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Petrol and diesel exhaust particles accelerate the horizontal transfer of plasmid-mediated antimicrobial resistance genes

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ABSTRACT

Particles exhausted from petrol and diesel consumptions are major components of urban air pollution that can be exposed to human via direct inhalation or other routes due to atmospheric deposition into water and soil. Antimicrobial resistance is one of the most serious threats to modern health care. However, how the petrol and diesel exhaust particles affect the development and spread of antimicrobial resistance genes (ARGs) in various environments remain largely unknown. This study investigated the effects and potential mechanisms of four representative petrol and diesel exhaust particles, namely 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil, on the horizontal transfer of ARGs between two opportunistic *Escherichia coli* (*E. coli*) strains, *E. coli* S17-1 (donor) and *E. coli* K12 (recipient). The results demonstrated that these four representative types of nano-scale particles induced concentration-dependent increases in conjugative transfer rates compared with the controls. The underlying mechanisms involved in the accelerated transfer of ARGs were also identified, including the generation of intracellular reactive oxygen species (ROS) and the consequent induction of oxidative stress, SOS response, changes in cell morphology, and the altered mRNA expression of membrane protein genes and those involved in the promotion of conjugative transfer. The findings provide new evidences and mechanistic insights into the antimicrobial resistance risks posed by petrol and diesel exhaust particles, and highlight the implications and need for stringent strategies on alternative fuels to mitigate air pollution and health risks.

1. Introduction

Particles exhausted from petrol and diesel consumptions in vehicle engines and stationary sources are major components of urban air pollution in China and worldwide (Xu et al., 2013; Hesterberg et al., 2010; MacIntyre et al., 2014; Jin et al., 2017). Nearly all these particles have dimensions < 1 μm ($PM_{1.0}$), and the vast majority of these are known as ultrafine particles (UFPs) with dimensions < 100 nm ($PM_{0.1}$) (Hesterberg et al., 2010; Durga et al., 2014). Numerous epidemiological studies and historical data indicate the severe health consequences of exposure to petrol and diesel exhaust particles, which are associated with the prevalence of respiratory tract infections, decreased lung function, and exacerbation of cardiovascular and cerebrovascular diseases (MacIntyre et al., 2014; Aguilera et al., 2013). Particularly, multidrug resistant pathogenic bacteria (also known as “superbugs”) are increasingly isolated from patients with respiratory tract infections during the haze weather, which can escalate the risks of worse clinical outcomes and even death (MacIntyre et al., 2014; WHO, 2014). In addition to the inhalation by human and animals, most of the petrol and diesel exhaust particles eventually deposit in the water and soil environments (He et al., 2014), which contain a high abundance and diversity of ARGs and antimicrobial resistant bacteria (ARB) (WHO, 2014; Andersson and Hughes, 2014; Bengtsson-Palme and Larsson, 2015; Chen et al., 2016; Zhu et al., 2013). However, how the petrol and diesel exhaust particles affects the development and dissemination of ARGs in various environments remain unknown at present.

Antimicrobial resistance is one of the most serious threats to modern health care (WHO, 2014; Q. Chen et al., 2015) and has been estimated to cause > 700,000 deaths yearly (O'Neill, 2014). The development and spread of antimicrobial resistance have been accelerated by the recruitment of ARGs into bacteria via de novo mutation (Andersson and Hughes, 2014; Lv et al., 2014) and by the horizontal transfer of mobile genetic elements (MGEs) (Andersson and Hughes, 2014; Bengtsson-Palme and Larsson, 2015), such as plasmids, transposons and integrons. Specifically, the horizontal transfer of ARGs was acknowledged as an important pathway to acquire and spread ARGs in various

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environments (Bengtsson-Palme and Larsson, 2015; Alekshun and Levy, 2007). Previous evidences suggested that the enhanced intracellular ROS production and the SOS response induced by antibiotics, disinfectants and disinfection by-products (DBPs) can be important mechanisms for horizontal transfer of ARGs (Andersson and Hughes, 2014; Beaber et al., 2004; Zhang et al., 2017). Nanomaterials (Qiu et al., 2012), ionic liquids (Wang et al., 2015), and disinfectants (Zhang et al., 2017) were previously found to promote conjugative transfer by increasing cell membrane permeability and altering the expression of conjugation-related genes.

The physical and chemical properties of petrol and diesel exhaust particles, which are major contributors to the ultrafine particles in urban smog, have been extensively studied (Yadav et al., 2010; Zhang et al., 2015; Durga et al., 2014; Wu et al., 2017). Previous findings showed that petrol and diesel exhaust particles had nano-scale structures and consisted of a carbon core coated with organic chemicals and metals (Yadav et al., 2010; Zhang et al., 2015; Wu et al., 2017), which could induce the ROS-mediated cytotoxicity and genetic toxicity at varying degrees in both prokaryotic and eukaryotic cells (Wu et al., 2017; Verma et al., 2015; Gerlofs-Nijland et al., 2013; Durga et al., 2014). Therefore, we hypothesize that petrol and diesel exhaust particles can accelerate the horizontal transfer of ARGs among bacteria via generating the intracellular ROS, triggering oxidative stress and the SOS response, damaging cell membrane structures, and altering the expression of genes involved in the conjugative transfer of ARGs.

To test this hypothesis, this study systematically investigated the effects of particles exhausted from four different types of petrol and diesel oils on the conjugative ARGs transfer between *Escherichia coli* (*E. coli*) strains, which are important opportunistic pathogens and can cause intestinal and respiratory tract diseases (WHO, 2014; Chmielarczyk et al., 2014; Katouli, 2010). The underlying mechanisms responsible for the accelerated transfer of ARGs were also identified, including the changes of the intracellular ROS, cell morphology, cell membrane permeability, oxidative stress, SOS response, and the mRNA expression of conjugative transfer-related genes. The findings provide evidence and mechanistic insights into the antimicrobial resistance risks posed by petrol and diesel exhaust particles, and highlight the implications and the need for stringent strategies on utilizing alternative fuels to reduce air pollution.

2. Material and methods

2.1. Chemicals and reagents

Petrol and diesel fuels used in this study were bought from petrol and diesel station in Shanghai, China. All the chemicals used in present study were reagent-grade. Tween 80, tryptone, yeast extract, and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Sigma-Aldrich, Shanghai, China). Agar was purchased from Fisher Scientific. Phosphate buffer saline (PBS; pH 7.4) and 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) were obtained from Invitrogen (Invitrogen, Shanghai, China). Kanamycin (Km), chloromycetin (Chl), and thiourea (CH_4N_2S, TU) were supplied from TCI (TCI, Tokyo, Japan). RNAisoPlus kit (Cat. No. D9108A) and reverse transcription kit (Cat. No. 2680A) were purchased from TaKaRa (TaKaRa, Dalian, China).

2.2. Combustion experiments and particle sampling

Four types of particle samples were collected from the combustion of four different petrol and diesel fuels, including 97 octane petrol, 93 octane petrol, light diesel oil, and marine heavy diesel oil, in a laboratory-scale burning chamber, which was described in detail in our previous research (Wu et al., 2017). The raw samples were then size-reduced in a ball mill, sieved to 200 nm, and sterilized by X-ray irradiation. Then, these particles samples were suspended in deionized water containing 0.01% Tween 80 as the dispersant. The stock solutions of each sample were prepared at a concentration of 10,000 mg/L and diluted to proper concentrations for exposure. The ranges of particles concentrations were selected based on the $PM_{2.5}$ levels that occur in urban air environments in the field traffic emission systems (Xu et al., 2014). The particles samples were ultrasonicated for at least 30 min before each experiment to minimize aggregation. The analysis of morphology characterization, elemental compositions, and chemical components like polycyclic aromatic hydrocarbons (PAHs) and heavy metals were described in detail in our previous publication (Wu et al., 2017).

2.3. Bacterial strains, plasmid and culture conditions

The donor *E. coli* S17-1 harbors the transferable pCM184-Cm plasmid that is controlled by RP4 DNA segment residing in the host's chromosome (Zhang et al., 2017). Plasmid pCM184-Cm carries the ampicillin (Amp), tetracycline (Tet), and Chl resistance genes, origin of transfer (*oriT*) and replication origin (*oriC*), but not the operons that codes for the DNA processing machinery and produces mating pair formation functions (Tra1 operon and Tra2 operon) (F. Chen et al., 2015). The RP4 DNA segment carries functional Tra1 operon and Tra2 operon, which regulate the transfer of mobilizable plasmid pCM184-Cm (7625 bp) between the donor and recipient bacteria (Samuels et al., 2000). *E. coli* S17-1 was cultured in Luria–Bertani (LB; pH 7.4) medium prepared with 10 g/L of tryptone, 5 g/L of yeast extract and 10 g/L of NaCl, containing 20 mg/L of Chl. The recipient *E. coli* K12 MG1655 harbors an un-transferable pUA139 plasmid carrying the Km resistance genes, and it was grown in LB culture medium containing 100 mg/L of Km. The donor and the recipient strains were both incubated in 37 °C for approximately 16–18 h, with shaking at 200 rpm prior to the conjugation experiments. The concentrations of bacteria were determined using the LB agar plate counting method, which used LB agar plates containing 20 mg/L of Chl for *E. coli* S17-1 and 100 mg/L of Km for *E. coli* K12. LB agar plates containing 20 mg/L of Chl and 100 mg/L of Km were adopted to select, verify and count the transconjugants after the conjugation process.

2.4. Cytotoxicity of petrol and diesel exhaust particles

The growth inhibition effects of four types of petrol and diesel exhaust particles on the donor (*E. coli* S17-1) and recipient (*E. coli* K12) were evaluated. The overnight-grown cultures of *E. coli* S17-1 and *E. coli* K12 strains were exposed to different concentrations of particles samples along with controls (without particles exposure, incubation in LB medium only) for 4 h. The concentrations of bacteria after particles treatment and controls were determined using the LB agar plate counting method as described above. Growth inhibition rates were determined against the controls in triplicates.

2.5. Evaluation of the conjugative transfer efficiencies of ARGs upon exposure to petrol and diesel exhaust particles

In this study, we used the optimized conjugation model to evaluate the horizontal transfer of ARGs between two *E. coli* strains (Zhang et al., 2017). Briefly, the suspension of the donor (*E. coli* S17-1) and recipient (*E. coli* K12) was treated with serial concentrations of exhaust particles collected from combustion of four types of petrol and diesel as described above, along with controls (without particles treatment). Following a 4 h exposure at 37 °C, the transconjugants were determined by using LB plates containing both 20 mg/L of Chl and 100 mg/L of Km at 37 °C after 24 h incubation. The conjugative transfer efficiency was determined by dividing the number of transconjugants by the total number of recipients. The individual recipient concentrations treated with various concentrations of particles were determined by using LB agar plates containing 100 mg/L of Km, as described above.