# Review on Genus Burkholderia

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**Abstract:** The *Burkholderia* (previously part of *Pseudomonas*) genus but overwhelming biochemical and chemical findings indicated the need to separate them from other Pseudomonas species, and hence, this new genus was created. This genus refers to a group of virtually ubiquitous Gram-negative, obligately aerobic, rod-shaped, oxidative, oxidase positive, catalase positive bacteria that are motile by means of single or multiple polar flagella, with the exception of *Burkholderia mallei* which is non-motile. Members belonging to the genus do not produce sheaths or prosthecae and are able to utilize poly-beta-hydroxybutyrate (PHB) for growth. The genus includes both animal and plant pathogens, as well as some environmentally important species. The conserved RNA structure Anti-hemB RNA motif is found in all known bacteria in this genus. Due to their antibiotic resistance and the high mortality rate from their associated diseases, *B. mallei* and *B. pseudomallei* are considered to be potential biological warfare agents, targeting livestock and humans.
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**Key word:** *Burkholderi, Mallei, pseudomallei*

## 1. Introduction

The genus was named after Walter H. Burkholder, plant pathologist at Cornell University. The first species to receive membership to the genus were transfers from the Pseudomonas genus, on the basis of various biochemical tests. Until recently, the *Burkholderia* genus was inclusive of all *Paraburkholderia* species. However, the *Paraburkholderia* genus is phylogenetically distinct, and can be distinguished from all *Burkholderia* species on the premise of molecular signatures that are uniquely found for each genus. (Sawana A, *et al.*, 2014).

**Taxonomy**

| | |
|---|---|
| Kingdom: | Bacteria |
| Phylum: | Proteobacteria |
| Class: | Betaproteobacteria |
| Order: | Burkholderiales |
| Family: | Burkholderiaceae |
| Genus: | ***Burkholderia*** |

*Burkholderia species* form a monophyletic group within the *Burkholderiales* order of the *Betaproteobacteria*. There are currently 48 validly named species that can be distinguished from neighboring genera (i.e. *Paraburkholderia*) and all other bacteria by conserved signature indles in a variety of proteins (Sawana A, *et al.*, 2014). These indles represent exclusive common ancestry shared among all *Burkholderia* species. Within the genus, there are three distinct monophyletic clusters. One group consists of all species belonging to the *Burkholderia cepacia* complex, another clade comprises *B. pseudomallei* and closely related species, and the last clade encompasses of most of the phytogenic species within the genus, including *B. glumae* and *B. gladioli*. Conserved signature indels have also been discovered that are specific for each of these subgroups within the genus that aid in demarcating members of this extremely large and diverse genus (Gupta RS, 2016).

*Burkholderia* is a genus of Proteobacteria almost exclusively saprophytes found in soil and water whose pathogenic members include *Burkholderia mallei*, responsible for glanders, a disease that occurs mostly in horses and related animals; *Burkholderia pseudomallei*, causative agent of melioidosis; and *Burkholderia cepacia*, an important pathogen of pulmonary infections in people with cystic fibrosis (Yabuuchi, E, *et al.*, 1992).

## 2. Pathogenesis and Pathogenicity

*B. pseudomallei* have a wide host range, including humans and causes melioidosi or pseudoglanderss. Infections are usually systemic and the manifestations depend on the extent and distribution of the lesions. The lesions are nodules that may suppurate and can form in any tissue, including the brain. Most infections are chronic but acute disease with terminal septicemia may occur. The toxins include a lethal factor with anticoagulant activity and a skin- necrotizing proteolytic agent (P.J. Quinn, *et al.*, 1994).

*B. mallei* causes glanders or farcy (the skin form) in the equidae, Humans and members of the cat family are susceptible with occasional infections in dogs,

goats, sheep and camels. Cattle, pigs, rats and birds are resistant to infection. Transmission occurs from infected animals via contaminated food and water and less commonly from aerosols and infection of wounds. Toxins are suspected in the pathogenesis but the mode of action is uncertain. Primary lesions occur at the point of entry with dissemination via the lymphatic system and bloodstream (P.J. Quinn, *et al*., 1994).

The disease can be acute or chronic and many infections are fatal if not treated at any early stage. Infection is characterized by the formation of tubercle-like nodules that frequently ulcerate. Lymphadenitis and lymphangitis that has ulcerated to the horse's skin - this is referred to as farcy (Figure 1).

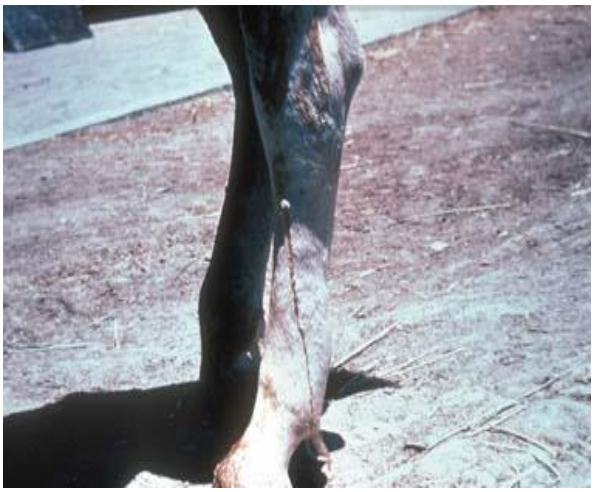

Figure 1. Lymphadenitis and lymphangitis

*B. pseudomallei* is an opportunistic pathogen and an environmental organism, it has no requirement to pass through an animal host to replicate. From the point of view of the bacterium, human infection is a developmental "dead end". Strains which cause disease in humans differ from those causing disease in other animals, by possessing certain genomic islands. It may have the ability to cause disease in humans because of DNA it has acquired from other microorganisms (Price EP, *et al.*, 2010). Its mutation rate is also high, and the organism continues to evolve even after infecting a host. *B. pseudomallei* are able to invade cells (intracellular pathogen) able to polymerise actin, and to spread from cell to cell, causing cell fusion and the formation of multinucleated giant cells. It possesses a uniquely fusogenic type-6 secretion system that is required for cell-cell spread and virulence in mammalian hosts (Toesca, I. J. *et al.*, 2014).

The bacterium also expresses a toxin called lethal factor 1 and the only organism identified that contains up to six different type-6 secretion systems. *B. pseudomallei* is intrinsically resistant to a large number of antimicrobial agents by virtue of its efflux pump mechanism. This mediates resistance to aminoglycosides (*AmrAB-OprA*), tetracycline, fluoroquinolones, and macrolides (*BpeAB-OprB*) (*Shalom* G. *et al.*, 2007). Disease and main hosts of the pathogenic burkholderia species are given below in table 1 (P.J.Quinn, *et al*., 1994).

Table 1. diseases and main hosts of the pathogenic *brukholderia* species

| Species | Host (s) | Disease |
|---|---|---|
| ***B. pseudomallei*** | Many animal species | Melioidosis (Pseudoglanders) |
| | Horses | The disease can mimic glanders |
| | Cattle | Acute and chronic forms with localization of lesions in lungs, joints and uterus |
| | Sheep | Arthritis and lymphangitis predominate |
| | Goats | Loss of condition, respiration and central nervous disturbances, arthritis and mastitis |
| | Pigs | As for goats but in addition diarrhea and abortion |
| | Dogs | Febrile disease with localizing suppurative foci |
| ***B.mallei*** | Horses and other equids | **Glanders**: acute from with high fever, mucopurulent nasal discharge, respiratory signs, septicemia and death within 2 weeks<br>**Chronic forms of glanders**<br>***pulmonary**: small nodules in lungs that break down and discharge B.mallei in to bronchioles<br>***cutaneous form**: farcy, which is a lymphangitis with ulcers along lymphatic vessels of the limbs and chest. The ulcers eventually heal leaving ‘star shaped’ scars. |
| | Humans, and other animals | Acute, septicaemic disease |
| ***B. cepacia*** | Human | cystic fibrosis |

## 3. Laboratory Diagnosis

*B. mallei* and *B. pseudomallei* are among the most dangerous bacteria to work with in laboratory. A biohazard cabinet must be used and all necessary safety procedures taken. Specimens taken varied and depend on the clinical signs and site of lesions (P.J. Quinn, *et al*., 1994).

### Direct Microscopy

Direct microscopy from specimens is of little diagnostic use as the *brukholderia* are medium sized, Gram negative rods with no other distinctive characteristics. A fluorescent antibody technique can be useful for *B. mallei* and *B. pseudomallei*. *In vitro*, *B. pseudomallei* optimal proliferation temperature is reported around 40 °C in neutral or slightly acidic environments (pH 6.8–7.0). The majority of strains are capable of fermentation of sugars without gas formation (most importantly, glucose and galactose; older cultures are reported to also metabolize maltose and starch). Bacteria produce both exo- and endotoxins. The role of the toxins identified in the process of melioidosis symptom development has not been fully elucidated (Haase A, *et al.*, 1997).

### Isolation

The *Burkholderia species* are non-fastidious and will grow on trypticase soy agar, blood agar and on less complex media. The growth of *B. mallei* is enhanced by 1% glycerol. A selective medium for *B. mallei* can be made by adding 1000 unit's polymyxin E, 1250 units' bacitracin and 0.25 mg actidione to 100 ml of trypticase soy agar. The cultures of *B. mallei* and *B. pseudomallei* are incubated aerobically at $37^{O}$C for 24-48 hours. *B. pseudomallei* is not fastidious and grows on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *Burkholderia cepacia* medium) may be used for selective isolation measures 2–5 μm in length and 0.4–0.8 μm in diameter and is capable of self-propulsion using flagella. The bacteria can grow in a number of artificial nutrient environments, especially betaine- and arginine-containing ones. Cultures typically become positive in 24 to 48 hours (this rapid growth rate differentiates the organism from *B. mallei*, which typically takes a minimum of 72 hours to grow (Peacock SJ, *et al.*, 2005).

### Identification

### Colonial morphology

*B. pseudomallei* Colonies growth varies from smooth and mucoid to rough with a dull, wrinkled, have a metallic appearance, and possess an earthy or musty odour. In the smooth form the colonies are round, low-convex, entire, shiny and grayish-yellow. After several days the colonies become opaque, yellowish- brown and umbonate (figure 6). Partial and later, complete haemolysis occurs on sheep blood agar. *B. pseudomalleus grows* on MacConkey agar, utilizing lactose (figure 7) but there is no growth on deoxycholate or salmonella- shigella agars. *B. mallei* growth is slower than that of *B.* pseudomallei but in 24-48 hours the colonies are 1-2 mm in diameter, smooth and white to cream. As the age, they become granular and yellowish or brown in colour. *B. mallei* are unable to grow on MacConkey agar (P.J. Quinn, *et al*., 1994).

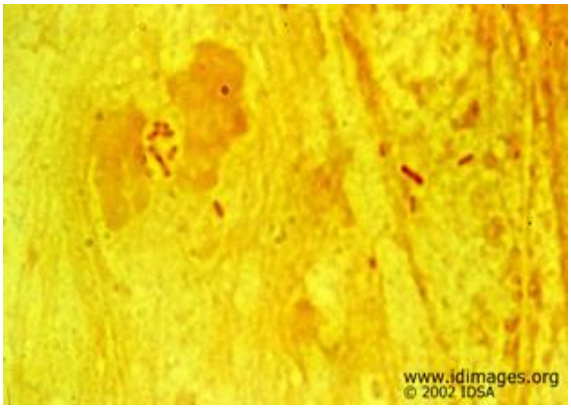

Figure 2. Gram stain showing bipolar staining of *Burkholderia pseudomallei*.

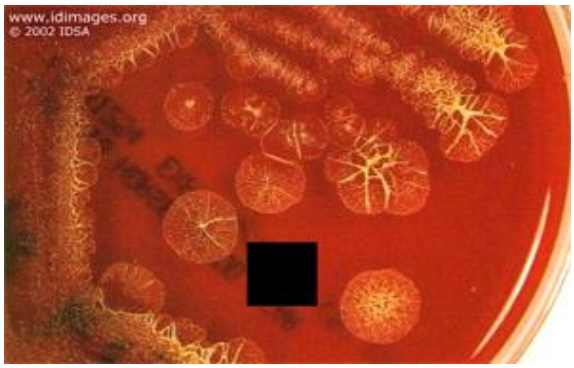

Figure 3. Culture of *Burkholderia pseudomallei*.

On Gram staining, *B. pseudomallei* is a Gram-negative rod with a characteristic "safety pin" appearance (bipolar staining). On sensitivity testing, the organism appears highly resistant (it is innately resistant to a large number of antibiotics including colistin and gentamicin) and that again differentiates it from *B. mallei*, which is in contrast, exquisitely sensitive to a large number of antibiotics. For environmental specimens only, differentiation from the nonpathogenic *B. thailandensis* using an arabinose test is necessary (*B. thailandensis* is never isolated from clinical specimens).

Laboratory identification of *B. pseudomallei* can be difficult, especially in Western countries where it is rarely seen. The large, wrinkled colonies look like environmental contaminants, so are often discarded as being of no clinical significance. Colony morphology is very variable and a single strain may display multiple colony types, so inexperienced laboratory staff may mistakenly believe the growth is not pure *(*Pumpuang, A *et al.,* 2011).

The organism grows more slowly than other bacteria that may be present in clinical specimens, and in specimens from non-sterile sites, is easily overgrown. Non sterile specimens should, therefore, be cultured in selective media (e.g., Ashdown's or *B. cepacia* medium) showing the characteristic cornflower head morphology (figure 5). For heavily contaminated samples, such as faces, a modified version of Ashdown's that includes norfloxacin, amoxicillin, and polymyxin B has been proposed. In blood culture, the BacT/ALERT MB system (normally used for culturing mycobacteria) by bioMérieux has been shown to have superior yields compared to conventional blood culture media (Jorakate P, *et al.,* 2015). Even when the isolate is recognised to be significant, commonly used identification systems may misidentify the organism as *Chromobacterium violaceum* or other non-fermenting, Gram-negative bacilli such as *Burkholderia cepacia* or *Pseudomonas aeruginosa*. Routine biochemical methods for identification of bacteria vary widely in their identification of this organism: the API 20NE system accurately identifies *B. pseudomallei* in 99% of cases, as does the automated Vitek 1 system, but the automated Vitek 2 system only identifies 19% of isolates (Amornchai P, *et al.,* 2007).

Figure 4. *B. pseudomallei* colonies on a blood agar plate.

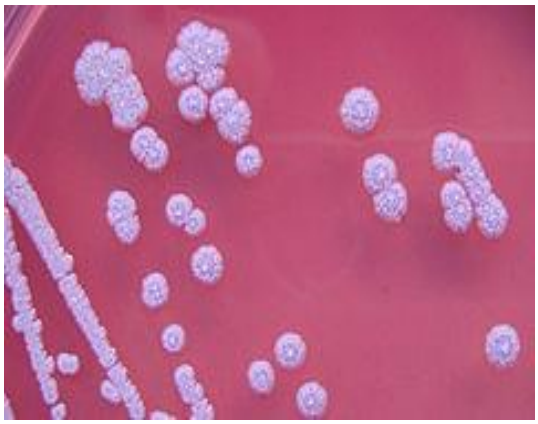

Figure 5. *B. pseudomallei* colonies on Ashdown's agar

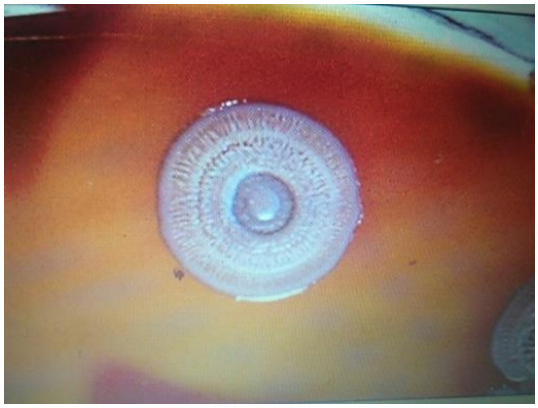

**Figure 6.** *B. pseudomallei* on Sheep blood agar

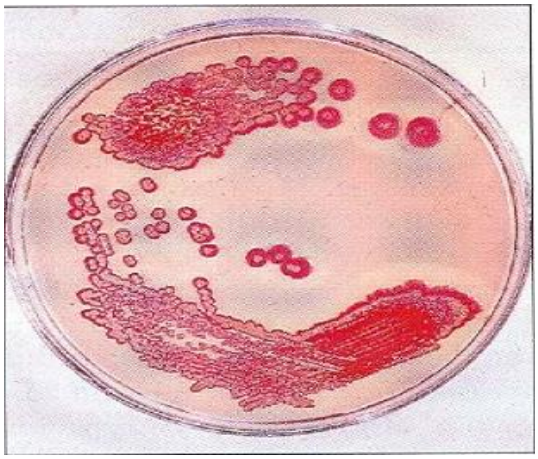

**Figure7.** *B. pseudomallei* on MacConkey agar

**Biochemical characteristics**

The characteristic colonial appearance, including odour and strong oxidase reaction will give a presumptive identification of *B. pseudomallei* and *B.mallei* are given in table 2 (P.J.Quinn, *et al*., 1994).

Table 2. Main characteristics of the pathogenic brukholderia species and pseudomonas species

| Characteristics | *B. pseudomallei* | *B.mallei* | *P.aeruginosa* |
|---|---|---|---|
| **Pigment produced** | -(but colonies become orange to cream) | -(but colonies become yellow to brown) | ++ |
| **Odour** | Putrid becoming earthy | - | Fruity, grape like |
| **Growth on MacConkey agar** | + | - | + |
| **Growth at 5OC** | - | - | - |
| **Growth at 42OC** | + | - | + |
| **Oxidation of glucose** | + | + | + |
| **Lactose** | + | - | - |
| **Arginine dihydrolase** | + | (+) | + |
| **Reductionof nitrate to nitrite** | + | + | + |
| **Reduction of nitrate to N2 gas** | + | - | +/- Variable |
| **Motility** | + | - | + |

**Immunological tests**

Melioidosis: complement- fixing and indirect haemagglutinating antibodies are produced after infection with *B. pseudomallei*. However the diagnosis of melioidosis depends more on the isolation and identification of the bacterium than on clinical findings and serological taste (P.J.Quinn, *et al*., 1994).

Glanders: both cell-mediated and antibody-mediated responses are elicited by infection with *B. mallei*. Complement fixation, agglutination, indirect haemagglutination and counter-immunoelectrophoresis tests are used in the diagnosis of glanders. False positive reactions may occur in areas where meliodosis is endemic as the serological tests may detect antibodies that cross react with those of *B. pseudomallei*. Mallein test are used to demonstrate the hypersensitivity developed after infection with *B. mallei*. Mallein is a glycoprotein extracted from the bacterium. In infected animals, subcutaneous inoculation of mallein (subcutaneous test) results in swelling at the injection site and fever (P.J. Quinn, *et al*., 1994).

Molecular methods (PCR) of diagnosis are possible, but not routinely available for clinical diagnosis. Fluorescence *in situ* hybridization has also been described, but has not been clinically validated, and it is not commercially available. In Thailand, a latex agglutination assay is widely used, while a rapid immunofluorescence technique is also available in a small number of centers (Wuthiekanun V, *et al.*, 2005).

**Animal Inoculation**

The straus reaction is seen in male guinea pigs inoculated intraperitoneally with infected material containing either *B. pseudomallei* or *B. mallei*. A localized peritonitis and a purulent inflammation of the testicular tunica vaginals develops in 2-3 days (P.J. Quinn, *et al*., 1994).

**Disinfection**

*B. pseudomallei* is susceptible to numerous disinfectants, including benzalkonium chloride, iodine, mercuric chloride, potassium permanganate, 1% sodiu *B. pseudomallei* is effectively killed by the commercial disinfectants, Perasafe and Virkon. The microorganism can also be destroyed by heating to above 74 °C for 10 min or by ultraviolet irradiation. *B. pseudomallei* are not reliably disinfected by chlorine (Howard, K. and Inglis, TJ. 2005).

**Antibiotic treatment and sensitivity testing**

The pattern of resistance to antimicrobials is distinctive, and helps to differentiate the organism from *P. aeruginosa*. The majority of *B. pseudomallei* isolates are intrinsically resistant to all aminoglycosides (via an efflux pump mechanism), but sensitive to co-amoxiclav: this pattern of resistance almost never occurs in *P. aeruginosa* and is helpful in identification. Unfortunately, the majority of strains in Sarawak, Borneo, are susceptible to aminoglycosides and macrolides, which means the conventional recommendations for isolation and identification do not apply there (Podin Y, *et al.*, (2013).

The antibiotic of choice is ceftazidime. While various antibiotics are active *in vitro* (e.g., chloramphenicol, doxycycline, co-trimoxazole), they have been proven to be inferior *in vivo* for the treatment of acute melioidosis. Disc diffusion tests are

unreliable when looking for co-trimoxazole resistance in *B pseudomallei* (they greatly overestimate resistance) and Etests or agar dilution tests should be used in preference (Wuthiekanun V, *et al.*, 2005). The actions of co-trimoxazole and doxycycline are antagonistic, which suggests these two drugs ought not to be used together. The organism is intrinsically resistant to gentamicin and colistin, and this fact is helpful in the identification of the organism. Kanamycin is used to kill *B. pseudomallei* in the laboratory, but the concentrations used are much higher than those achievable in humans (Trunck L. *et al*., 2009).

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