



Original Research Paper

Isolation and Evaluation of Biofilm-Forming Bacteria from Frequently Used Food-Contact Surfaces in a Food Microbiology Laboratory

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Abstract

Bacteria that form biofilms on frequently touched surfaces pose a persistent problem for food-handling and laboratory environments, since cells embedded within a self-produced polymeric matrix resist routine cleaning far more effectively than their free-floating counterparts. This study set out to isolate culturable bacteria from three heavily used surfaces in a food microbiology teaching laboratory a sink, a laboratory bench/platform, and a laminar air-flow (LAF) cabinet work surface and to screen the resulting isolates for biofilm-forming ability using simple, low-cost microbiological methods. Swabs collected from all three surfaces were enriched in nutrient broth and plated onto Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar and Potato Dextrose Agar. For each surface, one representative isolate was purified from each of Nutrient Agar (NA), MacConkey Agar (MAC) and Eosin Methylene Blue Agar (EMB) through two successive rounds of sub-culturing, giving nine surface-medium isolates in total, before being screened for biofilm formation using the crystal violet tube assay in triplicate ($n = 3$ independent tubes per isolate), with absorbance measured at 570 nm. All nine isolates produced a visible, stained film adherent to the glass wall after 48 hours, confirming that each was capable of forming a biofilm. Biomass was consistently highest for MAC isolates and EMB isolates and lowest for NA isolates at every surface, and sink-derived isolates produced more biofilm than bench/platform-derived isolates, which in turn exceeded laminar-cabinet-derived isolates, at every medium (mean OD₅₇₀ range 0.12–0.54 across the nine isolates). A one-way ANOVA confirmed that differences among the nine surface-medium groups were statistically significant ($F(8,18) = 2435.3$, $p < 0.001$). Taken together, these results show that biofilm-competent bacteria can be readily recovered from surfaces that undergo routine cleaning, that biofilm-forming capacity varies systematically by both isolation medium and originating surface, and that isolates from the same environment can differ substantially in how much biofilm they produce. Because isolates were not identified beyond their isolation medium and surface of origin, and no uninoculated negative-control tube was included to establish a validated optical-density cut-off, the findings should be read as a preliminary, though now replicated, screen rather than a definitive characterization; they nonetheless support the case for including biofilm-specific checks alongside conventional hygiene monitoring in food-handling and teaching laboratories.

Keywords: biofilm; crystal violet assay; food-contact surfaces; cross-contamination; surface associated bacteria; laboratory hygiene.

1. Introduction

Most discussions of food contamination focus on the food itself, yet a considerable share of contamination events can be traced instead to the surfaces that food, hands and equipment repeatedly come into contact with. Sinks, benches, cutting boards, taps and shared utensils can retain microorganisms even after cleaning, and these residual populations are then able to move back onto food, equipment or personnel, closing a contamination loop that a routine visual inspection is unlikely to catch [1,2]. Cross-contamination of this kind has been linked to numerous food-borne illness outbreaks and remains a persistent problem for the food industry, partly because the organisms responsible are often not simply suspended free in solution but organized into surface-attached, matrix-encased communities known as biofilms

[1–3].

Biofilm formation begins when planktonic bacteria attach to a surface, first reversibly and then irreversibly, before proliferating and secreting a matrix of extracellular polymeric substances (EPS) around the growing microcolony. This matrix, built mainly from polysaccharides, proteins, extracellular DNA and lipids, can account for anywhere between half and nearly all of a mature biofilm's organic mass, and it is largely what gives the community its mechanical stability and cohesion [4,5]. Rather than following one fixed sequence, biofilm development is now understood as a life cycle in which cells move between sessile and planktonic states depending on nutrient availability, shear forces and chemical signalling within the population (Figure 1) [5]. Once established, biofilm-embedded cells tolerate

desiccation, disinfectants and physical scrubbing far better than free-living cells do, which is precisely what allows them to survive repeated cleaning cycles and persist as a standing source of contamination [1,3,6,7].

Figure 1. Precise mechanism of biofilm formation on food-contact surfaces

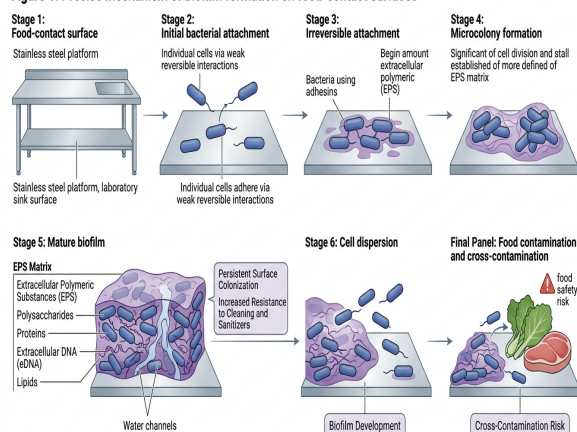


Figure 1. Precise mechanism of biofilm formation on a food-contact surface: initial reversible attachment of planktonic cells, irreversible attachment, EPS secretion, microcolony formation, maturation into a structured biofilm with water channels, and dispersion of cells to re-colonize new surfaces.

The food-safety consequences of this are well documented. Species of *Listeria*, *Salmonella*, *Escherichia* and *Staphylococcus* are all capable of forming biofilms on stainless steel, plastic, rubber and glass, materials common to almost any food-handling setting, and biofilm-associated strains of these organisms have repeatedly been linked to contamination events that persisted despite routine sanitation [3,6,8,9]. The literature on this subject points to a fairly consistent set of contributing factors, including surface material and roughness, moisture, nutrient availability, temperature, and how thoroughly and how often a given surface is cleaned [2,6,9,10].

Food microbiology teaching and diagnostic laboratories are not usually discussed alongside industrial food-processing facilities, but they share several of the same conditions: sinks, benches and shared equipment that are touched by multiple people throughout the day, exposed to moisture and organic residue, and cleaned only intermittently between sessions. Whether bacteria recovered from surfaces in this kind of academic setting are themselves capable of forming biofilms is a question that has received comparatively little direct attention. One accessible way to screen for this is the crystal violet tube or microtiter assay, in which biomass retained by a static or semi-static broth culture is stained, the bound dye is then solubilized, and the resulting absorbance serves as an indirect proxy for total biofilm mass. First described for staphylococcal adherence and later adapted across a much broader range of bacterial taxa, it remains a standard first-line phenotypic screen in both clinical and food microbiology [11,12].

This study had two specific aims: first, to isolate and

purify culturable bacteria from three frequently used surfaces in a food microbiology teaching laboratory (a sink, a laboratory bench/platform, and a laminar air-flow cabinet work surface), using standard selective and non-selective media; and second, to screen the resulting isolates, both qualitatively and quantitatively, for biofilm-forming capacity using the crystal violet tube assay in triplicate. The intention is to provide baseline evidence on biofilm-competent bacteria in this type of setting, to compare biofilm-forming capacity across surfaces as well as isolation media, and to help identify which surfaces and isolates would be worth pursuing in a larger study incorporating molecular identification and a validated biofilm-classification framework.

2. Materials and Methods

2.1 Study Site

This study was carried out in the Food Microbiology Laboratory, Department of Food Technology, Loyola Academy Degree and PG College, Secunderabad, Telangana, India. All isolation, purification and biofilm assay procedures followed aseptic technique under standard laboratory practice. Since the work involved only environmental surface sampling and no human or animal subjects, formal ethical clearance was not required.

2.2 Study Design

This was a laboratory-based, descriptive, preliminary study designed to isolate culturable bacteria from commonly touched laboratory surfaces and assess their biofilm-forming potential using conventional microbiological methods. Environmental swab samples were collected aseptically, enriched, cultured on selective and non-selective media, purified through two successive rounds of sub-culturing, and evaluated for biofilm formation using the crystal violet tube assay.

2.3 Sample Collection

Three food-contact surfaces within the Food Microbiology Laboratory were sampled: a sink surface (Sample 1), a laboratory bench/platform (Sample 2), and the working surface of a laminar air-flow (LAF) cabinet (Sample 3). Each surface was sampled with a sterile cotton swab moistened in sterile physiological saline (0.85% w/v NaCl), rubbed over an area of approximately 10 cm × 10 cm using horizontal, vertical and diagonal strokes to maximize microbial recovery, and transferred immediately into a separate, labelled sterile test tube containing 10 mL of sterile nutrient broth for enrichment and transport.

2.4 Preparation of Physiological Saline

Sterile physiological saline (0.85% w/v NaCl) was prepared by dissolving 8.5 g of sodium chloride in 1000 mL of distilled water, dispensing the solution into sterile containers, and autoclaving at 121 °C for 15 minutes. This saline was used throughout the study for sample collection and for preparing bacterial suspensions during sub-culturing.

2.5 Preparation of Culture Media

Four culture media were prepared according to the manufacturers' instructions for isolating and differentiating environmental microorganisms: Nutrient

Agar (NA), a general-purpose medium for a broad range of bacteria; MacConkey Agar (MAC), a selective medium for Gram-negative enteric bacteria that differentiates lactose fermenters; Eosin Methylene Blue Agar (EMB), a selective and differential medium for Gram-negative bacteria; and Potato Dextrose Agar (PDA), for isolating yeasts and filamentous fungi. Each dehydrated medium was suspended in distilled water, heated to complete dissolution, and sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Molten media were cooled to approximately 45–50 °C before being aseptically poured into sterile Petri plates within a laminar air-flow cabinet, then left to solidify and stored under refrigeration until use.

2.6 Isolation of Microorganisms

After enrichment in nutrient broth, each of the three samples was streaked aseptically onto NA, MAC, EMB and PDA using the quadrant streak technique to obtain well-separated colonies, giving twelve plates in total (four media × three surfaces), with Sample 1 (sink), Sample 2 (bench/platform) and Sample 3 (laminar cabinet) inoculated separately onto each medium. Inoculated NA, MAC and EMB plates were incubated aerobically at 37 °C for 24 h; PDA plates were incubated at room temperature for fungal isolation.

2.7 Purification of Bacterial Isolates

Purification of the primary isolates was carried out in two successive rounds. Owing to scheduling constraints in the teaching laboratory, the first round began 4–5 days after the initial 24 h incubation. Representative colonies from each of the nine primary NA, MAC and EMB plates (three surfaces × three media) were re-streaked, using the quadrant streak method, onto freshly prepared plates of the same original medium, that is, NA colonies onto NA, MAC colonies onto MAC, and EMB colonies onto EMB, in order to resolve the mixed primary cultures into morphologically uniform, well-isolated colonies.

For the second round, one representative colony was selected from each of the nine first-round purified plates (three surfaces × NA, MAC and EMB). Because fresh MacConkey and EMB agar were not available at this stage, all nine representative colonies were sub-cultured together, as separate single-colony streaks, onto freshly prepared Nutrient Agar plates; sterile physiological saline was used to prepare uniform bacterial suspensions before streaking. This second round produced nine final working isolates used for all subsequent analyses, each named according to its originating surface and isolation medium (the Sink, Platform and Laminar isolates on each of NA, MAC and EMB).

2.8 Preparation of Bacterial Inoculum

Nine sterile tubes of nutrient broth were labelled to correspond to the nine surface-medium isolates (Sink, Platform and Laminar isolates on NA, MAC and EMB). A single well-isolated colony from each purified culture was transferred aseptically into its corresponding broth using a sterile inoculating loop. Cultures were incubated aerobically with intermittent agitation on an orbital shaker (approximately 120 rpm) during the day to promote uniform planktonic growth and aeration, then moved to a static incubator at 37 °C overnight to give the cells a period of quiescence conducive to surface

Journal of Animal Environment, Vol. 18, No. 4s, Spring 2026 attachment; this alternating shaking/static cycle was repeated for two consecutive days to standardize the starting inoculum before it was used to inoculate the individually replicated tubes used for the biofilm assay itself (Section 2.9, Figure 2). As a methodological caveat, alternating shaking and static phases during inoculum preparation rather than a single standardized overnight culture grown to a target OD600 or 0.5 McFarland standard may itself promote some attachment before the assay begins, and could contribute to the variability observed between replicate tubes; this is addressed further in Section 4.5.



Figure 2. Cotton-plugged nutrient broth tubes during the alternating incubation cycle: (left) secured upright on the orbital shaker during the daytime agitation phase; (right) transferred to the static incubator (37 °C) overnight. Visible turbidity at the tube base reflects progressive bacterial growth.

2.9 Assessment of Biofilm Formation by the Crystal Violet Tube Assay

Biofilm formation was assessed using the crystal violet tube assay, adapted from the tissue-culture-tube method originally described by Christensen et al. [11] and later popularized in microtiter-plate form by O'Toole and Kolter [12]. For each of the nine surface-medium isolates, a loopful of the standardized inoculum (Section 2.8) was used to inoculate three independent tubes, each containing 5 mL of sterile nutrient broth in sterile borosilicate glass tubes ($n = 3$ replicate tubes per isolate, 27 tubes in total), and incubated for 48 h at 37 °C, with intermittent orbital agitation (approximately 120 rpm) during the day to encourage bacterial growth, surface attachment and biofilm formation. After incubation, the planktonic culture was decanted carefully without disturbing the adherent film on the tube wall, and the tubes were rinsed gently with sterile distilled water two to three times to remove loosely attached cells before air-drying.

The adherent biofilm was fixed with 2 mL of 90% ethanol for 10 minutes and air-dried at room temperature, then stained with 2 mL of 0.1% (w/v) crystal violet solution for 10–15 minutes. Excess stain was removed by gentle washing with sterile distilled water until the rinsate ran clear, followed by complete air-drying. The bound crystal violet was solubilized with 2 mL of 95% ethanol for 10 minutes, and the resulting solution was transferred to a clean cuvette for absorbance measurement at 570 nm

using a UV-visible spectrophotometer, against a reagent blank. Absorbance served as an indirect quantitative measure of total biofilm biomass, and the mean $\pm$ standard deviation of the three replicate tubes is reported for each isolate.

2.10 Data Presentation and Limitations of the Analytical Approach

Because molecular identification, such as 16S rRNA gene sequencing, and confirmatory biochemical panels were not performed in this study, the isolates are referred to throughout by their originating surface and isolation medium (e.g., Sink-MAC, Platform-MAC, Laminar-MAC isolates) rather than by a presumptive genus- or species-level name; confirmatory identification is recommended for future work. The biofilm assay was run on each of the nine surface-medium isolates in triplicate ($n = 3$ tubes per isolate), which allowed the mean and standard deviation to be calculated for each isolate and a one-way ANOVA to be used to test for overall differences among isolates (Section 3.5). However, no negative, uninoculated control tube was processed through the full fixation-staining-solubilization procedure, so a formal optical-density cut-off (ODc) and the corresponding strong/moderate/weak classification recommended by Stepanović et al. [13] still could not be calculated; results therefore remain descriptive absorbance values, with isolates compared to one another in relative terms rather than classified against a validated cut-off. The visual turbidity of broth cultures during the shaking/static incubation cycle (Section 3.3) was likewise assessed qualitatively, by direct observation and photographic record only; no optical density or colony-forming-unit measurement of the broth cultures was taken at these intermediate stages, so those descriptions should be read as a qualitative visual record rather than a quantified microbial count. These limitations, and what they mean for interpreting the results, are discussed further in Section 4.5.

3. Results

3.1 Primary Isolation of Bacteria from Food-Contact Surfaces

No visible bacterial growth was observed on any medium after approximately 20–22 h of incubation. Once the full 24 h period had elapsed, however, distinct bacterial growth was evident on Nutrient Agar, MacConkey Agar and EMB Agar at all three sampled surfaces (Figure 3). Nutrient Agar supported profuse, smooth, cream-colored colonies, consistent with successful recovery of culturable heterotrophic environmental bacteria. MacConkey Agar supported orange-pink, mucoid colonies consistent with lactose-fermenting Gram-negative organisms, while EMB Agar supported colonies with a dark, blue-black to purple appearance, consistent with Gram-negative growth on this differential medium. No appreciable fungal growth was observed on Potato Dextrose Agar throughout the incubation period, at any surface.

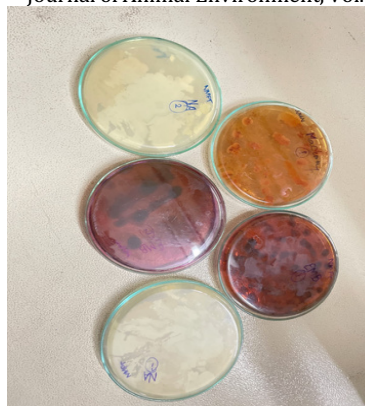


Figure 3. Primary isolation plates recovered from the sampled laboratory surfaces after 24 h of incubation, showing growth on Nutrient Agar (NA), MacConkey Agar and Eosin Methylene Blue (EMB) Agar.

3.2 Purification of Bacterial Isolates

In the first round of purification, repeated sub-culturing of representative colonies onto fresh plates of the same original medium (NA, MAC and EMB) produced dense, well-separated streaks on each medium, confirming successful recovery of viable isolates from the primary plates across all three surfaces (Figure 4 shows a representative set).

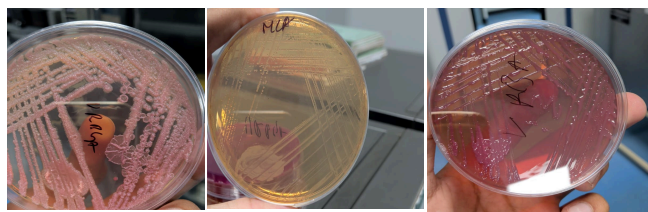


Figure 4. Representative first-round purification streaks, re-cultured on their original isolation media as labelled on the plates; the same procedure was applied to primary isolates from all three sampled surfaces.

In the second round, one representative colony from each of the nine first-round plates (three surfaces $\times$ NA, MAC and EMB) was combined and streaked separately onto Nutrient Agar. This yielded morphologically uniform, well-isolated colonies for all nine isolates, which appeared circular, with entire margins, a smooth, convex profile and a creamy-white appearance (Figure 5 shows a representative set). These nine final isolates the Sink, Platform and Laminar isolates on each of NA, MAC and EMB were retained for the broth growth and biofilm assays described below.

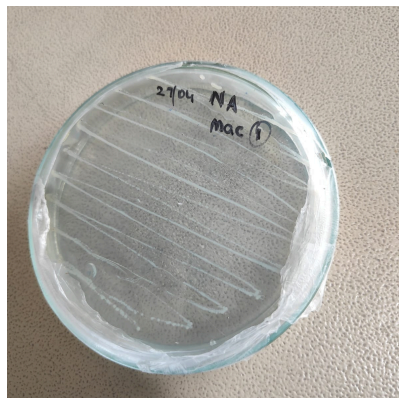


Figure 5. Representative second-round (final) purification streaks of the NA, MAC and EMB isolates on Nutrient Agar, showing uniform colony morphology; the same procedure was applied to isolates from all three sampled surfaces.

3.3 Growth of Purified Isolates in Nutrient Broth

Over the two-day alternating incubation cycle (daytime orbital shaking at ~120 rpm; overnight static incubation at 37 °C), broth cultures of all nine isolates became progressively more turbid, consistent with active bacterial growth (Figure 2; Table 1). By the end of the 48 h cycle, all nine cultures were visibly turbid with sedimented cell biomass apparent at the tube base after standing (Figure 6 shows representative tubes), confirming that actively growing cultures had been obtained for the biofilm assay. A visually discernible difference in turbidity between isolates suggested that their growth profiles also differed somewhat under these conditions.

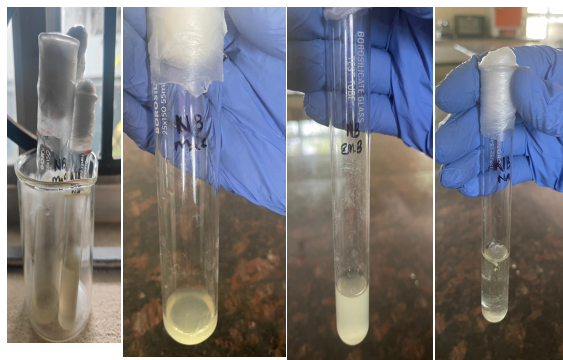


Figure 6. Turbidity and sedimented cell biomass in representative broth cultures at the end of the 48 h incubation cycle, indicating active bacterial growth.

Table 1. Qualitative visual assessment of broth turbidity across the two-day alternating incubation cycle. Assessments are based on direct visual observation and photographic record only; no optical density or colony-forming-unit measurement of the broth cultures was performed at these intermediate stages (see Section 2.10).

Incubation stage	Condition	Visual turbidity	Qualitative inference
Day 1 - daytime	Orbital shaking, ~120 rpm	Faint	Early active growth
Day 1 - overnight	Static incubation, 37 °C	Faint-moderate	Continued growth
Day 2 - daytime	Orbital shaking, ~120 rpm	Moderate	Progressive growth
Day 2 - overnight (final, 48 h)	Static incubation, 37 °C	Pronounced, with visible sediment	Actively grown culture, ready for biofilm assay

3.4 Qualitative Assessment of Biofilm Formation

After 48 h in the crystal violet tube assay, all nine isolates, across all three replicate tubes each, retained crystal violet stain as an adherent film on the inner wall and base of the test tube following washing, confirming their capacity for surface attachment and biofilm formation. Staining intensity differed visibly both by isolation medium and by originating surface: MAC isolates generally showed the most intense staining, followed by EMB isolates, with NA isolates showing comparatively lighter staining at every surface, and sink-derived isolates stained most intensely overall.

3.5 Quantitative Assessment of Biofilm Formation

Quantitative biofilm biomass, expressed as absorbance of the solubilized crystal violet stain at 570 nm against a reagent blank, is summarized as mean $\pm$ standard deviation ($n = 3$ independent tubes per isolate) in Table 2. Across all three surfaces, MAC isolates produced consistently high absorbance (Sink 0.538 ± 0.007 ; Platform 0.509 ± 0.002 ; Laminar 0.212 ± 0.004), EMB isolates were intermediate to high (Sink 0.484 ± 0.010 ; Platform 0.417 ± 0.005 ; Laminar 0.322 ± 0.003), and NA isolates were consistently the lowest at every surface (Sink 0.239 ± 0.006 ; Platform 0.220 ± 0.002 ; Laminar 0.122 ± 0.003). Sink-derived isolates produced the highest biofilm biomass at every medium, followed by platform-derived isolates, with laminar-cabinet-derived isolates the lowest; notably, the medium ranking itself shifted at the laminar cabinet, where the EMB isolate

outproduced the MAC isolate, reversing the MAC > EMB pattern seen at the other two surfaces. A one-way ANOVA across all nine surface-medium groups confirmed that these differences were highly significant ($F(8,18) = 2435.3$, $p < 0.001$); a two-way ANOVA further indicated significant main effects of surface ($F(2,18) = 3670.9$, $p < 0.001$) and medium ($F(2,18) = 5140.0$, $p < 0.001$), together with a significant surface $\times$ medium interaction ($F(4,18) = 465.1$, $p < 0.001$), consistent with the medium-ranking reversal observed at the laminar cabinet.

Table 2. Quantitative biofilm biomass of the nine surface-medium isolates, determined by the crystal violet tube assay (OD570, mean $\pm$ SD of $n = 3$ independent tubes per isolate).

Isolate	Surface origin / Isolation medium	OD570 (mean $\pm$ SD, $n = 3$)	Rank within surface*
Sink-MAC	Sink / MacConkey Agar	0.538 $\pm$ 0.007	Highest
Sink-EMB	Sink / Eosin Methylene Blue Agar	0.484 $\pm$ 0.010	Intermediate
Sink-NA	Sink / Nutrient Agar	0.239 $\pm$ 0.006	Lowest
Platform-MAC	Bench/Platform / MacConkey Agar	0.509 $\pm$ 0.002	Highest
Platform-EMB	Bench/Platform / Eosin Methylene Blue Agar	0.417 $\pm$ 0.005	Intermediate
Platform-NA	Bench/Platform / Nutrient Agar	0.220 $\pm$ 0.002	Lowest
Laminar-MAC	Laminar Cabinet / MacConkey Agar	0.212 $\pm$ 0.004	Intermediate
Laminar-EMB	Laminar Cabinet / Eosin Methylene Blue Agar	0.322 $\pm$ 0.003	Highest

Laminar-NA	Laminar Cabinet / Nutrient Agar	0.122 $\pm$ 0.003	Lowest
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*Rank reflects a relative comparison among the nine surface-medium isolates tested in this study only, and is not derived from a validated optical-density cut-off (ODc); see Section 2.10 and Section 4.5.

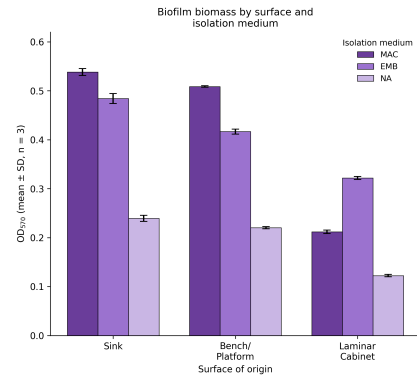


Figure 7. Quantitative assessment of biofilm formation by the nine surface-medium isolates using the crystal violet tube assay. Biofilm biomass was quantified by measuring absorbance at 570 nm (OD570); bars show mean $\pm$ SD ($n = 3$ independent tubes per isolate). Sink-derived isolates produced the highest absorbance at every medium, followed by platform-derived isolates, with laminar-cabinet-derived isolates the lowest; the medium ranking reverses at the laminar cabinet, where the EMB isolate exceeds the MAC isolate.

4. Discussion

4.1 Recovery of Culturable Bacteria from Laboratory Surfaces

Food-contact surfaces are routinely exposed to moisture, organic residue, aerosols and repeated hand contact, all of which favor microbial attachment and persistence even after cleaning [1,2,9]. Recovering culturable bacteria from all three sampled surfaces here indicates that viable organisms persist on these surfaces under ordinary laboratory conditions. The absence of visible colonies before 24 h, followed by clear growth once that period had passed, fits with the lag and early exponential phases typically needed for environmental isolates recovered at low density to reach a macroscopically visible colony size. The lack of fungal growth on Potato Dextrose Agar may reflect a genuinely low fungal load on the sampled surfaces, or simply incubation conditions that favored bacterial over fungal recovery during the period sampled.

4.2 Purification and Growth Characteristics

The uniform colony morphology obtained after two rounds of sub-culturing suggests that the purification procedure successfully resolved the mixed primary cultures into their component isolates, a necessary step before biofilm characterization can be considered reliable, since more than one organism in a culture can confound both colony morphology and biomass

measurements. The progressive turbidity seen in all nine broth cultures across the alternating shaking/static cycle (Table 1) confirms that the purified isolates were viable and metabolically active under this incubation regimen; the daytime agitation likely favored aeration and even planktonic growth, while the overnight static phase gave the cells periods of quiescence conducive to surface attachment, a prerequisite for biofilm initiation [4,5].

4.3 Biofilm-Forming Capacity of the Isolates

The crystal violet assay confirmed that all nine isolates could form an adherent biofilm on glass, consistent with the well-established observation that biofilm formation is a common, often near-constitutive trait among environmental heterotrophic bacteria [1,3]. The amount of biofilm formed, however, differed markedly both by isolation medium and by originating surface. At the sink and the bench/platform, MAC isolates produced the most biofilm, followed by EMB and then NA isolates; at the laminar cabinet this pattern shifted, with the EMB isolate outproducing the MAC isolate, while the NA isolate again produced the least biofilm at every surface. Averaged across media, sink-derived isolates consistently produced more biofilm biomass than platform-derived isolates, which in turn generally exceeded laminar-cabinet-derived isolates; a one-way ANOVA confirmed that differences among the nine surface-medium groups were highly significant ($F(8,18) = 2435.3$, $p < 0.001$), with significant main effects of surface ($F(2,18) = 3670.9$, $p < 0.001$) and medium ($F(2,18) = 5140.0$, $p < 0.001$) and a significant surface $\times$ medium interaction ($F(4,18) = 465.1$, $p < 0.001$) reflecting the medium-ranking reversal at the laminar cabinet. This kind of isolate-to-isolate and site-to-site variability, even among organisms recovered from the same laboratory, is widely reported and is generally put down to strain-specific differences in surface-adhesion mechanisms, EPS production, and the regulatory pathways governing the shift from reversible to irreversible attachment [1,4,5], as well as to differences in the microbial communities that colonize surfaces with different moisture, nutrient and traffic profiles [2,6,9,10]. That the sink, the most frequently wetted and organically loaded of the three surfaces, yielded the highest-biofilm-forming isolates at every medium is consistent with moisture and nutrient availability being key drivers of biofilm competence, while the comparatively lower biomass from the laminar cabinet may reflect its more controlled airflow and more frequent disinfection regime. From a food-safety standpoint this matters, because surfaces harboring even a minority of strong biofilm producers can carry a disproportionate contamination risk despite an otherwise low bacterial load, given how much more resistant biofilm-embedded cells are to routine sanitation compared with their planktonic counterparts [2,3,7,9].

4.4 Methodological Considerations for the Crystal Violet Assay

The crystal violet tube/microtiter assay is one of the most widely used phenotypic screens for biofilm formation, largely because it is inexpensive, technically undemanding, and works with equipment most laboratories already have [11,12]. It measures total retained biomass, live and dead cells together with the surrounding EPS matrix, rather than a direct count of viable adherent organisms, and absorbance readings can

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be sensitive to handling details such as how the planktonic supernatant is removed and how uniformly the staining and washing steps are carried out [14]. A formal, laboratory-specific correlation between crystal violet absorbance and biomass, along the lines recently proposed by Stephens et al. [15], would strengthen the quantitative interpretation of assays of this kind in future work.

4.5 Limitations

Several limitations of this study are worth stating plainly. The biofilm assay was performed in triplicate ($n = 3$ independent tubes per isolate) rather than as a single determination, which allowed descriptive statistics and a one-way and two-way ANOVA to be reported; however, all three replicate tubes for a given isolate were prepared from the same standardized inoculum batch (Section 2.8) rather than from fully independent culture batches grown on separate days, so the reported variability captures technical rather than full biological replication, and independent-batch replication remains a useful next step. No uninoculated negative-control tube was processed alongside the samples, so a formal optical-density cut-off (ODc) and the corresponding strong/moderate/weak classification recommended by Stepanović et al. [13] still could not be calculated; the relative comparisons drawn here should not be read as equivalent to a validated biofilm-production category. Isolate identity was established only to the level of the isolation medium and originating surface, without confirmatory biochemical panels or 16S rRNA sequencing, so the genus- or species-level identity of the nine isolates remains unresolved. The alternating shaking/static protocol used to prepare the standardized inoculum (Section 2.8) differs from the more conventional approach of growing a single standardized overnight culture before inoculating the assay itself, and may have allowed some attachment to begin before the 48 h assay period started, which is a possible source of the variability observed between replicate tubes and is worth revisiting in future protocol refinements. The turbidity assessment of broth cultures during the incubation cycle (Table 1) was qualitative and visual only, without corresponding optical density or colony-forming-unit data. A statistically powered follow-up study incorporating fully independent biological replicates, molecular identification, quantified growth-curve data and a validated ODc would address each of these remaining gaps and is a natural next step for this line of work.

5. Conclusion

This study shows that culturable, biofilm-competent bacteria can be readily recovered from frequently used food-contact surfaces in a food microbiology teaching laboratory using simple, conventional microbiological techniques. All nine isolates examined, spanning three surfaces (sink, bench/platform and laminar air-flow cabinet) and three isolation media (NA, MAC and EMB), were capable of forming an adherent biofilm within 48 h, with MAC and EMB isolates generally producing substantially more biofilm biomass than NA isolates, and sink-derived isolates consistently outperforming bench/platform- and laminar-cabinet-derived isolates at every medium (one-way ANOVA, $p < 0.001$). These results underline that biofilm-forming capacity can vary considerably both by the medium an organism is

recovered on and by the specific surface it originates from, even within a single, shared laboratory environment. These findings support extending routine surface-sanitation checks in food-handling and food-microbiology teaching laboratories beyond conventional colony-count monitoring to include biofilm-specific screening, with particular attention to frequently wetted, high-traffic surfaces such as sinks. Given the remaining gaps in this work, a larger study with fully independent biological replicates, a validated optical-density cut-off, and molecular identification of isolates would be a reasonable next step to confirm and build on these observations.

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Author Contributions

Dr. P. Kiran Kumari: Conceptualization, Supervision, Methodology, Review and editing.

Faaila Janis: Investigation, Sample collection, Laboratory experiments, Data analysis, Visualization, Writing – original draft, Writing – review and editing.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Statement

This study involved environmental sampling from laboratory food-contact surfaces only and did not involve human participants, animals, or clinical specimens. Ethical approval was therefore not required.

Declaration

The authors confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All authors have read and approved the final version of the manuscript.

Declaration of AI Assistance

During preparation of this manuscript, the authors used an AI-assisted writing and editing tool (Claude, Anthropic) to help with language editing, formatting, figure numbering, and cross-checking of the reported statistics against the underlying laboratory data. All sample collection, laboratory experiments, data generation and scientific interpretation are the authors' own work; the tool was not used to generate, alter, or invent any experimental result. The authors reviewed all AI-assisted edits, take full responsibility for the accuracy and integrity of the content, and confirm that its use is

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Abbreviations

EPS – Extracellular Polymeric Substances

EMB – Eosin Methylene Blue Agar

MAC – MacConkey Agar

NA – Nutrient Agar

OD – Optical Density

PDA – Potato Dextrose Agar

rpm – Revolutions per minute

UV-Vis – Ultraviolet-Visible Spectrophotometer

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