# THE HIGHLIGHTING OF MIGRATORY-TYPE COLONIES FOR PROTEUS

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REVIEW, RESEARCH ARTICLE

**Abstract**

*The most well-known characteristic of Proteus colonies is the formation of a "wave" or "swarming" pattern on solid media (such as agar). This is due to a property called "swarming," through which the bacteria move collectively across the surface of the medium, expanding and creating concentric rings. The colonies can rapidly increase in diameter due to this behavior. Proteus mirabilis has significantly higher motility than many other bacteria, allowing it to spread in waves on solid media. This movement is supported by a large number of pericellular flagella, which help the bacteria move in large groups. The swarming patterns of the colonies vary based on factors such as the composition of the culture medium, temperature, and humidity. In optimal conditions, Proteus colonies tend to appear more structured and organized, with concentric patterns that can be influenced by bacterial density and migration speed. Proteus colonies demonstrate high adaptability to various environmental conditions, which contributes to the bacterium's resistance in hostile environments and its survival in competitive conditions with other microorganisms. Proteus colonies are an important indicator in diagnosing urinary tract infections and other opportunistic infections, as the unique swarming patterns aid in quick identification in the laboratory. In the lab, these colony characteristics are particularly useful for identifying Proteus mirabilis and other species within the same genus, as each distinct form and characteristic movement is unique to this bacterial genus.*

**Keywords**: swarming colonies, environmental conditions, microorganisms

## INTRODUCTION

*Proteus* is a genus of Gram-negative bacteria in the family *Enterobacteriaceae*, best known for the species *Proteus mirabilis* and *Proteus vulgaris*. These bacteria are found in various environments, including soil, water, and the intestines of some animals and humans. Typically, *Proteus* bacteria are harmless, but certain species are pathogenic and can cause infections in humans, particularly urinary tract infections and wound-associated infections.

*Proteus* bacteria are rod-shaped bacilli equipped with flagella that give them impressive mobility, known as "swarming" motility. This motility facilitates their rapid movement and the formation of characteristic colonies on solid media, appearing as concentric rings. Swarming motility is a distinctive trait of *Proteus* bacteria. On solid culture media, such as agar, the bacteria form "wave-like" patterns due to their rapid and coordinated movement. This phenomenon occurs in stages, alternating between periods of intense swarming and rest phases.

*Proteus* produces the enzyme urease, which breaks down urea into ammonia and carbon dioxide. This ability plays an important role in diagnosing infections, as urease production contributes to the alkalinization of the environment, serving as a specific indicator in the laboratory for identifying these bacteria. Certain strains of *Proteus*, particularly *Proteus vulgaris*, have acquired resistance to multiple classes of antibiotics, including beta-lactams. This makes treating infections caused by *Proteus* challenging, especially in patients with compromised immune systems.

Species of the *Proteus* genus, particularly *Proteus mirabilis*, are often associated with urinary tract infections, especially in patients with long-term urinary catheter use. They can cause severe infections, such as pyelonephritis (a kidney infection), and may lead to the formation of urinary stones through ammonia production, which alkalinizes the urine. *Proteus* infections are common in the urinary tract, but these bacteria can also cause wound infections, otitis, and, in rare cases, septicemia. Diagnosis of these infections often relies on clinical symptoms and laboratory tests that identify the bacteria through specific properties, such as urease production and distinctive swarming patterns.

*Proteus mirabilis* is a Gram-negative bacterium from the *Enterobacteriaceae* family, known for its high motility and ability to colonize the urinary tract. It is one of the most common causes of urinary tract infections, especially in cases of complicated infections associated with urinary catheters. Typically, *Proteus mirabilis* lives in soil, water, and the intestines of some

animals and humans but can become pathogenic under certain circumstances. *Proteus mirabilis* is a rod-shaped bacillus with pericellular flagella that provide it with high motility. Due to the large number of flagella, *P. mirabilis* can move rapidly on moist surfaces, especially solid media, spreading in waves or concentric rings.

*Proteus mirabilis* exhibits a unique "swarming" behavior on solid media, where it forms wave-like patterns. This is a distinctive characteristic that facilitates its identification in the laboratory. Swarming occurs in cyclic stages: periods of rapid growth and migration are followed by resting phases. An important feature of this bacterium is the production of urease, an enzyme that breaks down urea into ammonia and carbon dioxide. This process leads to the alkalinization of the environment, creating conditions favorable for urinary stone formation. Alkalinization of the urine is a hallmark of *Proteus mirabilis* infections and aids in diagnosis. Ammonia-induced alkalinization promotes the precipitation of phosphate, calcium carbonate, and magnesium salts, which lead to the formation of urinary stones. These stones can cause blockages and severe inflammation, complicating urinary tract infections.

Although *Proteus mirabilis* is generally susceptible to many antibiotics, some strains have developed resistance to certain classes of drugs, including penicillins and cephalosporins. This can complicate treatment, especially in chronic or complicated infections.

## MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the medical analysis laboratory, S.C. Diaser, Oradea. To conduct the study, I also accessed the archive, recorded in the laboratory's computer program in S.C. Diaser, Oradea, as well as the computerized database of the unit.

In the laboratory, the identification of bacteria from the *Proteus* genus requires a specific set of materials and a methodology to confirm the presence of these bacteria and determine the species, particularly *Proteus mirabilis* and *Proteus vulgaris*, known for their implications in urinary tract infections and other infections. Here are details about the necessary materials and the identification method commonly used in the laboratory.

**Necessary materials**

**Culture media:**

- **MacConkey Agar:** This is a selective and differential medium used to isolate Gram-negative bacteria. *Proteus* grows on this medium without fermenting lactose, resulting in colorless colonies.
- **Blood Agar:** This medium is used to observe growth patterns and swarming motility, a characteristic of *Proteus mirabilis*.
- **TSI Agar (Triple Sugar Iron):** This medium allows for the observation of hydrogen sulfide ($H_2S$) production, gas formation, and sugar fermentation (glucose).
- **Urea Medium:** This is essential for detecting the production of the urease enzyme, which is a specific marker for *Proteus*.

**Biochemical reagents:**

- **Reagents for the urease test:** Indicate the presence of the urease enzyme by changing the color of the medium (usually to pink) due to the alkalinization caused by the breakdown of urea.
- **Reagents for the indole test:** Used to differentiate *Proteus mirabilis* (indole-negative) from *Proteus vulgaris* (indole-positive).

**Automated systems:**

- **VITEK system:** An automated bacterial identification and antibiogram system used in laboratories to confirm identifications.
- **MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry):** A modern technology for the rapid identification of bacteria based on the specific protein spectral pattern of each bacterial species.

**General equipment:**

- Test tubes and a bacterial loop for inoculation.
- An incubator set to 35-37°C.
- Microscopic examination lamps.

**Identification Method**

1. **Sample Collection and Inoculation:**
    - The clinical sample, in this case, urine, is collected using aseptic techniques and inoculated onto selective culture media, such as MacConkey agar and blood agar, using a bacterial loop to evenly

distribute the sample across the surface of the medium.
    - The plates are incubated at 35-37°C for 18-24 hours.
2. **Observation of Colonies:**
    - After incubation, the plates are checked for the presence of characteristic *Proteus* colonies. These colonies display a distinct "swarming" appearance on blood agar and appear colorless on MacConkey agar (as they do not ferment lactose).
    - Colonies with a swarming pattern and a pungent odor (characteristic of *Proteus mirabilis*) are suspected to belong to the *Proteus* genus.

**Biochemical Tests:**

- **Urease Test:** A sample from the colony is inoculated into a urea medium and incubated for 4-6 hours. *Proteus* will produce a positive reaction (typically a color change of the medium to pink) due to urease production.
- **TSI Test:** The colony is inoculated into a test tube with TSI medium and incubated for 18-24 hours. The appearance of $H_2S$ (which darkens the medium), gas formation, and glucose fermentation indicate the presence of *Proteus*.
- **Indole Test:** This test differentiates *Proteus mirabilis* (indole-negative) from *Proteus vulgaris* (indole-positive). The reagent is added after incubation, and a color change indicates a positive result.

**Antibiotic Sensitivity Test:**

- After identification, an antibiogram is performed to determine the sensitivity of *Proteus* to various antibiotics. This step is essential for guiding appropriate treatment of the infection, as certain strains may exhibit antibiotic resistance.

## RESULTS AND DISCUSSIONS

*Proteus mirabilis* and *Proteus vulgaris* are two important species within the genus *Proteus*, recognized as pathogenic agents in urinary tract infections. These bacteria have distinctive morphological, cultural, and biochemical characteristics that facilitate their identification in the laboratory.

In our study, both species, *Proteus mirabilis* and *Proteus vulgaris*, were found to be gram-negative, rod-shaped bacteria, measuring approximately 1-3 µm in length and 0.5-0.8 µm in width. The bacteria are highly motile due to the presence of numerous peritrichous flagella that are evenly distributed over the entire surface of the cell.

Both species are gram-negative and stain pink during Gram staining due to their thin peptidoglycan cell wall. Microscopic examination reveals them as pink-stained bacilli, either appearing individually or in irregular clusters.

It was noted that *Proteus mirabilis* and *Proteus vulgaris* grow well on standard bacterial culture media, such as nutrient agar, MacConkey agar, and blood agar. On solid media, such as blood agar and nutrient agar, both species exhibit swarming phenomena (collective movement), characterized by the formation of concentric rings resulting from the migration of bacterial cells.

On MacConkey agar, the colonies are non-lactose fermenters, appearing colorless or pale yellow. On blood agar, *Proteus* colonies may produce partial hemolysis, sometimes giving a transparent appearance around the colonies.

Regarding CLED medium, this medium is frequently used for the culture of bacteria from urine. In the case of *Proteus*, CLED prevents swarming, allowing the formation of isolated, round, and opaque colonies.

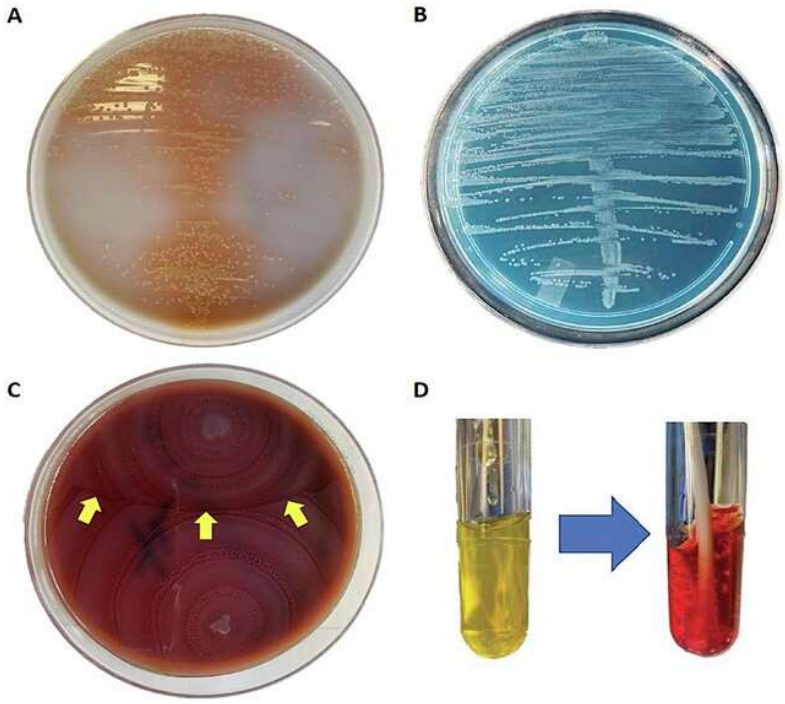


Fig.1. A) Proteus mirabilis culture in chromogenic medium giving rise to cream-colored colonies under a brown halo without swarming. (B) Growth translucent blue colonies in CLED medium. (C) Swarming phenomenon on blood agar. Yellow arrows indicate line of dienes. (D) Rapid urease reaction after 2 h causing a color change by the phenol indicator from yellow (pH: 6.6) to red (pH:8).
https://www.researchgate.net/publication/364423898_The_Brief_Case_Proteus_mirabilis_Causing_Coraliform_Lithiasis_and_Bacteremia_in_an_Elderly_Catheterized_Patient/figures?lo=1

*Proteus mirabilis* and *Proteus vulgaris* have similar biochemical profiles but also some important differences that help differentiate the two species.

Common biochemical tests that we conducted for both species include the urease test, where it was found that both species are urease-positive, producing the enzyme urease that breaks down urea into ammonia and carbon dioxide, leading to an increase in the pH of the medium.

The TSI (Triple Sugar Iron Agar) test revealed that both species ferment glucose, producing acid and gas. They also produce hydrogen sulfide ($H_2S$), which is visible by the black color of the medium due to the formation of ferrous sulfide precipitate.

We deduced that in the catalase test, both species are catalase-positive, releasing oxygen upon the addition of hydrogen peroxide. Regarding oxidase, it was found that both are oxidase-negative, a test useful for differentiating them from other gram-negative bacteria.

Other specific biochemical tests used in the study for differentiation are indole and carbohydrate fermentation. *Proteus vulgaris* is indole-positive, producing indole from tryptophan, which results in a positive reaction in the presence of indole reagents, such as Kovac's or Ehrlich's, turning pink-red. *Proteus mirabilis* is indole-negative and does not produce indole.

In terms of carbohydrate fermentation, both species ferment glucose but do not ferment lactose or sucrose, which is evident on selective media like MacConkey.

Table No. 1: Summary of the Differences Between *Proteus mirabilis* and *Proteus vulgaris*

| Characteristic | *Proteus mirabilis* | *Proteus vulgaris* |
|---|---|---|
| Indole Production | Indole- negative | Indole-positive |
| Urease Activity | Urease-positive | Urease-positive |
| Glucose Fermentation | Yes | Yes |
| Lactose Fermentation | No | No |
| Sucrose Fermentation | No | No |
| Catalase Test | Catalase-positive | Catalase-positive |
| Oxidase Test | Oxidase-negative | Oxidase-negative |

In the medical journal of Microbiology, P. G. H. Peerbooms, A. M. J. J. Verweij, and D. M. MacLaren, in their work "Uropathogenic Properties of *Proteus mirabilis* and *Proteus vulgaris*," highlighted that Krikler (1953) reported prevalences of 17% for *P. mirabilis* and 5% for *P. vulgaris* in the feces of healthy individuals. It is believed that urinary pathogens primarily originate from the intestine, and interestingly, *P. mirabilis* is disproportionately isolated more frequently from patients with urinary tract infections compared to *P. vulgaris*. For instance, Senior (1979) isolated 258 strains of *P. mirabilis* from infected urine, while only four strains of *P. vulgaris* were isolated, two of which were found alongside *P. mirabilis*. This epidemiological data suggests that *P. mirabilis* is more likely to cause urinary tract infections than *P. vulgaris*.

Since both species are genetically and biochemically very similar (Brenner et al., 1978), a comparative study of isolates from both species could provide valuable insights into the bacterial properties important in the pathogenesis of urinary tract infections. Therefore, they compared isolates from fecal samples of both species, taken from the same population, concerning several properties: experimental virulence in a mouse model, growth rates in urine and liquid media, production of hemolysins, hydrophobicity, sensitivity

## CONCLUSIONS

Regarding the results reported for *Proteus mirabilis* and *Proteus vulgaris*, it was found that they are gram-negative, rod-shaped bacteria. *Proteus mirabilis* and *Proteus vulgaris* grow well on common bacterial culture media, such as nutrient agar, MacConkey agar, and blood agar.

On solid media, such as blood agar and nutrient agar, both species exhibit the phenomenon of swarming, characterized by concentric rings that appear from the migration of bacterial cells. The TSI test highlighted that both species ferment glucose, producing acid and gas.

It was deduced that, in the catalase test, both species are catalase-positive, releasing oxygen upon the addition of hydrogen peroxide. In terms of oxidase, both were found to be oxidase-negative, a useful test to differentiate them from other gram-negative bacteria.

*Proteus vulgaris* is indole-positive, producing indole from tryptophan, which results in a positive reaction in the presence of indole reagents, such as Kovac's or Ehrlich's, turning pink-red. *Proteus mirabilis* is indole-negative and does not produce indole.

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