bacteria: New Insights using nano-SIMS and synchrotron UV fluorescence imaging. Frontiers in Microbiology, 9: 794

Ramos J L, Duque E, Godoy P, Segura A (1998). Efflux pumps involved in toluene tolerance in *Pseudomonas putida* DOT-T1E. Journal of Bacteriology, 180(13): 3323–3329

Ramos-González M I, Olson M, Gatenby A A, Mosqueda G, Manzanera M, Campos M J, Víchez S, Ramos J L (2002). Cross-regulation between a novel two-component signal transduction system for catabolism of toluene in *Pseudomonas mendocina* and the TodST system from *Pseudomonas putida.* Journal of Bacteriology, 184(24): 7062–7067

Rosenberg E (1993). Exploiting microbial growth on hydrocarbons:New markets. Trends in Biotechnology, 11(10): 419–424

Rosenberg M, Gutnick D, Rosenberg E (1980). Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. FEMS Microbiology Letters, 9(1): 29–33

Ross P L, Huang Y N, Marchese J N, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Pappin D J (2004). Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. Molecular & cellular proteomics: MCP, 3(12): 1154–1169

Schamfuss S, Neu T R, van der Meer J R, Tecon R, Harms H, Wick L Y (2013). Impact of mycelia on the accessibility of fluorene to PAH-degrading bacteria. Environmental Science & Technology, 47(13): 6908–6915

Sharma A, Kachroo D, Kumar R (2002). Time dependent influx and efflux of phenol by immobilized microbial consortium. Environmental Monitoring and Assessment, 76(2): 195–211

Stapleton J M, Mihelcic J R, Lueking D R (1994). Adsorption and desorption-kinetics of pyrene onto a great lakes sediment. Journal of Great Lakes Research, 20(3): 561–568

Stringfellow W T, Alvarez-Cohen L (1999). Evaluating the relationship between the sorption of PAHs to bacterial biomass and biodegradation. Water Research, 33(11): 2535–2544

Stucki G, Alexander M (1987). Role of dissolution rate and solubility in biodegradation of aromatic compounds. Applied and Environmental Microbiology, 53(2): 292–297

Su M, Li Y, Wang H, Diao S, Wang M (2016). Subcellular imaging and metabolic analysis of isotopically labeled carbon compounds in biological sample: An ion microprobe (Nano SIMS) study. Journal of Beijing Normal University (Nature and Science), 52(2): 223–227

Suszek-Łopatka B, Maliszewska-Kordybach B, Klimkowicz-Pawlas A, Smreczak B (2016). Influence of temperature on phenanthrene toxicity towards nitrifying bacteria in three soils with different properties. Environmental pollution, 216: 911–918

Taha M N, Krawinkel M B, Morlock G E (2015). High-performance thin-layer chromatography linked with (bio)assays and mass spectrometry: A suited method for discovery and quantification of bioactive components? Exemplarily shown for turmeric and milk thistle extracts. Journal of Chromatography. A, 1394: 137–147

Tejeda-Agredano M C, Gallego S, Vila J, Grifoll M, Ortega-Calvo J J, Cantos M (2013). Influence of the sunflower rhizosphere on the biodegradation of PAHs in soil. Soil Biology & Biochemistry, 57: 830–840

Thomas J M, Yordy J R, Amador J A, Alexander M (1986). Rates of dissolution and biodegradation of water-insoluble organic compounds. Applied and Environmental Microbiology, 52(2): 290–296

Tian W, Zhao J, Zhou Y, Qiao K, Jin X, Liu Q (2017). Effects of root exudates on gel-beads/reeds combination remediation of high molecular weight polycyclic aromatic hydrocarbons. Ecotoxicology and Environmental Safety, 135: 158–164

Tian X, Chen L, Wang J, Qiao J, Zhang W (2013). Quantitative proteomics reveals dynamic responses of *Synechocystis* sp. PCC 6803 to next-generation biofuel butanol. Journal of Proteomics, 78: 326–345

van Beilen J B, Eggink G, Enequist H, Bos R, Witholt B (1992). DNA sequence determination and functional characterization of the OCT-plasmid-encoded alkJKL genes of *Pseudomonas oleovorans*. Molecular Microbiology, 6(21): 3121–3136

van den Berg B, Black P N, Clemons W M Jr, Rapoport T A (2004). Crystal structure of the long-chain fatty acid transporter FadL. Science, 304(5676): 1506–1509

Vedovato N, Gadsby D C (2014). Route, mechanism, and implications of proton import during Na^+/K^+ exchange by native Na^+/K^+-ATPase pumps. The Journal of general physiology, 143(4): 449–464

Volkering F, Breure A M, Rulkens W H (1997). Microbiological aspects of surfactant use for biological soil remediation. Biodegradation, 8 (6): 401–417

Volkering F, Breure A M, van Andel J G, Rulkens W H (1995). Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. Applied and Environmental Microbiology, 61(5): 1699–1705

Wang H, Yang Y, Xu J, Kong D, Li Y (2019). iTRAQ-based comparative proteomic analysis of differentially expressed proteins in *Rhodococcus* sp. BAP-1 induced by fluoranthene. Ecotoxicology and Environmental Safety, 169: 282–291

Wang X D, Zhou M, Meng X R, Wang L, Huang D X (2016). Effect of protein on PVDF ultrafiltration membrane fouling behavior under different pH conditions: interface adhesion force and XDLVO theory analysis. Frontiers of Environmental Science & Engineering, 10(4): 12

Wang Y, Fang L, Lin L, Luan T, Tam N F Y (2014). Effects of low molecular-weight organic acids and dehydrogenase activity in rhizosphere sediments of mangrove plants on phytoremediation of polycyclic aromatic hydrocarbons. Chemosphere, 99: 152–159

Wang Y, Rawlings M, Gibson D T, Labbé D, Bergeron H, Brousseau R, Lau P C K (1995). Identification of a membrane protein and a truncated LysR-type regulator associated with the toluene degradation pathway in *Pseudomonas putida* F1. Molecular & general genetics: MGG, 246(5): 570–579

West C C, Harwell J H (1992). Surfactants and subsurface remediation. Environmental Science & Technology, 26(12): 2324–2330

Westgate S, Bell G, Halling P J (1995). Kinetics of uptake of organic liquid substrates by microbial cells: A method to distinguish interfacial contact and mass-transfer mechanisms. Biotechnology Letters, 17(10): 1013–1018

Wu L, Lin X, Wang F, Ye D, Xiao X, Wang S, Peng X (2006). OmpW and OmpV are required for NaCl regulation in *Photobacterium damsela.* Journal of Proteome Research, 5(9): 2250–2257

Xiao L, Qu X, Zhu D (2007). Biosorption of nonpolar hydrophobic organic compounds to *Escherichia coli* facilitated by metal and proton surface binding. Environmental Science & Technology, 41(8): 2750–2755

Xiao N, Liu R, Jin C X, Dai Y Y (2015). Efficiency of five ornamental plant species in the phytoremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil. Ecological Engineering, 75: 384–391

Xu Y, Sun G D, Jin J H, Liu Y, Luo M, Zhong Z P, Liu Z P (2014). Successful bioremediation of an aged and heavily contaminated soil using a microbial/plant combination strategy. Journal of Hazardous Materials, 264: 430–438

Yin X M, Liang X, Zhang R, Yu L, Xu G H, Zhou Q S, Zhan X H (2015). Impact of phenanthrene exposure on activities of nitrate reductase, phosphoenolpyruvate carboxylase, vacuolar H^+-pyrophosphatase and plasma membrane H^+-ATPase in roots of soybean, wheat and carrot. Environmental and Experimental Botany, 113: 59–66

Yun S H, Choi C W, Lee S Y, Lee Y G, Kwon J, Leem S H, Chung Y H, Kahng H Y, Kim S J, Kwon K K, Kim S I (2014). Proteomic characterization of plasmid pLA1 for biodegradation of polycyclic aromatic hydrocarbons in the marine bacterium, *Novosphingobium pentaromativorans* US6-1. PLoS One, 9(3): e90812

Zhang F, Zhong H, Han X, Guo Z, Yang W, Liu Y, Yang K, Zhuang Z, Wang S (2015). Proteomic profile of Aspergillus flavus in response to

water activity. Fungal Biology, 119(2-3): 114–124

Zhang H, Jiang X, Xiao W, Lu L (2014). Proteomic strategy for the analysis of the polychlorobiphenyl-degrading *cyanobacterium Anabaena* PD-1 exposed to Aroclor 1254. PLoS One, 9(3): 1–10

Zhang Y P, Wang F, Zhu X S, Zeng J, Zhao Q G, Jiang X (2015). Extracellular polymeric substances govern the development of biofilm and mass transfer of polycyclic aromatic hydrocarbons for improved biodegradation. Bioresource Technology, 193: 274–280

Zhao Y L, Zhou Y H, Chen J Q, Huang Q Y, Han Q, Liu B, Cheng G D, Li Y H (2015). Quantitative proteomic analysis of sub-MIC erythromycin inhibiting biofilm formation of *S. suis* in vitro. Journal of Proteomics, 116: 1–14

Zhong Q P, Tian J, Wang J, Fang X, Liao Z L (2018). iTRAQ-based proteomic analysis of the viable but nonculturable state of *Vibrio parahaemolyticus* ATCC 17802 induced by food preservative and low temperature. Food Control, 85: 369–375

Zhou J, Wang K, Xu S, Wu J, Liu P, Du G, Li J, Chen J (2015). Identification of membrane proteins associated with phenylpropanoid tolerance and transport in *Escherichia coli* BL21. Journal of Proteomics, 113: 15–28

Zi J, Zhang J, Wang Q, Zhou B, Zhong J, Zhang C, Qiu X, Wen B, Zhang S, Fu X, Lin L, Liu S (2013). Stress responsive proteins are actively regulated during rice (*Oryza sativa*) embryogenesis as indicated by quantitative proteomics analysis. PLoS One, 8(9): e74229