

# **Genus Pseudomonas**

# Gram-negative bacterium of the class y-proteobacteria

and family Pseudomonadaceae Pseudomonas is a ubiquitously distributed opportunistic pathogen, the major cause of nosocomial infections. Inhabits soil and water as well as animal, human, and plant-host-associated environments causes disease in plants, animals and humans, causing serious infections in immunocompromised patients, in some cases, the primary cause of death.

Its nutritional versatility, large number of virulence factors and high antibiotic resistance make this bacterium extremely difficult to eradicate from infected individuals, especially lung infections.

Genome Size: P. aeruginosa has a relatively large genome of 5.5–7 Mb.

## Pseudomonas aeruginosa

Gram-negative, rod-shaped, occurs singly, in pairs, or in short chains. Size: 0.5–0.8  $\times$  1.5–8  $\mu m$ , Non-spore forming Non-capsulated Motile - motile by polar flagella - monoflagellated bacterium

# **Growth and Cultural Characteristics**

*P. aeruginosa* has the ability to survive under a variety of environmental conditions. *P. aeruginosa* grows well at 25°C to 37°C, and its ability to grow at 42°C helps distinguish it from many other *Pseudomonas* species.

Organisms grow aerobically or anaerobically.

Most strains of *P. aeruginosa* produce one or more pigments, including **pyocyanin** (blue-green), **pyoverdine** (yellow-green and fluorescent), and **pyorubin** (red-brown).

Pyocyanin contributes to the persistence of *P. aeruginosa* in the lungs, also interferes with many mammalian cell functions, including cell respiration.

- Cetrimide Agar: The appearance of yellow-green to blue-colored colonies on cetrimide agar indicates the presence of *P. aeruginosa*.
- The colonies are medium-sized with irregular margins. The visual examination of the plates is performed by using ultraviolet light to detect the presence of fluorescein.
- **Cetrimide agar**, or pseudomonas agar Cetrimide, is a selective and differential medium used for the isolation and identification of Pseudomonas aeruginosa from water and clinical specimens.
- **Cetrimide** is a quaternary ammonium compound with bactericidal activity against a wide range Gram-positive and certain Gram-negative organisms, including species of *pseudomonas* other than *Pseudomonas* aeruginosa
- The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium



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Fig.: Pseudomonas aeruginosa on Cetrimide Agar (Source: Difco™ & BBL™ Manual, 2nd Edition)

## P. aeruginosa on Blood Agar

- *P. aeruginosa* produces mucoid-type colonies with a typical metallic sheen.
- β-hemolysis is observed on Blood Agar represented by the clear zone around the colonies.

## Nutrient Agar:

- *P. aeruginosa* forms large opaque and flat colonies with irregular margins and fruity or earthy odor.
- The colonies appear pigmented, but the color depends on the type of pigment produces. Usually, on NA, green-colored colonies can be seen due to the production of Pyoverdin pigment.



Fig.: Pseudomonas aeruginosa on Nutrient Agar

## Antigenicity

**P.** aeruginosa strains produce two distinct types of O antigen (O-Ag): a common polysaccharide antigen (A-band) composed of a homopolymer of d-rhamnose, and an O-specific antigen (B-band) composed of a heteropolymer of three to five distinct sugars in its repeat units. So far, *P. aeruginosa* isolates have been classified into 20 serotypes by the International Antigenic Typing Scheme (IATS).

The lipopolysaccharide (LPS) of *P. aeruginosa* is less toxic than that of other Gram-negative rods, facilitating its establishment of chronic infections by eliciting a low inflammatory response.



## Pathogenicity

Otitis and urinary tract infections in dogs, mastitis in dairy cows and endometritis in horses. Nosocomial infection-common in humans.

*Pseudomonas aeruginosa* is also a leading cause of **keratitis** and **corneal ulcers**. **Respiratory tract infections**.

*Pseudomonas aeruginosa* is one of the causative agents of **bovine mastitis**. Most strains of *Pseudomonas aeruginosa* have a type III secretion system that can induce an increase in the number of somatic cells count in the mastitic milk. In addition, most *Pseudomonas aeruginosa* strains can form biofilms, reducing the effectiveness of antibiotics.

## Diagnosis

## **1.** Isolation and identification:

**Material collection**: Swab from the lesion. Nasal secretion, Urine, etc. Cetrimide Agar, Nutrient Agar

Morphology: Gram Negative rods

MALDI-TOF MS assays:

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is used to map the protein spectrum of microorganisms. The mass spectry data of clinical microorganisms are compared with the standard protein database of known microorganisms to achieve the purpose of identification. Because of its rapid, accurate, sensitive, automated and high throughput, MALDI-TOF MS is an efficient microbial rapid identification technology used in clinical diagnosis, inability to ferment lactose, a positive oxidase reaction, fruity/mushy odor & ability to grow at 42° C.

## 2. Conventional PCR methods

The PCR technique is one of the most important inventions over the past 3 decades. In recent years, PCR-based assays for Pseudomonas aeruginosa also have developed progressively. Kingsford *et al.* established a PCR detection method for Pseudomonas aeruginosa that specifically targets the 16S rRNA gene. The method allows the detection of 1 pg chromosomal DNA or  $1 \times 10^5$  CFU mL<sup>-1</sup> of *Pseudomonas aeruginosa*.

## 3. ELISA

ELISAs were first used by Ueda et al. to detect immunoglobulin M (IgM) and immunoglobulin G (IgG) in horse serum against common serological *Pseudomonas aeruginosa* antigens (protease and elastase).

## 4. Immunofluorescence methods

Immunofluorescence methods do have a good sensitivity and specificity, thus, are considered to be among the most promising assays for pathogens such as Pseudomonas.

## References

Shoaib, M., Islam Aqib, A., Aamir Naseer, M., Ahmad Bhutta, Z., PU, W., Tanveer, Q., ... Hammad, M. (2022). Etiology of Bovine Mastitis. IntechOpen.

Ueda, Y., Sanai, Y., & Homma, J. Y. (1982). Enzyme-linked immunosorbent assay for detection of antibody to Pseudomonas aeruginosa and measurement of antibody titer in horse serum. American Journal of Veterinary Research, 43(1), 55-60.

Weihui Wu, Yongxin Jin, Fang Bai, Shouguang Jin.2015. Chapter 41 - Pseudomonas aeruginosa, Molecular Medical Microbiology (2, Edition), Academic Press.753-767.