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Culture-Based Isolation and Preliminary Characterization of Bacteria Associated with *Musca domestica* Collected from Diverse Environmental Habitats

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Abstract

The housefly, *Musca domestica*, is widely distributed across domestic, food-associated, livestock, and waste environments and is frequently exposed to microorganisms present in these habitats. The present study investigated the cultivable bacterial populations associated with *M. domestica* collected from diverse environmental locations. Houseflies were collected from eight sites, namely household, fruit shop, fish market, slaughterhouse, poultry farm, swine farm, dairy farm, and garbage site, and designated S1–S8, respectively. Samples were processed using serial dilution followed by culture-based isolation on nutrient agar using the spread plate technique. Bacterial growth was observed at 10^{-3}, 10^{-4}, and 10^{-5} dilution levels, with differences in colony density and morphology among samples. Morphologically distinct colonies were selected and purified by quadrant streaking. A total of 26 morphologically distinct bacterial isolates were obtained from the eight environmental sources. The variation in colony growth and morphology across sampling sites indicates the presence of diverse cultivable bacterial populations associated with environmentally exposed houseflies. The findings support the importance of culture-based investigation of *M. domestica* as a potential carrier of environmentally acquired bacteria and provide purified bacterial isolates for subsequent microbiological characterization.

1. Introduction

The common housefly, *Musca domestica* L., is a widespread synanthropic insect closely associated with human settlements, food premises, livestock facilities, animal waste, and decomposing organic matter. Its feeding and breeding behavior brings it into frequent contact with microorganisms present in diverse environmental substrates. As a result, *M. domestica* can acquire and transport microorganisms on its external body surfaces and within its digestive tract, making it an important organism for investigating

environmentally associated bacterial populations (Khamesipour *et al.,* 2018).

The bacterial communities associated with houseflies are influenced by the ecological conditions in which the flies live and feed. Previous investigations have demonstrated considerable bacterial diversity in *M. domestica* collected from different environmental habitats. Gupta *et al.* (2012) isolated 102 bacterial strains representing 22 genera from the gut of houseflies collected from diverse public environments, including garbage areas, markets, restaurants, hospitals, and human habitation. Their findings demonstrated that houseflies can harbor a broad range of bacterial taxa, including several genera containing potentially pathogenic species.

Environmental exposure also appears to influence the bacterial community associated with houseflies. Nayduch *et al*. (2017) demonstrated substantial bacterial diversity among houseflies collected from dairy farms and showed associations between fly-associated bacteria and the surrounding environmental sources. More recent studies have reported that habitat significantly influences bacterial community composition, with houseflies from agricultural, urban, and mixed environments carrying distinct bacterial assemblages (Onwugamba *et al.,* 2022). Culture-independent investigations have further shown that the external bacterial community of houseflies varies according to geographical location and habitat, whereas the internal bacterial community may be comparatively stable (Shukla *et al.,* 2019).

Although molecular approaches provide broader information on microbial communities, culture-dependent techniques remain valuable for recovering viable bacterial isolates for subsequent characterization. Therefore, the present study focused on the culture-based isolation and preliminary characterization of bacteria associated with *M. domestica* collected from eight contrasting environmental habitats, including domestic, food-associated, animal-processing, livestock-rearing, and waste-associated environments. The study aimed to document the cultivable bacterial population associated with houseflies from these different habitats and to obtain purified bacterial isolates for further investigation.

2. Materials and Methods

2.1 Study Area and Collection of *Musca domestica*

Houseflies were collected from eight different environmental locations representing domestic, food-associated, animal-processing, livestock-rearing, and waste-associated habitats. The sampling sites were designated as S1–S8: household (S1), fruit shop (S2), fish market (S3), slaughterhouse (S4), poultry farm (S5), swine farm (S6), dairy farm (S7), and garbage site (S8).

The collected adult houseflies were transferred individually into sterile containers, labelled according to their respective sampling sites, and transported to the laboratory for microbiological processing.

2.2 Serial Dilution and Bacterial Isolation

The collected *M. domestica* samples were processed to obtain microbial suspensions. Serial dilutions were performed from 10^{-1} to 10^{-5} using sterile diluent. The 10^{-3}, 10^{-4}, and 10^{-5} dilutions were selected for bacterial isolation.

Nutrient agar was prepared and sterilized by autoclaving at 121°C for 20 min. For samples S1–S4, 150 µL of the respective dilution was inoculated onto nutrient agar, whereas 100 µL was used for samples S5–S8. The inoculum was uniformly distributed using a sterile L-shaped spreader. The plates were incubated at 37°C for 24 h.

2.3 Purification and Preliminary Characterization

Following incubation, the bacterial colonies

were examined for visible morphological characteristics, including color, size, form, surface appearance, and distribution. Distinct colonies were selected and purified by quadrant streaking on nutrient agar. The purified cultures were maintained for further characterization.

3. Results

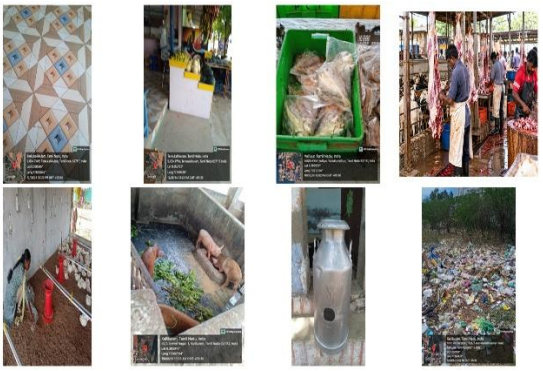
3.1 Collection of *Musca domestica*

Eight environmental sampling locations were successfully covered for the collection of *M. domestica*. The samples were designated S1–S8 according to their respective collection sites.

Sample	Collection site	Environmental category
S1	Household	Domestic
S2	Fruit shop	Food-associated
S3	Fish market	Food-market
S4	Slaughterhouse	Animal-processing
S5	Poultry farm	Poultry-rearing
S6	Swine farm	Livestock-rearing
S7	Dairy farm	Dairy-rearing
S8	Garbage site	Waste-associated

The selected sites represent markedly different environmental conditions and potential sources of microbial exposure.

Figure 1. Musca domestica samples were collected from different environmental sampling sites (S1–S8).



Eight sampling sites (S1–S8) representing different environmental habitats were successfully covered for the collection of *M. domestica*. The collected samples were clearly designated according to their sampling locations and were subsequently used for bacterial isolation and characterization

3.2 Serial Dilution

Serial dilutions of the collected *M. domestica* samples were performed from 10^{-1} to 10^{-5}. Progressive reduction in microbial density was observed across the dilution series, providing suitable dilutions for the recovery of discrete bacterial colonies.

The 10^{-3}, 10^{-4}, and 10^{-5} dilutions were subsequently used for culture-based bacterial isolation.

Figure 2. Serial dilution of *M. domestica* samples S1–S8 from 10^{-1} to 10^{-5} was performed.

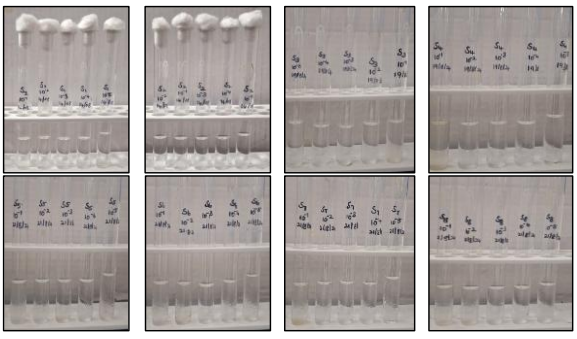


Figure 2 illustrates the serial dilution of the microbial suspension obtained from the collected *Musca domestica* samples at dilution levels ranging from 10^{-1} to 10^{-5}. The figure clearly represents the sequential dilution

process, in which the microbial concentration was progressively reduced from the initial suspension to the highest dilution. The prepared dilutions provided a suitable range of microbial concentrations for subsequent spread plate inoculation. This dilution process facilitated the reduction of microbial density and enabled the recovery of discrete bacterial colonies for further isolation and characterization of the isolated bacteriophages.

3.3 Culture-Based Isolation of Bacteria

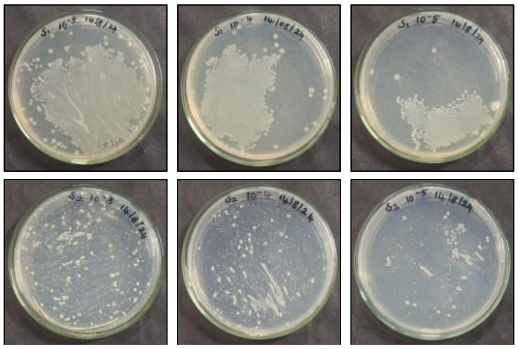
Bacterial growth was observed in all eight *M. domestica* samples at the tested dilution levels. Considerable variation was observed in the colony density and distribution among the sampling sites.

The S1 sample showed numerous colonies at 10^{-3}, with comparatively fewer colonies at higher dilutions. S2 also showed a progressive reduction in colony density with increasing dilution. S3 exhibited particularly abundant bacterial growth at 10^{-3} and 10^{-4}, whereas the 10^{-5} dilution provided more distinguishable colonies.

S4 showed pronounced bacterial growth at all three dilution levels. S5 demonstrated dense growth at 10^{-3} and progressively more discrete colonies at higher dilutions. S6 retained substantial bacterial growth even at 10^{-5} dilution. S7 showed extensive growth at lower dilutions and more distinguishable colonies at 10^{-5}. S8 exhibited dense and partially confluent growth at 10^{-3}, followed by more discrete colony development at higher dilutions.

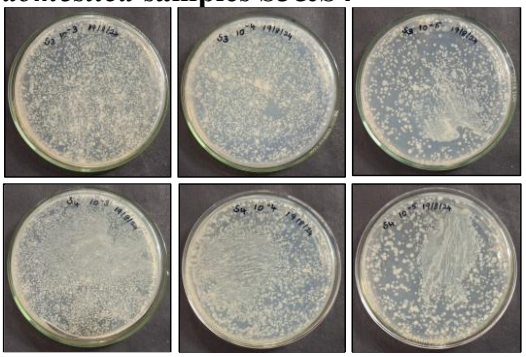
Differences were also observed in colony morphology, including variations in colony color, size, shape, surface appearance, and distribution.

Figures 3 Bacterial colonies isolated from *M. domestica* samples S1&S2



The S1 and S2 *Musca domestica* samples showed bacterial growth on nutrient agar plates at 10^{-3}, 10^{-4}, and 10^{-5} dilution levels (Figure 3). At 10^{-3} dilution, numerous colonies were observed, with variations in size and appearance. With increasing dilution, the colonies became progressively more separated, allowing individual colony forms to be distinguished. Both samples exhibited variations in colony size, shape, color, and surface appearance. The 10^{-5} dilution plates showed comparatively fewer and well-separated colonies, which were suitable for selection and further purification. Thus, the S1 and S2 samples demonstrated distinct cultivable bacterial growth across the tested dilution levels.

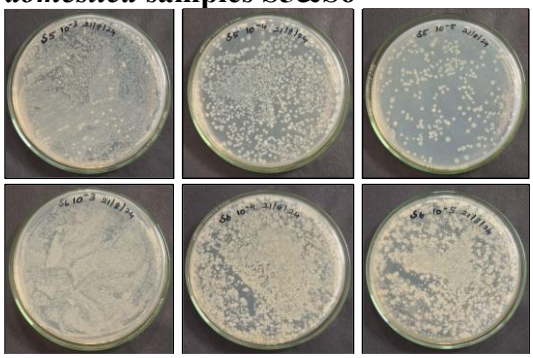
Figures 4 Bacterial colonies isolated from *M. domestica* samples S3&S4



The S3 and S4 *Musca domestica* samples exhibited abundant bacterial growth on nutrient agar plates at 10^{-3}, 10^{-4}, and 10^{-5} dilution levels (Figure 4). Dense and closely distributed colonies were observed at 10^{-3} and 10^{-4} dilutions, whereas the 10^{-5} plates showed comparatively more discrete colonies. Considerable variation in colony size, shape, form, and appearance was observed among the recovered bacterial colonies. Representative morphologically distinct colonies were selected

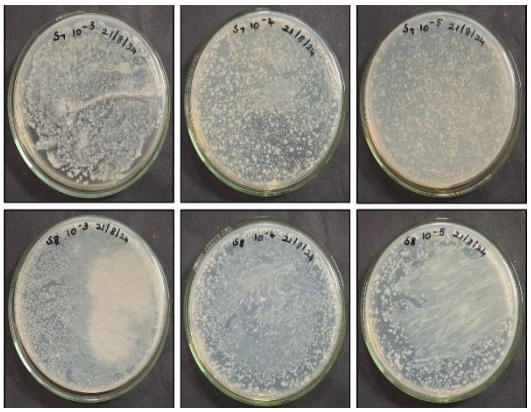
from different dilution levels for further purification and characterization. The observed colony distribution indicated substantial cultivable bacterial growth in both S3 and S4 samples. Thus, the S3 and S4 samples provided several distinguishable bacterial colonies for subsequent isolation and characterization.

Figures 5 Bacterial colonies isolated from *M. domestica* samples S5&S6



The S5 and S6 *Musca domestica* samples showed abundant bacterial growth on nutrient agar plates at 10^{-3}, 10^{-4}, and 10^{-5} dilutions (Figure 5). Dense and closely distributed colonies were observed at the 10^{-3} dilution, whereas comparatively discrete colonies were obtained at higher dilution levels. Both samples showed considerable variation in terms of colony size, shape, distribution, and appearance. Several morphologically distinct colonies were selected from different dilution levels for further purification and characterization. Notably, the S6 sample retained substantial bacterial growth even at 10^{-5} dilution, with distinguishable colonies available for selection. Thus, both S5 and S6 samples provided representative cultivable bacterial colonies for subsequent isolation and characterization.

Figures 6 Bacterial colonies isolated from *M. domestica* samples S7&S8



The S7 and S8 *Musca domestica* samples exhibited extensive bacterial growth on nutrient agar plates at 10^{-3}, 10^{-4}, and 10^{-5} dilution levels (Figure 5). Dense and closely distributed colonies were observed at 10^{-3} dilution, whereas the 10^{-4} plates showed numerous discrete colonies. At 10^{-5} dilution, comparatively smaller and well-separated colonies were observed, allowing individual colonies to be distinguished. Variation in colony size, shape, form, and distribution was evident among the bacterial colonies recovered from both samples. Representative morphologically distinct colonies were selected from different dilution levels for further purification and characterization. Thus, S7 and S8 provided distinct cultivable bacterial colonies across the tested dilution levels.

4. Discussion

The present study demonstrated the successful recovery of cultivable bacteria from *Musca domestica* collected from eight environmentally contrasting habitats: household, fruit shop, fish market, slaughterhouse, poultry farm, swine farm, dairy farm, and garbage site. Bacterial growth was observed from all sampling locations, although differences in colony density, distribution, and morphology were evident among the samples. The recovery of morphologically distinct colonies from all eight habitats resulted in 26 purified bacterial isolates. These findings support the ecological association of houseflies with diverse microbial environments and are consistent with the established role of *M. domestica* as a carrier of environmentally acquired bacteria.

The broad environmental distribution of the present sampling sites is particularly relevant to housefly-associated bacterial diversity. Houseflies commonly breed and feed in manure, decaying organic matter, refuse, and other microbe-rich substrates. Khamesipour *et al.* (2018), in a systematic review of pathogens associated with *M. domestica*, reported that houseflies collected from human habitation, animal farms, and other contaminated environments carried a wide range of bacterial pathogens. Their review identified more than 130 pathogens associated with houseflies, with bacteria representing the largest group. The present recovery of bacteria from household, food-market, animal-associated, and waste-associated sites is therefore consistent with the ecological behavior of the species.

The recovery of 26 morphologically distinct isolates also agrees with earlier culture-dependent investigations. Gupta *et al.* (2012) isolated 102 bacterial strains representing 22 genera from the gut of 65 houseflies collected from diverse public environments, including garbage dumps, public toilets, hospitals, restaurants, markets, and houses. Their study demonstrated that houseflies can harbor bacteria belonging to numerous taxonomic groups and included potentially pathogenic genera such as *Klebsiella*, *Aeromonas*, *Shigella*, *Morganella*, *Providencia,* and *Staphylococcus*. Although the number of isolates recovered in the present study was lower, the finding of multiple morphologically distinct cultivable isolates across several environmental habitats follows the same general pattern of bacterial heterogeneity.

The present observation of differences in bacterial growth among sampling sites may also reflect variation in environmental exposure. Samples obtained from the fish market, slaughterhouse, and livestock-associated environments showed pronounced bacterial growth, particularly at lower dilution levels, whereas higher dilution levels generally provided more discrete colonies. Such variation is biologically plausible because animal-associated and food-associated environments contain abundant organic substrates that support diverse microbial populations. Nayduch *et al.* (2017) reported diverse bacterial communities in houseflies associated with dairy farms and demonstrated relationships between fly-associated bacteria and environmental microbial sources.

A similar relationship between habitat and bacterial community composition was reported by Onwugamba *et al.* (2022), who compared houseflies from agricultural, urban, and mixed environments. Their results showed that bacterial community composition and diversity were influenced more strongly by habitat than by sampling time, and potential pathogenic taxa were particularly abundant in flies associated with agricultural and mixed environments. This supports the interpretation that the differences observed among the S1–S8 samples in the present study may be related to the distinct environmental conditions encountered by the flies.

The results are consistent with the larger-scale findings of Shukla *et al.* (2019), who examined houseflies from farms, homes, and hospitals in Belgium and Rwanda using culture-independent sequencing. They reported that the external bacterial community varied considerably with geographical location and habitat, while the internal bacterial community was comparatively consistent. Importantly, their study demonstrated that the external surface of the fly harbored greater bacterial diversity than the internal environment, highlighting the contribution of environmental exposure to housefly-associated microbial variation. The present culture-based study, although not designed to distinguish internal and external microbiota, similarly demonstrates that flies collected from different environmental settings can yield visibly different cultivable bacterial populations.

The observation of substantial bacterial growth even at a 10^{-5} dilution in some samples is

another notable finding. This indicates that the original microbial suspensions contained sufficient cultivable bacteria to remain detectable after extensive dilution. However, the present observations are qualitative because colony-forming unit values were not determined. Therefore, differences in visible colony density should be interpreted as evidence of variation in cultivable bacterial recovery rather than as quantitative differences in bacterial load.

The morphological diversity observed among the isolates further supports the recovery of a heterogeneous cultivable bacterial population. Differences in colony color, size, surface appearance, form, and distribution provided an initial basis for selecting distinct colonies for purification. Similar culture-dependent studies have used colony morphology as a preliminary screening step before biochemical and molecular identification. For example, a study of houseflies collected from slaughterhouses, garbage dumps, houses, hospitals, and eateries recovered six bacterial species, including *Salmonella*, *Proteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli,* and *Klebsiella pneumoniae*, demonstrating that morphologically selected housefly-associated isolates may subsequently reveal bacteria of medical importance (Adenusi *et al.,* 2020).

The present finding of 26 purified isolates should nevertheless be interpreted within the limitations of the culture-dependent methodology. Culture-based approaches recover only microorganisms capable of growing under the selected nutritional, temperature, and incubation conditions. Gupta *et al.* (2012) demonstrated this limitation by comparing culture-dependent and culture-independent approaches; their molecular analysis detected additional bacterial taxa that were not recovered by culture. Likewise, Shukla *et al.* (2019) reported a broader bacterial diversity through amplicon sequencing than would be expected from conventional cultivation alone. Thus, the 26 isolates obtained in the present study represent the cultivable bacterial fraction associated with the sampled houseflies rather than the complete microbiome.

Recent evidence further strengthens the ecological significance of housefly-associated bacteria. A large study of dairy farm houseflies reported that individual flies carried diverse bacterial communities encompassing taxa detected in cattle manure, along with additional bacteria potentially acquired from animals. The authors suggested that houseflies can act as carriers and sentinels of microbial threats in livestock environments (Nayduch *et al.,* 2024). Similarly, studies on houseflies from cattle farms have demonstrated that the bacterial community composition can vary according to farm type and geographical location (Ramos *et al.,* 2023). These findings provide further support for examining houseflies collected from multiple environmental sources rather than restricting the sampling to a single habitat.

Taken together, the present results and previous studies indicate that *M. domestica* can acquire a diverse cultivable bacterial population from the environments it frequents. The recovery of 26 morphologically distinct isolates from eight contrasting habitats provides useful preliminary evidence of bacterial heterogeneity associated with environmentally exposed houseflies. The purified isolates obtained in the present study provide a suitable basis for subsequent biochemical, molecular, and functional characterization, which is necessary to establish their taxonomic identity and biological significance.

5. Summary

The present investigation examined the cultivable bacteria associated with *M. domestica* collected from eight contrasting environmental habitats. Bacterial growth was successfully recovered from all sampling sites following serial dilution and spread plating on nutrient agar. Considerable variation in colony density, distribution, and morphology was observed among the samples. Morphologically distinct colonies were purified by quadrant streaking,

resulting in 26 purified bacterial isolates.

The findings demonstrate that houseflies collected from domestic, food-associated, animal-rearing, animal-processing, and waste-associated environments can serve as a source of diverse cultivable bacterial isolates. This study provides a culture-based foundation for subsequent bacterial identification and characterization.

6. Conclusion

The present study established that *Musca domestica* collected from diverse environmental habitats harbored a range of cultivable bacteria. The recovery and purification of 26 morphologically distinct bacterial isolates demonstrated the utility of houseflies as a source for investigating environmentally associated bacterial populations. Variation in bacterial growth and colony morphology among sampling sites further highlights the influence of environmental exposure on the cultivable bacterial fraction associated with houseflies. Further biochemical and molecular characterization of the purified isolates is necessary to establish their taxonomic identity and biological significance.

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