Jewel Journal of Scientific Research (JJSR) 10(2): 317–324, 2025
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
https://journals.fukashere.edu.ng/index.php/jjsr

Effects of Substrate, pH and Yeast Extract on Bacterial Growth in *Citrullus lanatus* Peel–Based Media

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Abstract
The search for cost-effective and sustainable microbial growth media has prompted interest in fruit peel–based substrates. This study investigated the growth of *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* on *Citrullus lanatus* (watermelon) peel–derived media, by examining the effects of substrate concentration, pH and yeast extract supplementation. Bacterial cultures were grown across a range of substrate concentrations (1–6%), pH values (6.5–7.2), and yeast extract levels (0–0.5%), and growth was measured in log CFU/ml. Optimal growth occurred at 5% substrate, near-neutral pH, and 0.2–0.4% yeast extract, with *B. subtilis* showing the highest proliferation. Growth declined slightly at 6% substrate concentration, and species-specific responses to yeast extract were observed. These findings indicate that *Citrullus lanatus* (watermelon) peel–derived media can effectively support robust bacterial growth hence, providing a sustainable and cost-effective alternative for industrial fermentation, probiotic production, bioremediation, and the valorization of agricultural waste.

Keywords: Fruit peel–based media; *Citrullus lanatus*; bacterial growth; substrate concentration; pH; yeast extract supplementation; sustainable culture media; agro-waste valorization.
Received: 7th Sept, 2025 *Accepted:* 21th Nov, 2025 *Published Online:* 26th Dec, 2025

Introduction

The rising cost and limited availability of conventional microbiological culture media have stimulated interest in alternative substrates derived from agricultural residues (Jadhav *et al*., 2018; Vargas *et al*., 2024). Fruit peels constitute a major fraction of fruit-processing waste and are abundantly available in many regions. They are rich in carbohydrates, minerals, and organic compounds, making them suitable for supporting microbial growth, as well as making them attractive candidates for formulating low-cost culture media (Sagar *et al*., 2018).

Several studies have demonstrated the successful use of plant-derived substrates as alternative nutrient sources for microbial cultivation. Agricultural residues such as fruit peels, vegetable wastes, and other plant-based by-products have been widely investigated because their rich carbohydrate, mineral, and organic nutrient content can support microbial growth and partially substitute conventional culture media (Ajila *et al*., 2012; Ravindran & Jaiswal, 2016; Sagar *et al*., 2018; Vargas *et al*., 2024). Among these substrates, fruit peels have attracted considerable interest because they are abundant and contain a wide range of nutrients. In particular, *Citrullus lanatus* (watermelon) peel has recently gained attention as a promising material for the formulation of alternative microbial culture media (Hasanin and Hashem, 2020; Nirmala *et al*., 2025)

However, while the capacity of these media to support growth is established, there is limited information on how variations in formulation

such as substrate concentration, pH, and supplementation affect performance across different bacterial isolates. Therefore, systematic evaluation of these factors is essential for optimizing media performance. Optimizing substrate concentration, adjusting pH or supplementing with growth-promoting additives such as yeast extract can enhance bacterial proliferation and improve consistency outcomes across different isolates. Accordingly, this study aimed to evaluate the effects of these factors on the growth of some selected isolates in watermelon peel–based media, providing practical insights into the development of sustainable, low-cost alternatives to conventional culture substrates.

Materials and Methods

Bacterial Isolates

Three bacterial isolates were used in this study: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. These isolates were selected based on their superior growth performance in a previous investigation evaluating ten bacterial isolates on fruit peel-based media (banana, orange, watermelon, pineapple) individually and in combinations. Stock cultures were maintained on Nutrient Agar (NA) slants at 4°C (Cheesbrough, 2006).

Preparation of Fruit Peel–Based Media

Watermelon peels were thoroughly washed, dried in an oven at 40˚C, and milled into fine powder using a laboratory grinder. The powders were extracted by boiling in distilled water to obtain aqueous extracts, which were subsequently sieved to remove insoluble residues (Jadhav *et al*., 2018). Agar was added as a solidifying agent, and the media were sterilized by autoclaving at 121 °C for 15 min.

Standardization of Bacterial Inoculum

Each bacterial isolate was subcultured on nutrient agar and incubated at 37 °C for 18–24 h. Well-isolated colonies were aseptically transferred into sterile normal saline and homogenized to obtain uniform suspensions. The turbidity of each suspension was adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL (Cheesbrough, 2006). The standardized inocula were further diluted with sterile normal saline to obtain a final concentration of 1.0×10^6 CFU/mL for consistent inoculation and reliable colony enumeration.

Effect of Substrate Concentration

The effect of substrate concentration was evaluated by preparing watermelon peel basal medium at concentrations: of 1%, 2%, 3%, 4%, 5% and 6% (w/v). Nutrient Agar without additional substrate served as the control. Media were sterilized by autoclaving at 121 °C for 15 minutes, poured into petri dishes pre-inoculated with the standardized bacterial suspensions, and gently swirled to ensure even distribution. Plates were incubated at 37 °C for 24 hours, after which bacterial growth was quantified as log colony-forming units per milliliter (log CFU/mL).

Effect of pH

The effect of pH on bacterial growth was evaluated by adjusting the pH (range 6.5 to 7.2) of watermelon peel media prior to sterilization using sterile 1 M HCl or 1 M NaOH (Park, Chua, & Lee, 2023). The pH was verified using a calibrated pH meter. After autoclaving, media were dispensed into pre inoculated petri dishes and incubated at 37°C. Growth responses were assessed across the different pH conditions and quantified as log colony-forming units per milliliter (log CFU/mL) respectively.

Effect of Yeast Extract Supplementation

The effect of yeast extract concentration was evaluated by supplementing watermelon peel medium with varying concentrations of yeast extract: 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%. Nutrient Agar served as the control, and watermelon peel agar without supplement served as the negative control. Media were sterilized, poured, into pre inoculated petri dishes, and incubated at 37°C. (Cheesbrough, 2006).

Growth Assessment and Statistical Analysis

Bacterial growth was assessed based on colony counts and expressed as log colony-forming units per milliliter (Log CFU/mL). All experiments were conducted in triplicate, and results were expressed as mean ± standard error. Statistical analysis was performed using

two-way analysis of variance (ANOVA), with significance set at $p < 0.05$.

Results

The growth response of the three bacterial isolates to varying substrate concentrations, pH levels, and yeast extract supplementation is summarized in Figure 1–3.

Effect of substrate concentration

All bacterial isolates exhibited a progressive increase in growth as substrate concentration increased from 1% to 5%, reaching near-maximum levels at 5% before slightly declining at 6%. *B. subtilis* consistently showed the highest growth across all concentrations, achieving 7.96 ± 0.00 log CFU/ml at 5%, which was significantly higher than the other isolates ($p < 0.05$). *P. aeruginosa* and *S. aureus* displayed similar growth patterns, with peak values of 7.91 ± 0.01 log CFU/ml and 7.90 ± 0.01 log CFU/ml, respectively, also at 5% substrate concentration. Two-way ANOVA revealed that both media concentration and bacterial isolate, as well as their interaction, had significant effects on growth ($P < 0.0001$). Among these, the bacterial isolates were the most dominant source of variation ($F(5, 36) = 3129$), followed by media concentration ($F(2, 36) = 252.5$), while the significant interaction between isolates and concentration ($F(10, 36) = 44.32$) indicated that the response to substrate levels varied depending on the specific isolate.

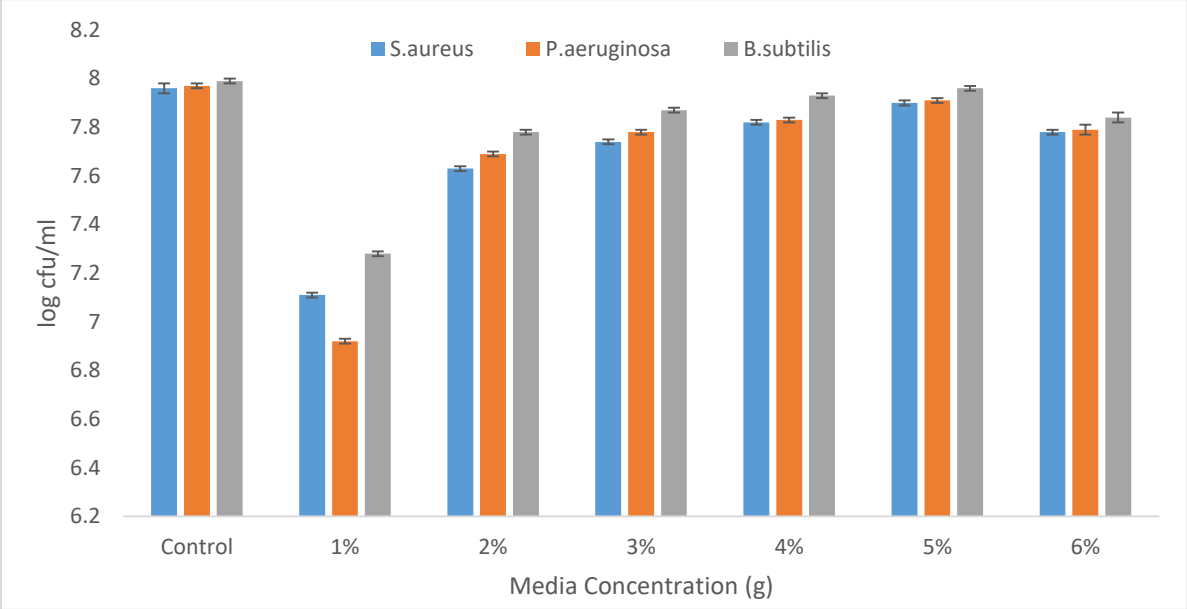

Figure 1. Effect of varying media concentrations on bacterial growth (mean log CFU/mL ± SEM, n = 3)

Effect of pH

All bacterial isolates exhibited robust growth across the tested pH range, with *B. subtilis* maintaining the highest overall growth, reaching 7.99 ± 0.00 log CFU/ml in the control. *S. aureus* and *P. aeruginosa* achieved 7.96 ± 0.00 and 7.97 ± 0.01 log CFU/ml, respectively. Statistical analysis showed that *B. subtilis* consistently outperformed the other isolates across all pH conditions tested ($p < 0.05$). Two-way ANOVA indicated that pH was the primary factor influencing bacterial growth ($F(6, 42)$, $p < 0.0001$), while the type of isolate also contributed significantly to the observed variation ($F(2, 42) = 248.0$, $p < 0.0001$).

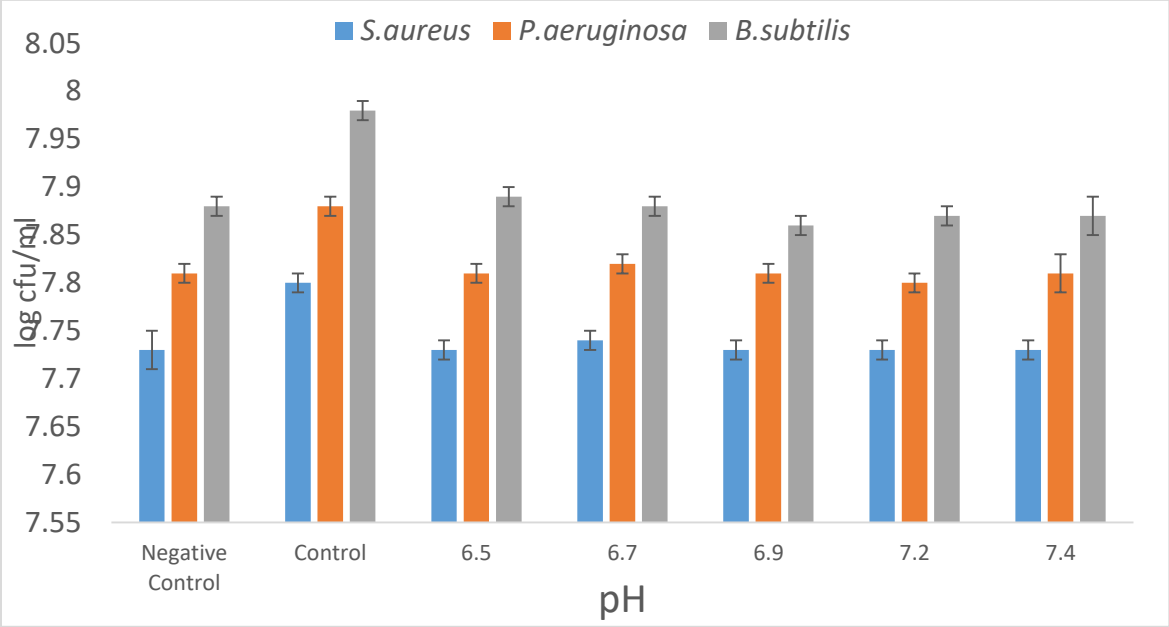

Figure 2: Effect of varying pH on bacterial growth (mean log CFU/mL ± SEM, n = 3)

Effect of Yeast Extract Supplementation

All isolates exhibited enhanced growth with increasing yeast extract concentration, reaching optimal responses that varied by isolate. *B. subtilis* achieved its maximum growth at 0.4% yeast extract (8.00 ± 0.02 log CFU/ml), significantly higher than the control (7.86 ± 0.01 log CFU/ml). *P. aeruginosa* showed peak growth at 0.5% (7.96 ± 0.01 log CFU/ml), while *S. aureus* reached its maximum at 0.4% (7.94 ± 0.01 log CFU/ml). Two-way ANOVA revealed that yeast extract concentration was the most influential factor, accounting for 60.14% of the total variation ($F(6, 42) = 1468$, $p < 0.0001$), with isolate type contributing 18.11% ($F(2, 42) = 1326$, $p < 0.0001$). The interaction between isolate type and yeast extract concentration explained 21.46% of the variation ($F(12, 42) = 261.9$, $p < 0.0001$), indicating that isolates responded differently to supplementation. While yeast extract generally promoted microbial growth, the magnitude of its effect depended on both the nutrient concentration and the specific bacterial isolate.

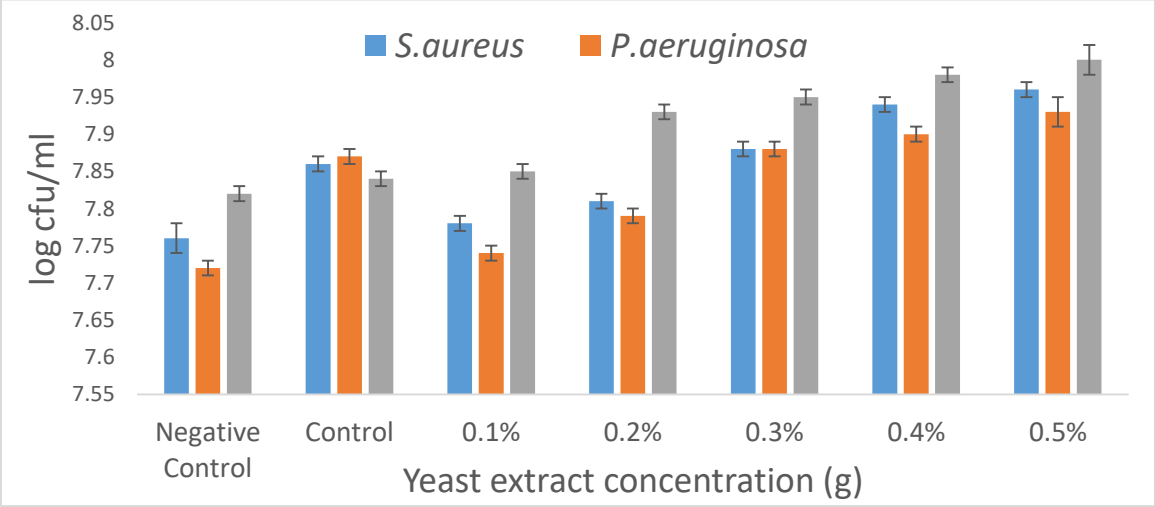

Figure 3: Effect of Yeast extract in varying concentrations on bacterial growth (mean log CFU/mL ± SEM, n = 3)

Discussion

Substrate concentration emerged as the principal determinant of bacterial growth in the watermelon peel–based media, with a clear concentration-dependent response observed across all isolates. Growth increased progressively up to 5% substrate, after which a slight but consistent decline occurred at 6%. This pattern reflects fundamental principles of bacterial growth kinetics. In accordance with the Monod model, microbial growth rate increases with substrate availability until an optimal threshold is approached, beyond which additional substrate does not proportionally enhance growth and may instead exert inhibitory effects (Monod, 1949). The increase up to 5% suggests efficient nutrient assimilation within this range, whereas the reduction at 6% indicates the onset of substrate inhibition. At lower concentrations, growth was likely constrained by limited availability of utilizable carbon and associated nutrients. Conversely, elevated substrate levels may have imposed osmotic stress, disrupting cellular homeostasis and metabolic efficiency (Csonka, 1989; Zheng *et al*., 2024). High concentrations may also alter enzymatic activity through regulatory or kinetic constraints, thereby affecting metabolic flux (Kovarova-Kovar & Egli, 1998). The consistent decline observed at 6% across *B. subtilis*, *P. aeruginosa* and *S. aureus* suggests that this concentration approaches a general physiological threshold rather than a species-specific limitation. Overall, these findings emphasize the need to carefully optimize substrate concentration so that sufficient nutrients are available without creating conditions that may inhibit microbial growth in plant-derived media. Among the isolates tested, *B. subtilis* consistently showed higher growth across the different concentrations. This may be attributed to its well-known metabolic versatility and strong ability to adapt to environmental stress (Zhao & Lin, 2024). As a soil-associated organism, *B. subtilis* possesses extensive regulatory networks and secretes extracellular hydrolytic enzymes that facilitate the breakdown of complex organic substrates (Priest, 1977; Sonenshein, 2007; Akinsemolu *et al*., 2024). These physiological traits likely enhance its ability to efficiently utilize nutrients present in fruit peel–based media, thereby supporting improved growth performance compared with the other isolates.

pH also influenced bacterial growth performance, with near-neutral conditions generally supporting more consistent proliferation than acidic or alkaline environments. All three isolates maintained robust growth across the tested range, with minimal variation, indicating that standard laboratory pH conditions (approximately 7.0) are physiologically suitable. This observation is consistent with the ecological adaptability of *S. aureus*, which colonizes human skin and mucosal surfaces characterized by mildly acidic to near-neutral pH (Tong *et al*., 2015); *P. aeruginosa*, noted for its broad environmental versatility across soil, water, and host-associated niches (Moradali *et al*., 2017); and *B. subtilis*, a soil-dwelling organism routinely exposed to fluctuating physicochemical conditions, including variable pH (Kovács, 2019). The ability of these isolates to maintain stable growth across the tested pH range reflects efficient pH homeostasis mechanisms. Bacteria regulate intracellular pH through proton-translocating ATPases, modulation of membrane permeability, and cytoplasmic buffering systems, thereby preserving enzymatic functionality and metabolic balance (Padan *et al*., 2005). The limited variation observed suggests that these regulatory systems were not physiologically challenged within the experimental range. These findings carry practical relevance for watermelon peel–based media formulation. Because watermelon peel extracts contain natural organic acids (e.g., citric, malic, succinic), which contribute to their acidic character, unadjusted media may inadvertently restrict bacterial proliferation despite adequate nutrient availability (Krajewska *et al*., 2025). However, the demonstrated tolerance near neutrality indicates that maintaining standard laboratory pH conditions is sufficient to support optimal growth, potentially reducing the need for stringent pH control in applied or industrial settings.

Yeast extract supplementation markedly enhanced bacterial growth across the tested media, likely due to the provision of readily assimilable amino acids, vitamins, and growth factors that are partially deficient in plant-derived substrates. The three isolates, however, exhibited distinct responses. *B. subtilis* showed the major increase, rising from 7.85 to 8.00 log CFU/ml at the optimal concentration which is a modest 0.15 log increase that corresponds to a ~1.4-fold rise in viable cells. Although *B. subtilis* can synthesize most amino acids, the additional peptides and vitamins in yeast extract reduce the metabolic burden of de novo biosynthesis (Sonenshein, 2007; Tao *et al*., 2023). *P. aeruginosa*, a metabolic generalist, also benefited, reaching maximal growth at 0.5% yeast extract, reflecting efficient utilization of exogenous amino acids and iron-containing nutrients (Palmer *et al*., 2007; Wijesinghe *et al*., 2019). In contrast, *S. aureus* displayed a narrower response and even decreased growth at 0.1% supplementation, consistent with its auxotrophic requirements for certain amino acids and vitamins, which make it sensitive to metabolic imbalances at suboptimal levels (Krishnan *et al*., 2022; Hu *et al*., 2025).

The substantial interaction effect (21.46% of variation) underscores that the optimal yeast extract concentration is species-specific: 0.4% for *B. subtilis* and *S. aureus* and 0.5% for *P. aeruginosa*. This finding highlights that "one-size-fits-all" nitrogen supplementation strategies may underperform when applied to mixed cultures or when maximizing yields for particular organisms.

These conditions have important implications for industrial fermentation, where improved biomass can enhance the yield of enzymes, bioplastics and other microbial metabolites. They are also relevant for probiotic production, as optimized media can help achieve higher viable cell counts required for therapeutic applications. In addition, such conditions support bioremediation efforts by promoting the growth of degradative bacteria capable of utilizing waste substrates. Furthermore, they contribute to fruit peel valorization by enabling the development of cost-effective, nutrient-rich culture media derived from agricultural by-products.

Conclusion

Watermelon peel–based media effectively supported the growth of *B. subtilis*, *P. aeruginosa* and *S. aureus* when optimized for substrate concentration (5%), near-neutral pH, and low yeast extract supplementation (0.2–0.4%). Among the tested isolates, *B. subtilis* exhibited the strongest growth response, likely reflecting its metabolic versatility and ability to utilize complex plant-derived substrates. These findings demonstrate the feasibility of fruit peel–derived media as a low-cost and sustainable alternative to conventional culture media, with potential applications in industrial fermentation, probiotic production, bioremediation and the valorization of agricultural waste.

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