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RESEARCH ARTICLE

Isolation and identification of latex-degrading bacteria

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Abstract - Latex-degrading bacteria have contrasting potential to offer sustainable waste biodegradation solutions while simultaneously threatening manufacturing productivity, product shelf-life, and overall quality. This study aimed to isolate, identify, and characterize latex-degrading bacteria to achieve a deeper understanding of their degradation abilities. Bacterial strains were isolated from raw latex and soil samples from the Bukit Kuantan Research Station of the Malaysia Rubber Board using latex-overlay agar and a three-quadrant streaking method to yield single colonies. Identification was carried out using conventional biochemical methods, including Gram staining, catalase testing, and Indole, Methyl Red, Voges-Proskauer, and Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) tests, alongside Sanger DNA sequencing. To characterize degradation activity, liquid natural latex was incubated with the isolated bacteria for seven days at 37 °C. The resulting molecular and physical changes were quantified using (Fourier transform infrared spectroscopy) FTIR spectroscopy, total solid content (TSC), and DRC analyses. A total of 10 distinct bacterial colonies exhibiting clearing zones on the latex overlay were selected for identification. Morphological and biochemical screening determined that six isolates were Gram-negative and four were Gram-positive, while all 10 isolates uniformly tested catalase-positive and indole-negative. Further identification of two out of ten bacterial isolates by Sanger sequencing identified as *Lysinibacillus xylanilyticus* and *Stenotrophomonas geniculata*. FTIR analysis verified successful latex degradation, displaying extra minor peaks of C-C and C-O stretching compared to the untreated negative control. Degradation was further validated by physical measurements, which resulted in reduced post-degradation TSC and DRC values of 20.64% and 16.61%, respectively. The study successfully isolated ten bacterial strains from raw latex and soil, and confirmed the specific latex-degrading capacities through comprehensive biochemical, molecular, and physical characterization.

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1. Introduction

The natural rubber industry has long been a vital part of the global economy due to its supply of renewable, biodegradable materials for a wide range of products. However, latex-degrading bacteria present both beneficial and detrimental effects on the natural rubber industry. They offer eco-friendly, sustainable solutions for the biodegradation of latex waste, potentially reducing pollution and waste disposal costs. However, their presence in manufacturing processes can adversely affect the quality, shelf-life, and composition of latex products, challenging the industry by reducing product quality, increasing production costs, and lowering productivity [1]. While numerous studies have identified prevalent bacterial species and their modes of action, a knowledge gap remains regarding the fundamental mechanisms of latex degradation and effective control measures [2]. Natural rubber latex contains approximately 90% cis-1,4-polyisoprene, with the remaining 10% comprising proteins, carbohydrates, and lipids [3]. Latex is extracted as a viscous fluid via cuts through the laticiferous system located between the cambium and outer bark of rubber-bearing trees. Natural rubber is a biopolymer found in over 2000 plant species and some fungi. Latex is a key material used in industrial products such as medical gloves and car tyres. Vulcanisation, a process involving sulphur cross-linking, enhances the elasticity and durability of rubber, making it suitable for 68% of car tyre production and 42% of general rubber goods. Natural rubber latex contains about 25-30% polyisoprene, 50-70% water, and small amounts of proteins, carbohydrates, lipids, and inorganic components [4]. Its molecular weight (Mw) reflects the average polyisoprene chain length. Due to its sticky, viscous, and temperature-sensitive nature, raw latex cannot be used directly in manufacturing but requires processing into durable rubber products [5]. Söhngen and Fol [6] demonstrated microbial assimilation of the hydrocarbon chains of rubber, with notable strains such as *Nocardia*, *Streptomyces*, and *Gordonia* isolated from soil, sewage, and deteriorating car tyres. These microorganisms metabolise rubber as their sole carbon source, often forming colonies that degrade rubber efficiently. Gram-positive bacteria in latex primarily include *Bacillus*, *Streptomyces*, *Lactococcus*, and *Micrococcus* [7] - [8]. Subsequent studies identified several microorganisms, particularly *Actinomycetes*, capable of degrading poly(cis-1,4-isoprene) in rubber [9]-[10]. This study aims to isolate, identify, and characterise latex-degrading bacteria to better understand their ability to degrade latex. Isolation was conducted using latex-overlay agar and streaking techniques, while bacterial identification involved Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) tests and Sanger sequencing. Characterisation of degradation activity utilised Fourier transform infrared spectroscopy (FTIR), total solid content (TSC), dry rubber content (DRC), and SEM analyses. This research seeks to address the challenges posed by latex-degrading bacteria while exploring their potential applications in sustainable waste management. Understanding these

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bacteria can contribute to innovative solutions for the natural rubber industry, ensuring its long-term viability and adaptability to global market demands.

2. Materials and Methods

2.1 Collection of Raw Latex

Fresh latex and soil samples were collected from the Bukit Kuantan Research Station, Malaysia Rubber Board. The samples were stored in sterilised 50 mL tubes at room temperature.

2.2 Isolation of Bacteria

Bacterial isolation was performed on latex overlay agar according to a modified protocol of [11]. Latex overlay agar was prepared by first allowing the nutrient agar to solidify at the bottom of a Petri dish, then covering it with agar supplemented with 0.2% (v/v) purified latex mixed with diluted latex. To prepare 150 mL of nutrient agar, 20 g of nutrient agar powder was added to a beaker, then filled with distilled water to a final volume of 1000 mL. The mixture was stirred well to prevent clumping and to enhance the solubility of the agar in the distilled water. The agar was then sterilised by autoclaving it at 121 °C for 20 minutes. After autoclaving, it was cooled in running water, then carefully poured into Petri dishes to avoid air bubbles. The dishes were covered with lids and allowed to solidify. The solidified agar plates were stored at 4 °C for future use. Next, a serial dilution of the latex was prepared by transferring a known volume of the latex sample into a tube labelled Tube 1. An appropriate amount of diluent was added to the tube to achieve the desired dilution ratio, and the mixture was thoroughly vortexed. A small aliquot of the first dilution was transferred to the second tube to prepare the second dilution, and this process was repeated to prepare subsequent dilutions. The diluted latex was mixed with nutrient agar supplemented with 2% (v/v) purified latex and poured as the top layer onto the latex overlay agar. The plates were then inverted and incubated at 37 °C for 3 days. Bacterial colonies were picked and streaked onto new nutrient agar plates to obtain single colonies, using the three-quadrant streaking method. The single colonies obtained were used for identification purposes.

2.3 Identification of Latex-Degrading Bacteria

Identification of latex-degrading bacteria was performed using conventional biochemical tests, including IMViC tests and Gram staining. Sanger DNA sequencing was used to confirm the identity of the isolated bacteria.

2.3.1 IMViC test

The IMViC tests and catalase test were performed according to [12]. The tryptophan broth, MR-VP broth and citrate agar slant were inoculated with bacterial isolates and incubated overnight at 37 °C. For the indole test, a few drops of Kovac's reagent were added to the tryptophan broth, and a positive result was indicated by a pink-red ring forming at the top of the solution. For the methyl red test, five drops of the methyl red indicator were added to the MR-VP broth, and a positive outcome was indicated by the development of a red colour. For the Voges-Proskauer test, 0.2 mL of 40% KOH and 0.6 mL of alpha-naphthol were added to the MR-VP broth, and the mixture was vortexed. A positive outcome was indicated by the development of a red colour. For the catalase test, 1–2 mL of hydrogen peroxide was poured into a test tube, and a few colonies of bacterial isolates were inoculated into it using a sterile glass rod. The production of air bubbles indicated a positive result.

2.3.2 Gram-staining

Gram staining was performed according to [13]. A pure culture's heat-fixed bacterial smear was prepared. After that, the smear was covered with crystal violet dye and left to stain for a minute. The crystal violet dye was removed with cold water after a minute. After that, Gram's iodine solution mordant was poured onto the microscope glass slide with the bacterial smear and allowed to sit for a minute. After that, safranin decolouriser was used to remove the mordant. After adding more safranin decolouriser and staining for 20 to 50 seconds, the mixture was rinsed with cold water. The slides were allowed to air-dry. A light microscope with 10× magnification was used to view the slide. After applying immersion oil, the slides were examined at a magnification of 100×. Gram-negative bacteria absorbed the pink colour and retained the counterstain, whereas Gram-positive bacteria were stained purple.

2.3.3 DNA extraction, quantification and quality assessment

Bacterial isolates were extracted, and the extracted genomic DNA was quantified according to [14]. The Dneasy® Blood & Tissue Kit was used to extract DNA in accordance with the manufacturer's instructions. Gel electrophoresis was used to assess the quality of genomic deoxyribonucleic acid (gDNA), and the Qubit 4 Fluorometer was used to quantify gDNA. DNA quality and size were assessed using agarose gel electrophoresis. If it exceeds 20 ng/µl, the DNA is deemed high quality and can proceed to the next phase. The gDNA was stored at –20 °C for extended periods.

2.3.4 Sanger DNA sequencing

Sanger DNA sequencing was used to verify the identity of the selected pure bacterial isolates. The extracted DNA were sent to Patriot Biotech Sdn. Bhd for DNA sequencing. The V3 region of the DNA is the target of the primers used in PCR. The sequencing results were compared with known sequences in the database using the Basic Local Alignment Search Tool (BLAST) on the National Centre for Biotechnology Information website.

2.4 Characterisation of degradation of latex by Latex-Degrading Bacteria isolates

Characterisation of latex degradation was performed using Fourier transform infrared spectroscopy, total solid content and dry rubber content.

2.4.1 Fourier transform infrared spectroscopy

The FTIR analysis of latex was performed according to [15]. Several steps were followed to analyse samples of latex-degrading bacteria using FTIR spectroscopy in Attenuated Total Reflection (ATR) mode. First, the FTIR was prepared and calibrated in ATR mode, ensuring that the ATR crystal surface was clean and free of contaminants. Next, a sample containing latex-degrading bacteria was carefully dropped onto the ATR crystal surface. The crystal was gently lowered to contact the sample droplet, applying slight pressure to spread the latex uniformly across the crystal surface. Before capturing the sample spectrum, the background signal was measured to account for any contributions from the crystal itself by scanning with the ATR crystal in the absence of the sample. Then, the FTIR spectrum was obtained using the ATR crystal in contact with the latex sample, with the spectral measurement spanning the designated infrared wavelength range. This measurement provided essential data for analysis by capturing the interaction between the latex sample and infrared light at the ATR crystal interface. The obtained spectrum was analysed using corresponding software and displayed as a graph. The graph, showing specific peaks or bands, was used to determine whether the latex-degrading bacteria cleaved the latex composition.

2.4.2 Total solid content

The total solid content of the latex was determined according to [16]. A 40 mL volume of latex was added to a 50 mL conical flask, which was sealed with a rubber stopper. The empty Petri dish and its cover were weighed, and the weight was recorded. Approximately 5 to 7 g of latex was transferred to the Petri dish, covered, and weighed again, with the weight recorded. The latex was spread evenly on the Petri dish and placed in an oven without a cover at 100°C for 2 to 3 hours or until it lost its whiteness. After drying, the Petri dish was removed from the oven and placed in a desiccator for a few minutes to cool. Then, the dried latex, along with the Petri dish's cover, was weighed, and the weight was recorded. The experiment was performed in duplicate.

2.4.3 Dry rubber content

The dry rubber content of the latex was determined as described in [17]. An empty Petri dish was weighed. A few drops of collected latex were added to the Petri dish, and the weight was measured and recorded. A 1% (v/v) acetic alcohol solution, composed of 1% (v/v) acetic acid and 1% (v/v) ethyl alcohol, was carefully poured into the Petri dish to surround the latex, allowing it to coagulate. After a set period, the latex coagulated to form a flattened coagulum. The coagulum was gently pressed with a flat-bottomed glass stopper, taking extra care to avoid tearing or overlapping. The acid mixture was then discarded, and the sample was thoroughly rinsed with distilled water. The sample was dried in a steam bath for 15 minutes, resulting in a clear, spot-free sample. The sample was immediately placed in an oven at 70°C for 30 minutes, then cooled in a desiccator for 5 minutes. The sample was weighed along with the Petri dish, and the weight was recorded. The experiment was performed in duplicate.

3. Results and Discussion

Fresh latex and soil samples collected from the Bukit Kuantan Research Station of the Malaysian Rubber Board were used to screen for latex-degrading bacteria on latex-overlay agar plates. A total of 10 bacterial colonies with distinct morphological traits and clearing zones on the latex overlay were selected for pure culture. Tables 1 and 2 show the colony and bacterial morphology of the bacterial isolates. All bacteria have entire colony margins, with variations in colour, shape, and elevation. Bacterial isolates 1, 2, 4, 6, 7, and 8 were Gram-negative, while bacterial isolates 3, 5, 9, and 10 were Gram-positive. Bacterial isolates 1, 3, 4, 8, 9, and 10 exhibited bacillus morphology, whereas bacterial isolates 2, 5, 6, and 7 exhibited coccus morphology.

Table 1. The colony morphology of bacterial isolates

Bacteria Isolates	Colony Size	Colony Form	Colony Margin	Colony Elevation	Colony Colour
1	Moderate	Irregular	Entire	Raised	Yellowish
2	Moderate	Round	Entire	Convex	Creamy Yellowish
3	Punctiform	Punctiform	Entire	Flat	Pinky Red
4	Large	Irregular	Entire	Flat	Creamy White
5	Moderate	Round	Entire	Raised	Creamy White
6	Moderate	Round	Entire	Raised	Yellow
7	Large	Irregular	Entire	Raised	White
8	Punctiform	Round	Entire	Convex	Yellow
9	Moderate	Round	Entire	Flat	Pinky Red
10	Punctiform	Round	Entire	Raised	Pink

Biochemical tests revealed uniform negative indole test results across all isolates. Bacteria 5, 8, and 9 tested negative for both Voges-Proskauer (VP) and Methyl Red (MR), while only Bacteria 2 tested positive for VP. All isolates were

catalase-positive (Table 3). Two distinct isolates (1 and 2) were selected for Sanger sequencing and identified as *Lysinibacillus xylanilyticus*, and *Stenotrophomonas geniculata*, respectively. Colonies have complete margins, are round, opaque, and dark yellow in colour. Other than that, Lysinibacillus sp. has the remarkable ability to break down low-density polyethene (LDPE). *Lysinibacillus xylanilyticus* is closely related to *Bacillus* sp. [18]. *Lysinibacillus xylanilyticus* can degrade xylan and polyethene. Gram-negative *Stenotrophomonas geniculata* is closely related to *Stenotrophomonas* species, which are related to *Xanthomonas* sp. It has been identified as an antifungal biocontrol bacterium and is well known for its ability to stimulate plant growth [19]. To determine the degradation activity of the bacterial isolates, the liquid natural latex was incubated with the isolates for 7 days at 37 °C, and the degradation activity was characterised using FTIR, DSC, and TSC. The molecular changes in the latex were quantified by using FTIR. The FTIR spectrum analysis of the natural latex negative control after 168 hours without bacterial degradation is displayed in Figure 1. The FTIR spectra of the natural latex after 168 hours of bacterial degradation by bacterial isolates 1 and 2 are shown in Figures 3 and 4, respectively.

Table 2. Gram-staining results of bacterial isolates

Bacteria Isolates	Gram Staining	Morphology (1000× magnification)
1	-	Bacillus
2	-	Cocci
3	+	Bacillus
4	-	Bacillus
5	+	Cocci
6	-	Cocci
7	-	Cocci
8	-	Bacillus
9	+	Bacillus
10	+	Bacillus

Table 3. Summary of biochemical tests results

Bacteria Isolates	Indole test	MR test	VP test	Catalase test
1	-	+	-	+
2	-	+	+	+
3	-	+	-	+
4	-	+	-	+
5	-	-	-	+
6	-	+	-	+
7	-	+	-	+
8	-	-	-	+
9	-	-	-	+
10	-	+	-	+

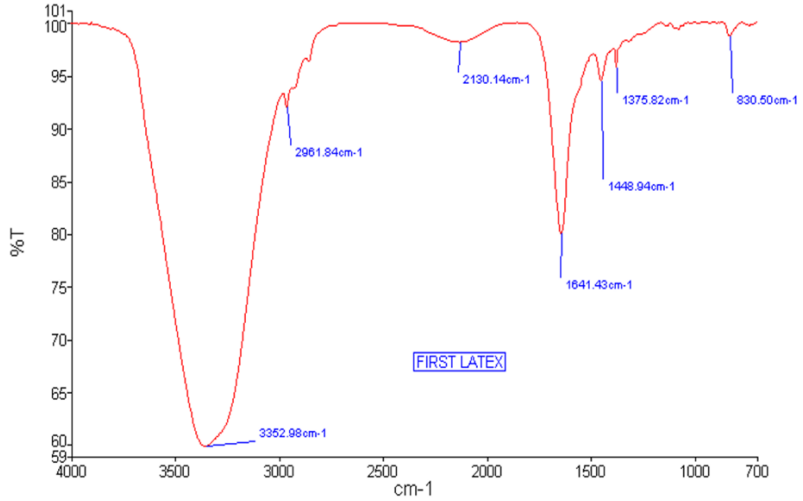

Figure 1. FTIR analysis of the negative control of liquid natural latex

The presence of O-H group stretching was indicated by several prominent peaks that showed a strong and broad appearance in the absorption range of 3550 to 3200 $cm{-}1$ and 3200 to 2700 $cm{-}1$. In substances that were mostly from

the alcohol class, these unique characteristics were suggestive of intermolecular bonding. This is because ammonia, which is alkaline, was used to treat the fresh liquid latex samples to prevent them from coagulating [20]. Furthermore, all latex samples showed consistent medium-intensity peaks around 1639 $cm{-}1$, indicating the presence of either N-H bending or C=C group stretching. While N-H bending was characteristic of amine compounds, C=C group stretching could happen in compounds with conjugated alkene or cyclic alkene structures. The FTIR spectrum showed the distinctive unsaturated C=C bond of natural rubber, particularly in the cis position, while the NH group could have come from proteins in the raw latex or from the ammonia preservatives used to prolong the shelf life of the liquid raw latex. Additionally, the FTIR analysis revealed that the structure of raw latex contains C-H bending and C-O stretching, as evidenced by the recognised FTIR spectrum. Nonetheless, a faint peak was observed at 2140 to 2100 cm-1, indicating the presence of 2140 to 2100 $cm{-}1$. These characteristics suggested the existence of monosubstituted compounds, primarily those in the alkyne class. Compared with the negative control, the FTIR spectra of latex degraded by bacterial isolate 1 show additional minor peaks corresponding to C=C and C=O stretching. This shows that the raw latex was degraded by the bacteria. The DRC of freshly tapped natural rubber latex typically ranges from 25 to 35%. Numerous factors, including the climate and the timing and intensity of tapping, affect this variation. Typically, the TSC number is greater than the DRC number. This experiment also showed that the TSC number exceeds the DRC number. Since latex-degrading bacteria are present and break down latex, the TSC and DRC numbers should be lower than those of normal fresh latex. The TSC and DRC values for bacterial degradation were 20.64% and 16.61%, respectively, supporting that the latex was degraded by the bacteria.

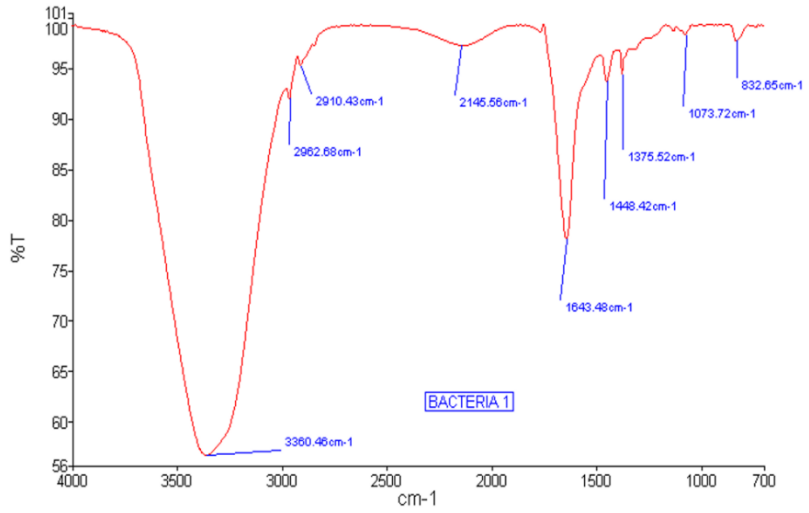

Figure 2. FTIR analysis of natural latex incubated with Isolate 1

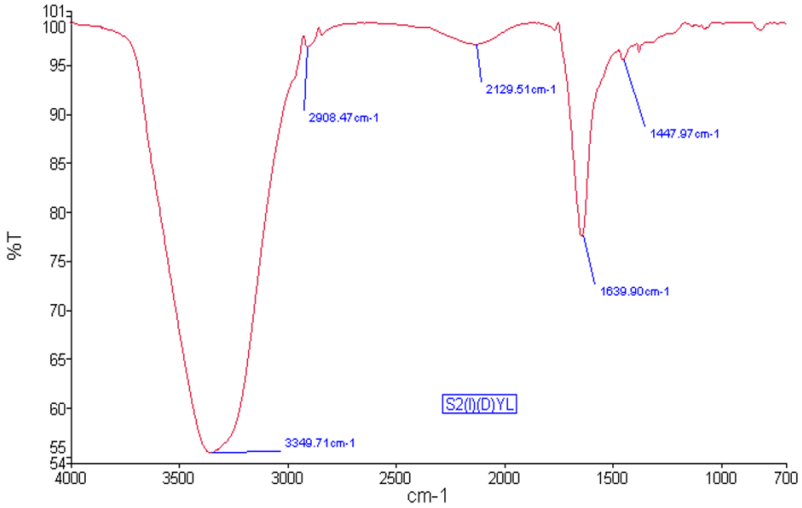

Figure 3. FTIR analysis of natural latex incubated with Isolate 2

4. Conclusions

This study successfully isolated, identified, and characterised 2 distinct bacterial strains capable of degrading natural rubber latex, utilising raw latex and soil samples collected from the Bukit Kuantan Research Station of the Malaysia Rubber Board. Through comprehensive morphological and biochemical screening, the ten isolates were categorised as six Gram-negative and four Gram-positive strains, all of which proved to be catalase-positive and indole-negative. Two of the 10 isolates were selected to be identified using Sanger DNA sequencing, and the isolates were identified as

Lysinibacillus xylanilyticus and *Stenotrophomonas geniculata*. The latex-degrading ability of these 2 isolated bacteria was conclusively validated by FTIR spectroscopy, TSC and DRC. The molecular alterations in the latex were confirmed by FTIR spectroscopy, which revealed additional minor peaks corresponding to C=C and C=O stretching in the degraded samples compared with the untreated negative control. Furthermore, physical degradation was substantiated by significant reductions in TSC and DRC, which decreased to 20.64% and 16.61%, respectively. The findings of this study carry a significant dual impact on the global natural rubber industry. On the one hand, identifying the specific activities of these latex-degrading bacteria offers a promising pathway for eco-friendly, sustainable waste biodegradation solutions. The use of bacteria with latex-degrading capabilities can help mitigate environmental pollution and significantly reduce latex waste disposal costs. Conversely, the isolation of these bacteria directly from raw latex underscores a critical operational challenge for manufacturers. The unchecked presence of these microbes threatens manufacturing productivity by compromising product shelf life, altering latex composition, and ultimately increasing production costs. To bridge existing knowledge gaps and ensure the long-term viability of the rubber industry, the latex-degradation mechanisms by the bacteria need to be elucidated to identify the specific enzymatic pathways utilised by *Lysinibacillus xylanilyticus* and *Stenotrophomonas geniculata* to cleave polyisoprene chains. Future studies should optimise the cultivation of latex-degrading bacteria in controlled bioreactor environments to adapt their degradation capabilities for industrial-scale rubber waste management.

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Declaration of Competing Interests
The authors declare no conflicts of interest.

CRediT Author Contribution Statement
K.N. Chin : Formal analysis; Data curation; Investigation; and Writing – original draft; Resources.
N.S. Adzahar: Formal analysis; Data curation; Investigation; and Writing – original draft; Resources.
Z. Roji: Conceptualisation; Resources
T.C. Lee: Conceptualisation; Formal analysis; Visualisation; Supervision; and Writing – original draft; Resources.

Availability of Data and Materials
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Statement
This study did not involve human participants or animal subjects. Ethical approval was therefore not required for this research.

Generative Artificial Intelligence Disclosure
AI-based tools were used solely for language editing and grammar refinement. No AI content generation or data interpretation was performed. All intellectual contributions are original and solely attributable to the authors.

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