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**Original Article**

# Effects of tobacco smoke on the expression of virulence genes in *Escherichia coli*

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## Abstract

It is widely acknowledged that smoking exacerbates the severity of infectious diseases. A presumed mechanism involves the damage inflicted by tobacco smoke on the organs of host organisms. In this study, an alternative hypothesis was explored: smoking enhances the virulence of bacteria. This possibility was investigated using *Escherichia coli* as the model bacteria and *Drosophila* as the host organism. Our inquiry focused on the potential gene expression changes in *E. coli* subsequent to exposure to tobacco smoke extracts. Analysis of the transcription promoter activity of genes encoding proteins within the *E. coli* two-component system, a regulatory machinery governing gene expression, revealed the suppression of thirteen out of 23 promoters in response to tobacco smoke extracts. Subsequently, *Drosophila* was infected with *E. coli* exposed to tobacco smoke extracts or left untreated. Interestingly, there were no significant differences observed in the survival periods of *Drosophila* following infection with *E. coli*, whether treated or untreated with tobacco smoke extracts. Contrary to the initial hypothesis, the findings suggest that while tobacco smoke extracts alter gene expression in *E. coli*, these changes do not appear to impact bacterial virulence. Although this study has illuminated the influence of tobacco smoke extracts on the gene expression of *E. coli*, further analyses are necessary to elucidate the implications of these changes. Nevertheless, the results imply that smoking affects not only host organisms but may also exert influence on invading bacteria.

**Keywords**: Fruit flies, *in vivo*, tobacco, *E. coli*, virulence

## Introduction

Bacteria possess a sophisticated mechanism for sensing environmental changes, modulating gene expression, and responding to the surrounding conditions to ensure their survival. So far, extensive research has been conducted to understand the environmental response of bacteria to variations in temperature [1], pH [2], metal ions [3], and environmental pollutants [4,5]. The regulation of gene expression in bacteria is governed by two primary mechanisms: the regulation of transcription efficiency via bacterial information pathways and the determination of genes to be transcribed. This determination is executed by a prokaryotic-specific sigma factor, a subunit of the RNA polymerase [6]. Taking the example of *Escherichia coli*, a Gram-negative model bacterium, the RNA polymerase comprises a core subunit responsible for RNA synthesis activity and a sigma factor that binds to DNA transcription promoter sequences, with seven distinct types identified [7]. During a well-nourished logarithmic growth phase, one type of sigma factor, sigma 70, predominates and regulates the transcription of genes essential for survival [6]. However,



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exposure to a stressful environment induces the expression of the remaining six types, leading to the synthesis of proteins not previously expressed, thereby altering the physiological state [8]. Previous research within our group has demonstrated an increase in the abundance of the sigma factor sigma 38 during host infection [9]. This induction prompts the expression of bacterial catalase, thereby avoiding bactericidal action by reactive oxygen species (ROS) post-phagocytosis by macrophages, enabling the continuation of the infection.

On another front, the mechanism through which bacteria sense environmental changes involves a two-component regulatory system. This system comprises two types of proteins: membrane receptor histidine kinases, localized at the plasma membrane, referred to as sensor kinases, and transcriptional regulators, known as response regulators. Both components function in specific combinations, forming a regulatory network to orchestrate bacterial responses to environmental cues [10,11]. It is well-established that two-component regulatory systems play a pivotal role in positively or negatively regulating the expression of specific genes, each essential for distinct biological functions. Upon recognition of information molecules by membrane receptors, two-component regulatory systems undergo autophosphorylation, facilitating the transfer of phosphate groups to transcription factors functioning in pairs. This process induces a change in the activity of transcription factors, leading to alterations in the transcription efficiency of the regulated gene cluster and subsequently influencing the expressed protein repertoire [12].

The *E. coli* two-component regulatory system included an intricate network comprising 27 sensor kinases and 34 response regulators, potentially forming numerous different combinations of sensor kinases and response regulators [13,14]. Each specific *E. coli* two-component regulatory system encompasses regulon genes—groups of genes whose expression is intricately controlled and regulates their transcription in either a positive or negative direction. Certain types of *E. coli* two-component regulatory systems have been identified and classified, such as metabolic, respiratory, stress response, and transport systems [13,14]. Consequently, knowledge of the pathways through which activity changes occur allows for predictions regarding altered gene expression and cellular responses to a certain extent. Our previous study has a comprehensive analyzed of highly active two-component regulatory systems in *E. coli*, utilizing a model infection system where *E. coli* was injected into the body cavity of *Drosophila* as the host [15]. The study has identified pathways crucial for maintaining infection and exerting toxicity within the host organism.

Prior investigations into bacterial infections have revealed that the interactions between bacteria and the host result in subsequent modifications in gene expression for both parties involved [16,17]. These cumulative alterations significantly influence the pathogenesis of the infection [16,17]. When bacteria invade a host, they encounter host defense provided by immune cells, and in response, they employ strategies to resist, survive, and may alter their properties to become toxic or establish an infection [18]. Although environmental agents may contribute to this process, few studies have explored their involvement in detail.

Tobacco smoke encompasses various compounds, including carbonyls such as formaldehyde and acetaldehyde, nitrogen oxides, and benzo[*a*]pyrene, alongside typical substances like nicotine and tar [19]. These components constitute a complex array of more than 4000 substances in tobacco, with over 200 identified as toxic chemicals [19]. Common elements, such as nicotine, tobacco smoke components, and tobacco smoke chemicals, contribute to the intricate composition of tobacco [19]. Notably, nicotine, tar, and carbon monoxide stand out as prevalent constituents of tobacco smoke, though numerous components with unidentified structures and contents persist. These substances are known to contribute to diseases such as periodontal disease and heart disease [20,21].

Smoking exposes cells in the oral cavity and gastrointestinal tract to elevated concentrations of tobacco smoke. Periodontal disease, a prevalent oral infection, exhibits a causal relationship with smoking habits, leading to increased disease severity. The prevailing interpretation suggests that chemicals in tobacco smoke induce inflammation and damage to oral tissues, exacerbating periodontal disease when periodontopathic bacteria encounter immune cells and body fluid components [22]. Conversely, smoking introduces bacteria to tobacco smoke, potentially altering their gene expression through the aforementioned pathways. This alteration may, in turn, influence their toxicity and harm to the host. Given the extensive diversity of chemicals, including

unidentified substances, present in different ratios in tobacco and variations in types and amounts of substances in mainstream smoke, sidestream smoke, and the gaseous component, comprehensive analyses utilizing whole tobacco smoke extracts are imperative to investigate the impact of tobacco smoke on changes in gene expression and bacterial properties.

As previously mentioned, we have implemented an assay system utilizing *Drosophila* as a host and *E. coli* and *Staphylococcus aureus* as Gram-negative and Gram-positive bacterial models, respectively, for studying bacterial infections. *Drosophila melanogaster*, with a generation lasting approximately 10 days and proves to be an easily reared insect [9,15,23-25]. This characteristic makes it conducive for conducting bacterial infection and drug administration experiments with minimal sample amounts.

Given the evolutionary conservation of basic innate immune mechanisms in *Drosophila* and mammals [26], including humans, it is highly probable that insights gained from the *Drosophila* infection system are applicable to mammalian infections. The innate immune system of *Drosophila* encompasses a humoral response, marked by the production of antimicrobial substances, and a cellular response, represented by phagocytosis and bactericidal action of macrophages [26,27]. In the humoral response, the intracellular information pathway is activated by the recognition of cell wall components from Gram-positive or Gram-negative bacteria by specific receptors. The receptors, information molecules, and transcription factors comprising this pathway are shared between *Drosophila* and humans [26].

*Drosophila* possesses specialized phagocytes analogous to mammalian macrophages [27]. In these phagocytes, receptors recognizing bacterial cell wall components are localized on the plasma membrane, leading to cytoskeletal reorganization through intracellular signaling pathways. This results in the internalization of bacteria into macrophages. Once inside macrophages, bacteria are transported to lysosomes, where they face degradation by hydrolytic enzymes under acidic conditions and exposure to radicals like reactive oxygen species and nitric oxide, ultimately leading to their killing, degradation, and elimination [27]. A previous study has identified common bacterial phagocytic receptors in both humans and *Drosophila* [28]. Furthermore, our study has demonstrated that bacteria possess mechanisms to evade phagocytic elimination by altering gene expression [23,29]. These findings highlight the conserved nature of host-pathogen interactions across species and provide valuable insights into bacterial evasion strategies within the host immune system.

Based on the aforementioned, we hypothesized that tobacco smoke could modify gene expression in bacteria, thereby altering the nature of their toxicity and immune resistance to the host. To investigate this, a comprehensive analysis was conducted to identify gene expression regulators affected by the presence of tobacco smoke and to elucidate their impact on host toxicity. The Gram-negative bacterium *E. coli* was selected as the model organism due to its well-established genome analysis and extensively studied gene expression regulation mechanisms. *E. coli* has a genome size of 4.64 Mb and 4,600 genes, and as mentioned, the two-component regulatory system of the signaling pathway, its sub-regulatory genes and the sigma factor of the RNA polymerase have been identified. In addition, information and resources necessary for analysis were considered optimal for this research purpose (National Institute of Genetics: https://www.nig.ac.jp/nig/ja/). Simultaneously, *D. melanogaster* served as the host organism to explore the interplay between host toxicity and the immune response during *E. coli* infection. The utilization of *Drosophila* as a host provides valuable insights due to the evolutionary conservation of innate immune mechanisms, making it a relevant model for studying interactions between bacteria and host organisms, including the potential effects of tobacco smoke on gene expression and immune responses.

## Methods

### Fly stocks

*D. melanogaster*, *Oregon R* strain (Kyorin-Fly; Kyorin University, Tokyo, Japan), was utilized as the experimental organism. In this study, a mutant strain of *D. melanogaster* with a disruption in the IMD pathway, $imd^{1}$ [30], was used as a fly model with suppressed activity in the humoral pathway.

### *Escherichia coli*

*E. coli*, strain BW25113, derived from strain K-12, was used as the parental strain. The *E. coli* strains were obtained from the Keio Collection of the National Resource Centre of the National Institute of Genetics [31]. Plasmid pBR322, in which the transcription promoter sequence of the gene controlled by the two-component control system was inserted, was provided by Dr Akira Ishihama and Dr Kaneyoshi Yamamoto of Hosei University, Japan. This plasmid has a gene encoding the green fluorescence protein (GFP) downstream of the gene's transcription promoter sequence. This plasmid was transformed and maintained in the parental strain of *E. coli* strain BW25113. These strains expressed GFP driven by the promoters of genes coding for sensor kinases and response regulators of 23 *E. coli* two-component system (TCS): IT1568/rpoS-GFP, K030 phoB-GFP, K052 ylcA/c/GFP and K052 ylcA/c/GFP. K052 ylcA/cusR-GFP, K053 ylcB/cusC-GFP, K239 acrD-GFP, K428 cheY-GFP, K429 yedV-GFP, K430 yehT-GFP, K520 spy-GFP, K633 galU-GFP, K782 torC-GFP, K069 kdpA-GFP, K214 atoD-GFP, K391 dcuB-GFP, K693 narG-GFP, K436 glnG-GFP, K441 creD-GFP, K107 phoP-GFP, K227 evgA-GFP, K365 cpxR-GFP, K373 zraS-GFP, K426 kdpE-GFP, K427 phoQ-GFP, K431 evgS-GFP and K433 qseC-GFP [15]. These *E. coli* strains were incubated in Luria-Bertani (LB) medium for 18–20 h with shaking, washed once with phosphate-buffered saline (PBS) and used for each experiment.

### Collection of tobacco smoke and preparation of tobacco extracts

The ISO 4387 method, published by the International Organization for Standardization for extracting nicotine and other substances, served as the designated collection method when simulating a human smoking model. Briefly, this approach involved attaching a cigarette (a local brand with 12 mg tar, 1.0 mg nicotine) to the end of the smoking device and pumping at a rate of puff volume of 35 mL, a puff duration of two seconds and a puff interval of 60 seconds. The entire procedure, involving five cigarettes, was performed, and the particulate matter of mainstream smoke was collected on Cambridge glass fiber filter (Borgwaldt KCGmbH). The particulate matter of side stream smoke was collected on a glass fiber filter through a fishtail chimney at a flow rate of 3.0 L/min. The filters from which the tobacco smoke was extracted and collected were cut in half. One was soaked in 15 mL of PBS and shaken overnight, and the resulting liquid was used as the water-soluble extract. The other was soaked in 15 mL of isopropanol and shaken overnight, after which the isopropanol was volatilized and dissolved in DMSO to form an organic extract.

### *Drosophila* viability analysis

Overnight shaking cultures of *E. coli* (full growth) were washed with PBS, 5% (w/v) of the tobacco smoke extracts were added and left at room temperature for five min. The tobacco smoke extract was then diluted with PBS to achieve the desired concentration. The body cavities of adult male *Drosophila* (3–7 days after eclosion) were injected with PBS containing *E. coli* or PBS as a negative control per animal using a nitrogen gas-controlled microinjector (NARISHIGE/IM300) [15]. The survival rate was calculated by placing 15–20 flies per vial containing food and counting the number of flies alive after rearing at 29°C. This survival rate served as an indicative measure of the impact of *E. coli* on the host.

### Analysis of gene transcription promoter activity

*E. coli* BW25113 strains carrying the plasmids were subjected to shaking and incubation in LB medium at 37°C for the stationary phase. Following incubation, the bacteria were collected by centrifugation, and the bacterial pellet was then resuspended in PBS. Subsequently, the bacterial suspension and tobacco extract were combined in a 96-well microplate, resulting in a final concentration of 0.5% (w/v), and the mixture was left at room temperature for five min. The fluorescence intensity of GFP was quantified using a microplate reader (TEKAN SPARK-10). This process allowed for the measurement of GFP fluorescence as an indicator of gene expression modulation in response to the interaction between the bacterial suspension and tobacco extract extracts.

### Measurement of *E. coli* growth

Bacteria incubated in LB medium were measured by optical density (OD) at 600 nm wave light for growth rate in a liquid medium and colony forming on LB agar medium.

### Reverse transcriptase-quantitative PCR

The mRNA levels of antimicrobial peptides were assessed using reverse transcriptase-quantitative PCR (RT-qPCR) following established protocols [23,24]. Briefly, total RNA extracted from flies served as the template for reverse transcription to generate cDNA. The resulting cDNA was then subjected to real-time PCR to amplify specific sequences corresponding to attacin, diptericin, and ribosomal protein 49 (rp49) mRNA. The signals from attacin and diptericin mRNA were normalized to those from rp49 mRNA, serving as an internal control. The primer sequences used for PCR were as follows: for diptericin mRNA, 5′-GCTGCGCAATCGCTTCTACT-3′ (forward) and 5′-TGGTGGAGTGGGCTTCATG-3′ (reverse); for attacin mRNA, 5′-CCCGGAGTGAAGGATG-3′ (forward) and 5′-GTTGCTGTGCGTCAAG-3′ (reverse); and for rp49 mRNA, 5′-GACGCTTCAAGGGACAGTATCTG-3′ (forward) and 5′-AAACGCGGTTCTGCATGAG-3′ (reverse).

### Data analysis

Results from quantitative analyses are expressed as the mean ± SD of the data from at least three independent experiments. Statistical analyses were performed using the log-rank test (Kaplan–Meier method) for the data from an assay for fly survival or a two-tailed Student t-test for all other data. The $p$-values are indicated in the corresponding figures or figure legends and any $p<0.05$ were considered significant.

## Results

### Effect of tobacco smoke extracts on the TCS activity in *E. coli*

Tobacco smoke was collected using a dedicated collection device and for every five tobaccos, 0.0398±0.0004 g (average from five times collection) of tobacco smoke could be recovered from mainstream smoke, and 0.3352±0.003 g (average from five times collection) from sidestream smoke. In the context of the TCS of *E. coli* that are responsible for sensing environmental changes, 23 TCSs were analyzed for positively regulated genes. Transcriptional promoter assays utilizing GFP as a reporter were conducted for these genes. The TCSs and the indicator genes can be seen in each panel of **Figure 1**. This established transcription promoter assay system, utilizing GFP as a reporter, detected changes in gene expression of the *E. coli* two-component control system during fly infection and in fly body fluid components.

Plasmid-retaining bacteria for the analysis of 23 TCSs were cultured to steady state, co-cultured with tobacco mainstream or sidestream smoke (extracted using isopropanol), and GFP fluorescence intensities were measured. This resulted in the identification of 13 genes with altered transcriptional promoter intensities, as shown in **Figure 1**. The affected genes, whose activity was reduced by isopropanol-extracted components of tobacco main stream smoke and side stream smoke include AtoS-AtoC (*atoD*), NtrB-NtrC (*glnG*), CheA-CheB (*cheY*), YedV-YedW (*yedV*), CusS-CusR (*cusC*), EnvZ-OmpR (*ompC*), phoR-PhoB (*phoB*), ArcB-ArcA (*focA*), CpxA-CpxR (*cpxR*), BaeS-BaeR (*spy*), YedV-YedW-RssB (*galU*), RstA-RstB (*narG*), and EvgA-EvgS (*evgA*) (**Figure 1**). These genes are involved in various functions, including stress response, transport, metabolism, and functions of unknown specificity. Furthermore, it was observed that the types of genes affected were consistent between mainstream and sidestream smoke extracts, though the degree of change varied. GFP fluorescence intensity was significantly reduced when exposed to sidestream smoke extract, whereas only NtrB-NtrC remained unchanged in main stream smoke extract, indicating a differential impact between the two.

### No effect of tobacco smoke on bacterial growth

In this experiment, 5% (v/v) of tobacco side stream smoke PBS extract, isopropanol extract, or DMSO (solvent control) was added to *E. coli* BW25113 strain, incubated for five minutes, and subsequently cultured in LB liquid medium for the duration indicated in **Figure 2**.

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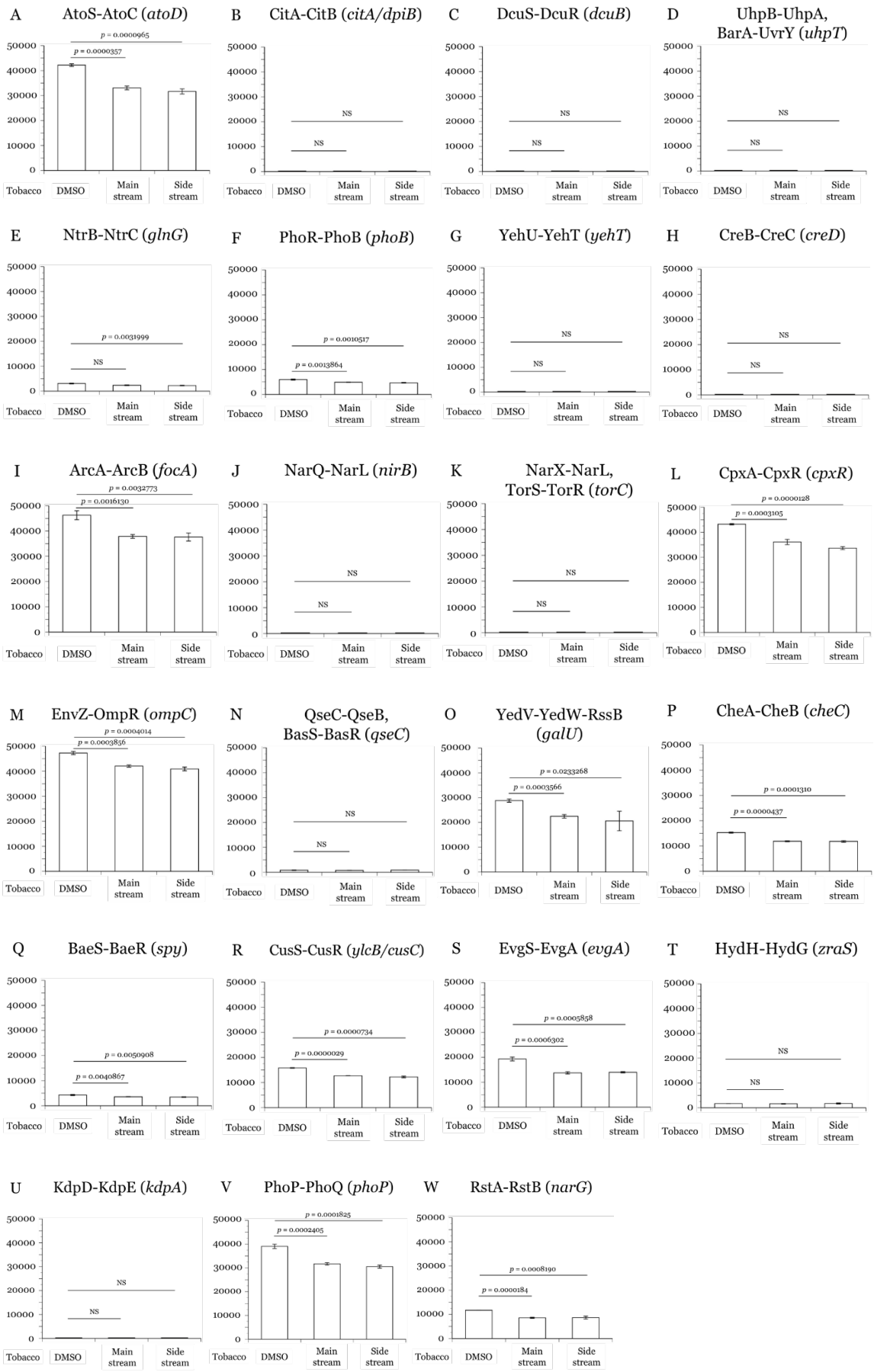


Figure 1. Thirteen two-component systems (TCSs) of *E. coli* are altered in the presence of tobacco smoke extracts. Transcriptional promoter assays utilizing green fluorescence protein (GFP) as a reporter were used to analyze a total of 23 TCSs (A-W) for positively regulated genes.

Both the absorbance at 600 nm (**Figure 2A**) and the number of colony-forming bacteria, determined by coating on LB agar medium (**Figure 2B**), were measured. There was no discernible difference in the turbidity of the liquid medium or the number of colony-forming bacteria on the agar medium attributable to the presence of tobacco smoke extracts. It was unequivocally observed that 5% (v/v) of tobacco smoke extracts had no discernible impact on the growth of *E. coli*.

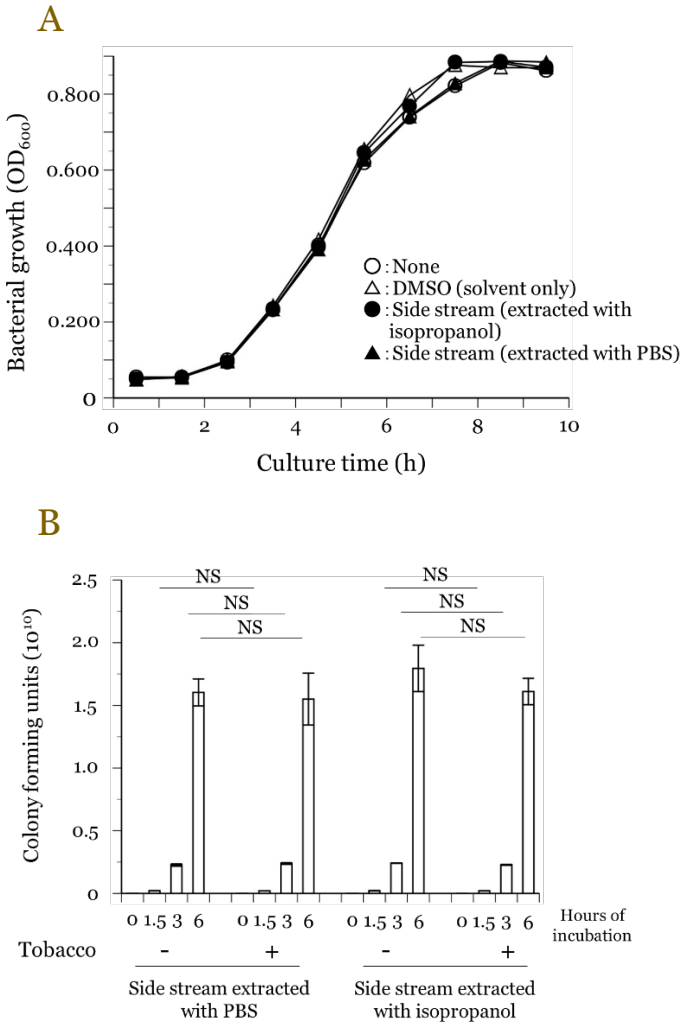


Figure 2. The presence of tobacco smoke extracts did not result in any apparent impact on the growth of *E. coli*. A total of 5% (v/v) of tobacco side stream phosphate-buffered saline (PBS) extract, isopropanol extract, or DMSO (solvent control) were added to the *E. coli* BW25113 strain, allowing it to stand for five minutes, and subsequently cultured in Luria-Bertani (LB) medium for the duration specified in the figures. Both the absorbance at 600 nm (A) and the number of colony-forming bacteria, determined by coating on LB agar medium (B), were measured. NS, non-significant.

## No effect of tobacco smoke extracts on bacterial virulence in flies

The *E. coli* BW25113 strain was exposed to 5% (v/v) of tobacco side stream smoke PBS extract, isopropanol extract, or solvent, and the mixtures were incubated for five minutes. In a separate experimental setup, latex beads, serving as a phagocytosis inhibitor, were injected into the abdomens of *imd* mutant flies ($imd^1$), and the flies were examined under a microscope. Survival rates were calculated for different time intervals, as indicated in **Figure 3**. Over time, wildtype flies (**Figure 3A**), *imd* mutant flies (**Figure 3B**) and phagocytosis-inhibited *imd* mutant flies (**Figure 3C**) succumbed to the injection of *E. coli*, with no observed difference in survival rates based on the presence or absence of tobacco smoke extract.

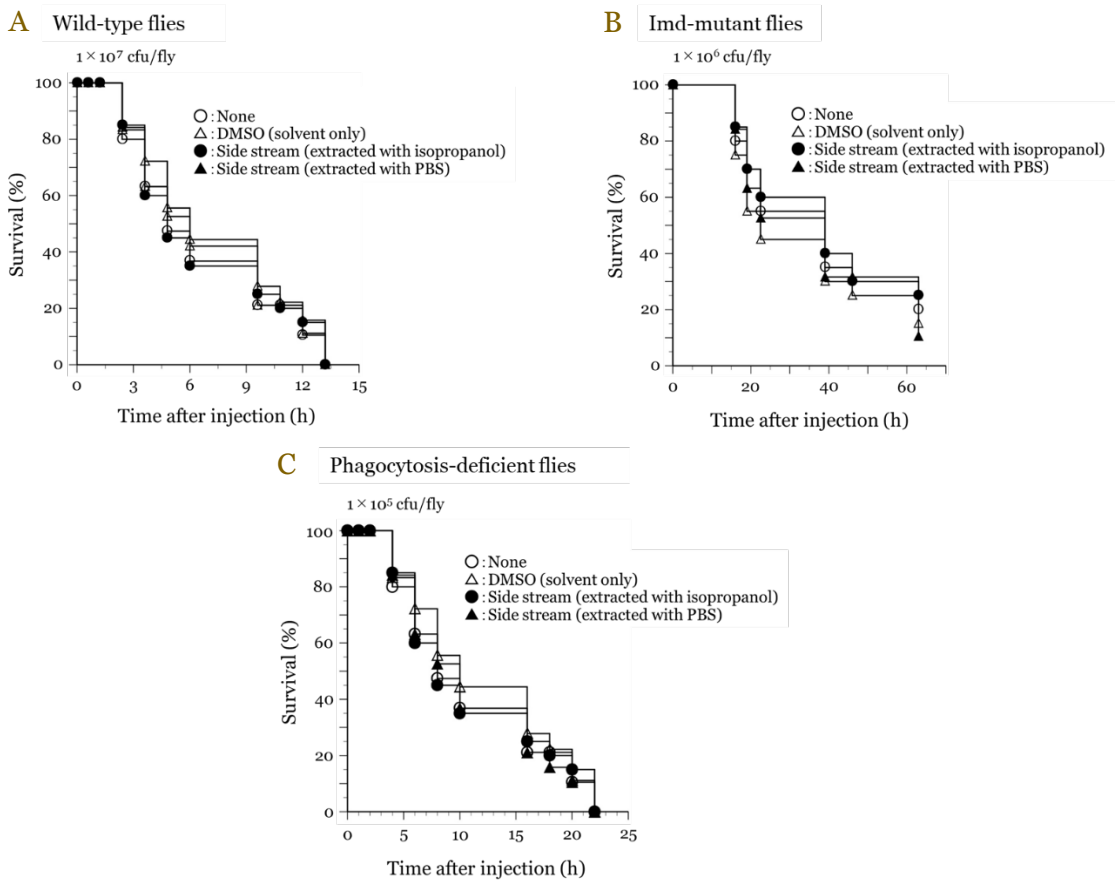


Figure 3. Tobacco smoke extracts did not affect the virulence of *E. coli*. The *E. coli* BW25113 strain was treated with 5% (v/v) of tobacco side stream smoke phosphate-buffered saline (PBS) extract, isopropanol extract, or DMSO (solvent control), followed by a 5-minute incubation, prior to injection to the abdomen of flies. Inhibition of phagocytosis was achieved by the injection of latex beads as a phagocytosis inhibitor into the abdomens of *imd* mutant flies. Microscopic examinations were conducted, and survival rates were calculated on the wildtype *Oregon R* (A), *imd* mutant (B), or phagocytosis-inhibited *imd* mutant (C) flies at various time intervals as specified in the figures.

Furthermore, the evaluation of antimicrobial peptide (AMP) expression was conducted in *Oregon R* flies following injection with *E. coli* previously exposed to tobacco smoke extracts. Apparently, tobacco smoke extracts did not upregulate the expression of diptericin (**Figure 4A**) and attacin (**Figure 4B**), two AMPs known to be induced by Gram-negative bacteria such as *E. coli*. Hence, the components of tobacco smoke extracts did not appear to alter the virulence of *E. coli* to the host.

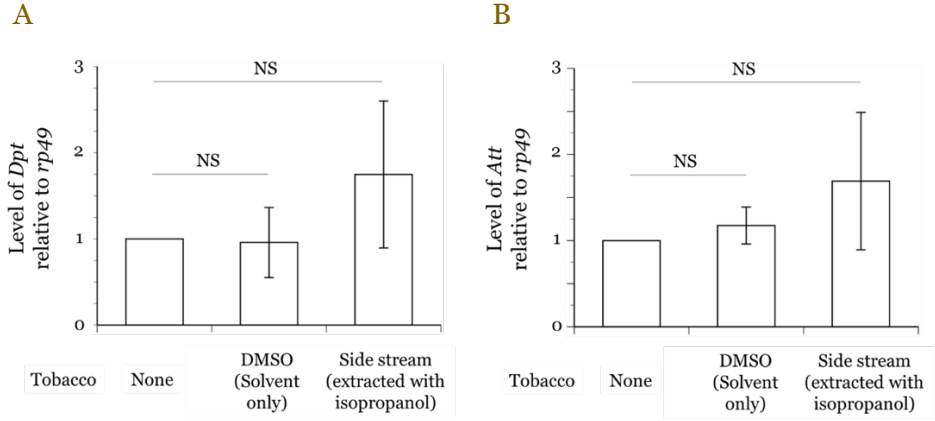


Figure 4. Tobacco smoke extracts did not influence the expression of diptericin (A) and attacin (B). NS, non-significant.

### No influence of tobacco smoke extracts on bacterial sensitivity to low pH and $H_2O_2$

We next assessed whether tobacco smoke components can influence the sensitivity of bacteria to several stressors, such as low pH and hydrogen peroxide. These stressors were used to mimic the conditions of sterilization by the lysosomal acidic environment within macrophages or superoxide produced by macrophages. Upon the addition of isopropanol extract (5%) of tobacco smoke extracts to *E. coli* strain BW25113, two separate experiments were conducted. In **Figure 5A**, the bacteria were cultured in LB medium adjusted to pH 4.5 using benzoic acid, while in **Figure 5B**, the *E. coli* were cultured in LB medium to which hydrogen peroxide was added. Subsequently, the bacteria were spread on agar medium, and the number of colony-forming bacteria was determined. In the presence of tobacco smoke extracts, there was no observed difference in the degree of colony formation between the two experimental conditions. The resistance to low pH and active oxygen did not appear to be influenced by the presence of tobacco smoke extracts.

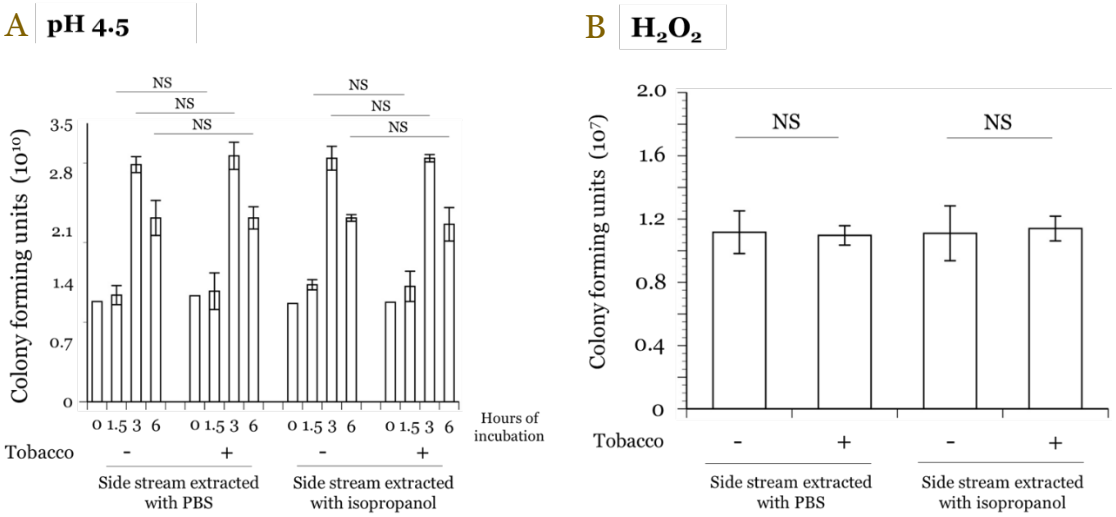


Figure 5. There is no effect of tobacco smoke extracts on the bacterial sensitivity to low pH and hydrogen peroxide. Evaluation of *E. coli* BW25113 strain responses to isopropanol extract (5%) of tobacco smoke under different conditions. In (A), *E. coli* were cultured in Luria-Bertani (LB) medium adjusted to pH 4.5 using benzoic acid for the duration specified in the figures, and in (B), *E. coli* were cultured in LB medium with the addition of hydrogen peroxide to a final concentration of 20 mM. The resulting bacterial colonies were quantified after spreading on agar medium.

## Discussion

In our present study, we have demonstrated that tobacco smoke components can alter gene expression in *E. coli* without observable changes in bacterial virulence. This presents an intriguing aspect of host-pathogen interactions in the context of smoking. Although the study did not demonstrate a direct link between altered gene expression and increased bacterial virulence in the *Drosophila* model, it does underscore the complex influence of tobacco smoke components on both host organisms and invading bacteria.

The absence of any discernible impact on bacterial growth in *E. coli*, as evidenced by consistent absorbance at 600 nm and comparable colony-forming units, following exposure to 5% (v/v) of tobacco smoke extracts is a noteworthy observation. This result contradicts the notion that tobacco smoke components may influence the growth dynamics of *E. coli* under the experimental conditions, at least the ones employed in this study. Several factors could contribute to these findings. Firstly, the concentration of tobacco smoke components utilized in the experiment may not be sufficient to induce detectable changes in bacterial growth. It is plausible that higher concentrations or prolonged exposure durations might be necessary to elicit a measurable effect. Additionally, *E. coli* BW25113, being a well-characterized laboratory strain, may possess inherent resilience or adaptive mechanisms that mitigate the impact of tobacco smoke components on its growth. The use of specific assays to investigate potential alterations in

metabolic pathways, stress response, or regulatory mechanisms within *E. coli* could provide a more detailed understanding of how tobacco smoke components may influence bacterial physiology without affecting overall growth. Further experiments exploring different concentrations, exposure durations, and bacterial strains may unveil nuances in the relationship between tobacco smoke components and bacterial behavior.

The observed lack of impact on bacterial virulence in *Drosophila* following exposure to 5% (v/v) of tobacco smoke extracts is a significant outcome that warrants discussion. Despite the potential alterations in gene expression identified earlier, these changes did not translate into measurable effects on the virulence of *E. coli* in the fly host. One possible interpretation of these results is that the specific concentration of tobacco components applied in this study might not be sufficient to modulate the virulence factors of *E. coli* in a way that is detectable within the experimental timeframe. Virulence in bacteria is a multifaceted trait influenced by various factors, and subtle changes in gene expression may not always correlate directly with alterations in virulence [32,33]. It is plausible that a more nuanced investigation, possibly at different concentrations or with extended exposure periods, could reveal differential effects on specific virulence determinants. The use of latex beads as a phagocytosis inhibitor [34] in *imd* mutant flies introduces an additional layer of complexity. While this approach allows for the assessment of bacterial virulence independent of phagocytosis, it also narrows the focus to direct bacterial effects on the host. The absence of significant differences in survival rates between groups suggests that the tobacco smoke components did not influence the virulence of *E. coli* in this experimental context.

Our data indicated that tobacco smoke components have an impact on gene expression in *E. coli*, yet thus far, no alterations in bacterial virulence have been identified. Several interpretations and implications arise from our observations, as explained above. In addition, the lack of a straightforward correlation between gene expression changes and increased virulence suggests that the effects of tobacco smoke components on bacteria may be more subtle or context-dependent than initially hypothesized. Bacterial virulence is a multifaceted trait influenced by various factors, and the study's experimental design might not capture all relevant aspects of this intricate relationship.

While the study did not reveal a discernible effect of tobacco smoke extracts on bacterial virulence in the *Drosophila* model, it is essential to approach these results with a nuanced perspective. Future research directions may involve exploring different concentrations, exposure durations, and employing more detailed assays to unravel the intricate dynamics of host-pathogen interactions in the presence of tobacco smoke components. The focus on growth alone may overlook potential changes in other aspects of bacterial physiology, such as gene expression, virulence factors, or stress responses. Additionally, the implications of these findings in the context of host-pathogen interactions should be investigated further, as the complex interplay between tobacco smoke components, bacterial behavior, and host responses merits thorough exploration.

## Conclusions

Tobacco smoke extracts possesses the capability to modify gene expression in *E. coli*, yet without discernible alterations in bacterial virulence. This unveils a compelling facet of host-pathogen dynamics within the framework of smoking. While this study did not establish a direct association between modified gene expression and heightened bacterial virulence in the *Drosophila* model, it underscores the intricate impact of tobacco smoke components on both host organisms and invading bacteria.

### Ethics approval

Not required.

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### Competing interests

The authors declare that there are no conflicts of interest.

### Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

## How to cite

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