財團法人明日醫學基金會專題研究計畫申請書

一、基本資料： 申請條碼：

本申請案所需經費(單選)	■A 類(執行計畫所需經費) ☐B 類(研究主持費，限人文處計畫，不須填寫表 C002 及 C004 至 C009)				
計畫類別(單選)	■一般型研究計畫 ☐特約研究計畫 ☐新進人員研究計畫 ☐其他				
研究型別	■個別型計畫 ☐整合型計畫				
申請機構/系所(單位)	長庚大學醫學院微生物及免疫學科				
本計畫主持人姓名	賴志河	職稱	教授	身分證號碼	
本計畫名稱	中文	開發具改善空氣汙染誘導呼吸道發炎之益生菌			
	英文	Development of functional probiotics to alleviate air pollution-related respiratory inflammation			
整合型總計畫名稱					
整合型總計畫主持人				身分證號碼	
全程執行期限	自民國 115 年 1 月 1 日起至民國 115 年 12 月 31 日				
研究學門(請參考本申請書所附之學門專長分類表填寫)	學門代碼	名稱(如為其他類，請自行填寫學門)			
研究性質	■純基礎研究 ☐導向性基礎研究 ☐應用研究 ☐技術發展				
本計畫是否為國際合作計畫	■否； ☐是，合作國家：_____，請加填表 I001～I003				
本計畫是否申請海洋研究船	■否； ☐是，請務必填寫表 C014。				
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三、主要研究人力：

（一）請依照「主持人」、「共同主持人」、「協同研究人員」及「博士後研究」等類別之順序分別填寫。

類 別	姓名	服務機構/系所	職稱	在本研究計畫內擔任之具體工作性質、項目及範圍	*每週平均投入工作時數比率(%)
主持人	賴志河	長庚大學/醫學院/微生物及免疫學科	教授	研究之規劃及推動、實驗設計、整理數據、撰寫研究成果與發表論文	70%

※註：每週平均投入工作時數比率係填寫每人每週平均投入本計畫工作時數佔其每週全部工作時間之比率，以百分比表示（例如：50%即表示該研究人員每週投入本計畫研究工作之時數佔其每週全部工時之百分五十）。

Abstract

Ambient fine particulate matter with an aerodynamic diameter of ≤ 2.5 μm (PM2.5) is a significant environmental pollutant that contributes to the development of respiratory diseases. Exposure to PM2.5 induces airway inflammation and compromises innate immune defenses, increasing susceptibility to microbial infections. Given the important role of macrophages in respiratory immunity, this project aims to evaluate probiotic candidates for their potential to attenuate PM2.5-induced inflammatory responses. The study will (i) screen probiotic strains using macrophage-based models, (ii) validate their protective efficacy and underlying mechanisms in PM2.5-exposed murine models, and (iii) establish *Drosophila* models to assess probiotics in reducing PM2.5 accumulation. Through an extensive study, this research aims to identify probiotic interventions that can ameliorate PM2.5-associated respiratory inflammation and enhance host defense, ultimately facilitating their translational and industrial applications.

Keywords: PM2.5; probiotics; respiratory inflammation; immune response

1. Introduction

1.1. Air pollution and particulate matter

Particulate matter (PM) with aerodynamic diameter ≤ 2.5 μm (PM2.5) is known to significantly contribute to airway inflammation [1]. A recent study from Taiwan reported that the traffic-related emissions and coal combustion were the major sources of PM2.5, which particularly spread in the western coastal area of central Taiwan [2]. Most importantly, a recent longitudinal study in Taiwan has demonstrated that long-term exposure to PM2.5 is associated with an increased risk of chronic obstructive pulmonary disease (COPD) [3]. Even low PM2.5 exposure levels also pose certain public health risks [4]. Therefore, understanding the effects of PM2.5 on respiratory diseases will be invaluable for developing novel strategies to improve human health.

1.2. PM2.5 exposure exacerbates respiratory inflammation

Mounting evidence suggests that exposure to ambient pollution particles impairs pulmonary function and increases the risk of infectious diseases [4]. Furthermore, ambient particles have been detected in airway macrophages within the septum of healthy individuals [5], and their impact on human immune responses has been reported [6]. Exposure to PM2.5 imbalanced M1 and M2 macrophage polarizations, which in turn dysregulated immune response and caused airway inflammation [7]. These lines of evidence suggest that ambient particles impact the respiratory innate defense, increasing susceptibility to bacterial infections.

1.3. Significance

Despite the evidence that PM2.5 poses adverse health risks, new treatments for alleviating PM2.5-induced impairment of the innate immune defense remain to be studied. **This study proposes the following experiments to develop novel probiotics to mitigate PM2.5-induced respiratory inflammation. The results from this research will undoubtedly be important for the development of potent products that protect against PM2.5-associated airway inflammation, promoting their industrial and translational applications.**

2. Research design

2.1. Macrophage-based models for screening potential probiotics

Macrophage cell models will be employed to evaluate candidate samples for their potential to inhibit PM2.5-induced inflammation. The probiotic samples provided by GenMont Biotech Inc. will be screened using RAW264.7 macrophages (ATCC TIB-71). Cells will be pretreated with each sample, followed by exposure to PM2.5 (5 or 20 μg/mL; ≤ 2.5 μm, National Institute of Standards and Technology, MD, USA) for 24 h. Cell viability will be determined to exclude cytotoxic effects. The levels of proinflammatory cytokines (IL-1β and TNF-α) in the culture supernatant will be quantified using ELISA (R&D Systems) [8]. Nitric oxide production will be measured with Griess reagent (Sigma-Aldrich), and inducible nitric oxide synthase (iNOS) protein expression will be analyzed by western blotting [9]. This screening will identify probiotic candidates capable of reducing PM2.5-induced inflammatory responses in macrophages, paving the way for subsequent in vivo validation.

2.2. Translational study by using murine models

We will then select the most promising candidates to investigate their protective activity in PM2.5-exposed murine models (Fig. 1). In this study, male BALB/c mice aged 4-6 weeks will be used and maintained at the animal center. Bronchoalveolar lavage fluid (BALF) will be collected for analysis of inflammatory cell infiltration and cytokine production (IL-1β and TNF-α) using ELISA. Lung tissues will be harvested for histopathological examination (H&E staining) and immunohistochemical analysis of iNOS and macrophage markers (F4/80). Total protein extracts from lung tissues will be subjected to western blot analysis to assess the expression of key inflammatory mediators. PM2.5 exposure is reported to alter bacterial composition in the nasal pathway, airway, and gut. We recently reported that exposure to PM2.5 causes bacterial community dysbiosis and exacerbates inflammatory diseases in the respiratory tract [10]. However, whether the potent candidates that alter PM2.5 levels influence the gut microbiota community is unclear. For this purpose, stool samples will be collected from each group, and their microbiota profiles will be analyzed.

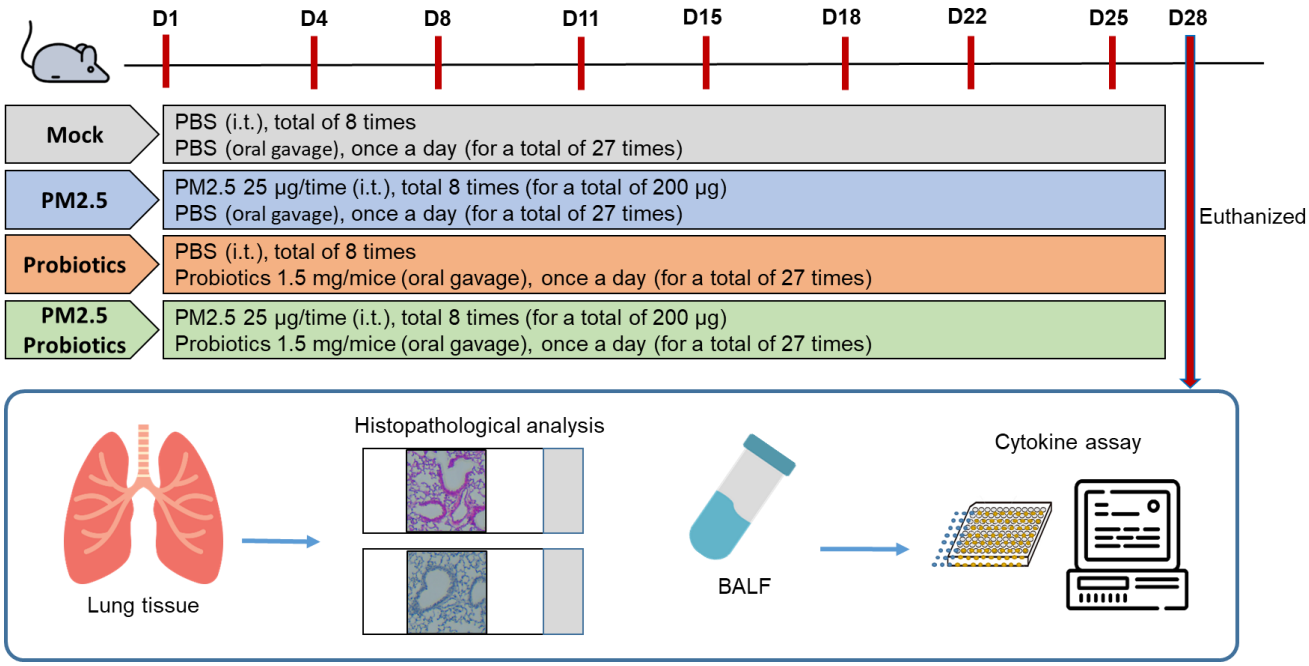

Fig. 1. Experimental design and establishment of murine models for evaluating probiotic effects. Mice will receive intratracheal administrations of PM2.5 from day 1 to day 25 (total dose: 200 μg). In parallel, probiotics (1.5 mg/mouse) will be administered orally once daily for 27 days. On day 28, mice will be euthanized, and bronchoalveolar lavage fluid (BALF) will be collected for cytokine analysis. Lung tissues will be harvested for histopathological examination.

2.3. Establishing *Drosophila* models to assess probiotics in reducing PM2.5 accumulation

To evaluate the potential of probiotic candidates to reduce PM2.5 accumulation *in vivo*, *Drosophila melanogaster* will be used as a model organism by collaborating with co-PI (Professor Chia-Lin Wu 吳嘉霖教授). Adult flies will be exposed to PM2.5 via food supplementation or inhalation for a defined period. Concurrently, selected probiotic candidates identified from murine studies will be administered through the diet, as described in our recent study [11]. The

experimental design will include control (no probiotic) and treatment groups (probiotic-administered) to assess the effects on PM2.5 accumulation and clearance. Quantitative measurement of PM2.5 in the flies will be performed using inductively coupled plasma mass spectrometry (ICP-MS) or equivalent particle detection methods. In addition, oxidative stress and inflammation markers in the flies will be evaluated to assess physiological responses to PM2.5 exposure and probiotic intervention. This *Drosophila* model will provide rapid, cost-effective insights into the capacity of probiotic candidates to reduce PM2.5 accumulation and mitigate associated systemic stress, supporting translational relevance for mammalian models.

3. Preliminary results

3.1. Characterization of PM2.5 and its effects on innate immune response

To examine the potential effects of PM2.5 on macrophage activity, we utilized standard reference material obtained from the National Institute of Standards and Technology (MD, USA) [7]. The particle size of PM2.5 was first characterized using a field-emission scanning electron microscope (JSM-7500F, JEOL, Japan). As shown in Fig. 2A, the majority of the particles displayed diameters smaller than 2.5 μm, confirming their classification as fine particulate matter. Next, cells were treated with low (5 μg/mL) or high (20 μg/mL) concentrations of PM2.5 for 2 h. As shown in Fig. 2B, neither concentration significantly affected cell viability. When macrophages were exposed to 20 μg/mL PM2.5 for 24 h, light microscopy revealed visible particulate deposits within the cytoplasm, indicating successful cellular uptake and embedding of PM2.5 (Fig. 3). Based on these findings, PM2.5 concentrations of 5 μg/mL and 20 μg/mL will be selected for subsequent experiments to assess macrophage responses.

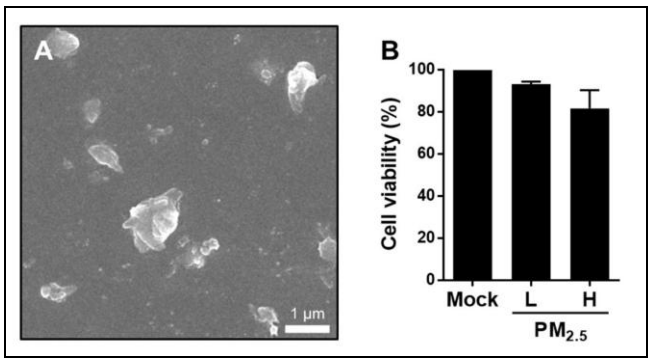

Fig. 2. Characterization of PM2.5 and its effects on macrophage viability. (A) Field-emission scanning electron microscopy (FE-SEM) image showing the morphology and size distribution of PM2.5 particles (< 2.5 μm). (B) Effects of PM2.5 exposure on RAW264.7 macrophage viability after 2 h treatment with 5 μg/mL or 20 μg/mL. Scale bar: 1 μm.

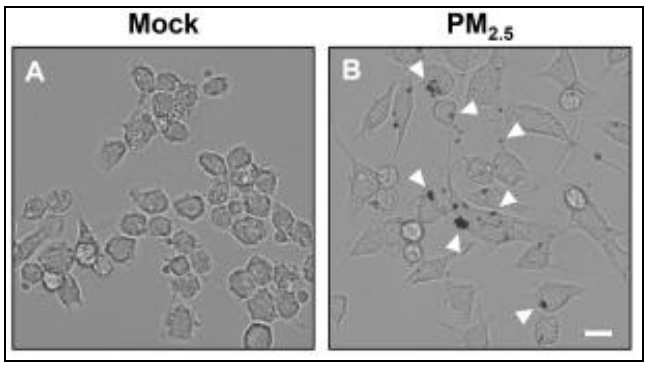

Fig. 3. Deposition of PM2.5 in the cytoplasm of macrophages. RAW264.7 cells were (A) mock-treated or (B) treated with 20 μg/mL PM2.5 for 24 h and observed under light microscopy. Arrowheads indicate macrophages containing phagocytosed PM2.5 particles. Scale bar: 5 μm.

3.2 $PM_{2.5}$ impairs phagocytosis of bacteria by macrophages

To assess whether PM2.5 exposure affects macrophage phagocytic function, we evaluated phagocytosis using antibody-coated fluorescent latex beads. As shown in Fig. 4A, treatment with a low concentration of PM2.5 (5 μg/mL) slightly reduced the internalization of FITC-conjugated latex beads by RAW264.7 macrophages, whereas exposure to a higher concentration (20 μg/mL) markedly suppressed bead uptake. These findings indicate that PM2.5 compromises macrophage phagocytic capacity in a dose-dependent manner. To further confirm this effect using a biologically relevant target, bacterial internalization was assessed by gentamicin protection assay. Consistently, macrophages treated with PM2.5 exhibited significantly reduced bacterial uptake compared with untreated controls (Fig. 4B). These results collectively demonstrate that exposure to PM2.5 impairs macrophage phagocytic activity and diminishes bacterial clearance efficiency.

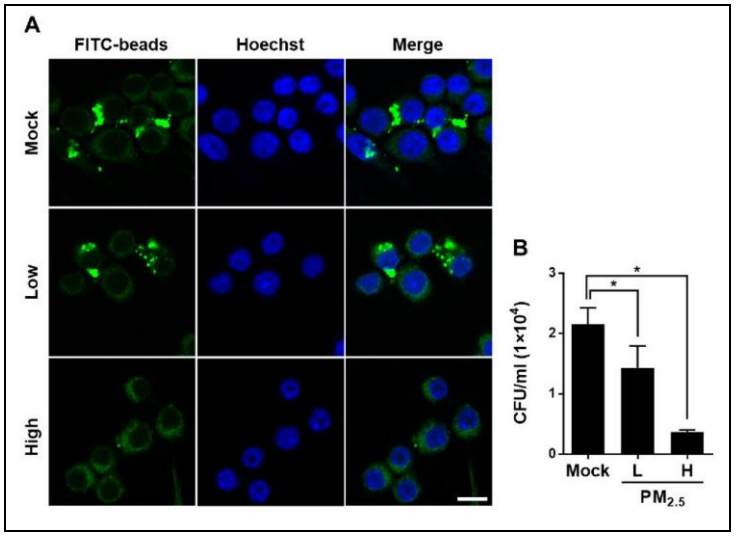

Fig. 4. PM2.5 suppresses macrophage phagocytosis during bacterial infection. (A) RAW264.7 cells were pretreated with 5 or 20 μg/mL PM2.5 for 2 h, then incubated with fluorescent latex beads for 3 h. Nuclei were stained with Hoechst 33342 and imaged by confocal microscopy. Scale bar: 10 μm. (B) Macrophages were exposed to PM2.5 for 2 h and infected with pneumococci (MOI = 100) for 2 h. Intracellular bacteria were quantified using a gentamicin protection assay and expressed as CFU ($P < 0.05$).

4. Anticipative results

The primary objective of this study is to identify probiotics that can mitigate PM2.5-induced respiratory inflammation. The findings are expected to inform the development of strategies to mitigate air pollution, particularly PM2.5. The anticipated outcomes are as follows:

4.1. Our results will first screen the tested samples and demonstrate that the potential candidates inhibit inflammatory mediators, alleviating PM2.5-elicited inflammation.

4.2. Using PM2.5-exposed murine models, the protective efficacy of selected candidates will be confirmed, translating *in vitro* results into physiologically relevant outcomes.

4.3. These results from performing *Drosophila* studies will identify potent probiotic strains capable of mitigating PM2.5 accumulation and validating their biological impacts.

This study is expected to generate -2 high-quality publications. Furthermore, the identified probiotic candidates will have potential for industrial development to alleviate PM2.5-induced respiratory inflammation. With the strong support of Tomorrow Medical Foundation, this project will advance both preventive strategies and the development of effective interventions for respiratory diseases associated with air pollution, promoting translational and industrial applications.

5. References

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