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Original Research

A Comprehensive Review of Burkholderia sp.

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Abstract:

This comprehensive review gives a systematic overview of the genus Burkholderia by its taxonomy, drug resistance, various aspects of pathogenicity in humans and animals as well as diagnostics methods and phylogeny. Initially, Pseudomonas cepacia was discovered to include Burkholderia but now this genus contains a lot of species and each new one has different roles, such as strict ecological niche ecology, plant symbiosis, and pathogenicity towards plants, animals and humans. Progress of molecular biology area has dramatically improved the discrimination and diagnosis of the Burkholderia that established its genetic diversity and quite intricate responses to the host(s). The description here explores the disease transmission ways of Burkillerdiaceraillea species, that have serious clinical consequences meaning for the humans and animals including B. cepacia complexa, B. pseudomallei, and B. mallei species. There will be the emergence of antimicrobial resistance in the trans-Burkholderia reservoirs which require extensive efforts over time through the research that will provide an ideal therapeutic approach. In addition to this analysis, the chapter discusses purely evolutionary relationships inside Burkholderia generes. With this in mind, we can detect accurate species identification of the genus to investigate the epidemiology, develop targeted treatments and prevent outbreaks.

Keywords: Burkholderia, pathogenicity, phylogeny, genetic diversity

1. Overview of Burkholderia

Burkholderia was initially described as including Pseudomonas cepacia and similar bacteria that belonged to rRNA homology group II (Elery, 2023). The genus was named after John W. Burkholder, who studied P. cepacia bacteria extensively. Over time, many species have been reclassified under this taxon, leading to its inclusion of a wide range of bacteria (Duong, 2022). However, the distinction between Burkholderia and other rRNA homology groups and genera like Ralstonia was never clearly defined until the proposal to split Burkholderia into two genera (Loeven, 2022).

Burkholderia is a large taxon with over 60 species and Paraburkholderia has around 10 species. They have varied characteristics including different

colony sizes, motility, and metabolic abilities. Some species can fix nitrogen. (Bellés-Sancho et al., 2023).

Burkholderia is a genetically diverse proteobacteria found in various environments, including acidic soils and the rhizosphere of plants. It plays different ecological roles, degrades pollution, and can harm crop plants (Bach et al.2022). It has been split into Burkholderia and Paraburkholderia genera due to gene sequence data. Some species initially classified as Burkholderia are now grouped under Ralstonia and Pandorea due to genotypic differences. (Mullins & Mahenthiralingam, 2021).

1.1. General characteristics

Members of the Burkholderia Homo Microbiota belong to the Gram-negative, aerobic rods grouping. While they mitigate some of the effects of nitrate, it is not entirely eliminated. The 16S rRNA sequence is the foundation for evolutionary novel strain categorization. But just recently, we have four complexes known that are designated as B. cepacia complex, B. pseudomallei complex, B. cocovenenans complex, and plant-associated complex, respectively. B. cepacia is a unique kind and can be both potently pathogenic or non-pathogenic. B. pseudomallei, a soil saprophyte, is highly prevalent in the Southeast Asia and Northern Australia regions and is responsible for melioidosis. Besides influencing the type of corn, pathogens can also control the corn appearance such as yellow rot. Legume-associated consortium may establish a symbiotic interplay with plant rhizosphere and lead to an higher crop productivity decrease due to soil nitrogen. (Maki et al., 2022) (Espinosa-Victoria et al., 2020).

Among all the bacteria genera, Burkholderia is one of the most versatile bacteria, since it is able to survive in any surroundings. It involves the mixtures of pathogenic and nonpathogenic strains respectively so that the plants and animals can also be invaded. Non-pathogenic strains have been shown to re-source the oblique structures of dissimilar aromatic compounds. The indeterminate species was aligned to the branch and a crotch type genus was then created with B. cepacia (previously known as P. cepacia but differentiated by genus) as its type species. (Pal et al., 2022).

1.2. Taxonomy and classification

Recent technologies have set a new Burkholderia taxonomy classification scheme, through a molecular presence, and the combination of phage and ribosomal 16S rRNA analysis. Among others, the genus comprises no less than 28 valid species that can be added with the capturing of other species through further re-search effort. Burkolderia, on the other hand, can be more used by molecular sources since it readily can be cultivateted and DNA is available (Jin et al., 2020). At the beginning, 16S rRNA gene sequencing helped to make the difference between this bacterium and other different strand of bacteria from beta subclass of proteobacteria. However, recent taxonomic markers have emphasized the importance of classifying Burkholderia alongside other bacteria using all available information. (Pratama et al., 2020)

Domain: Bacteria
Phylum: Pseudomonadota,
Class: Betaproteobacteria,
Order: Burkholderiales,
Family: Burkholderiaceae,
Genus: Burkholderia

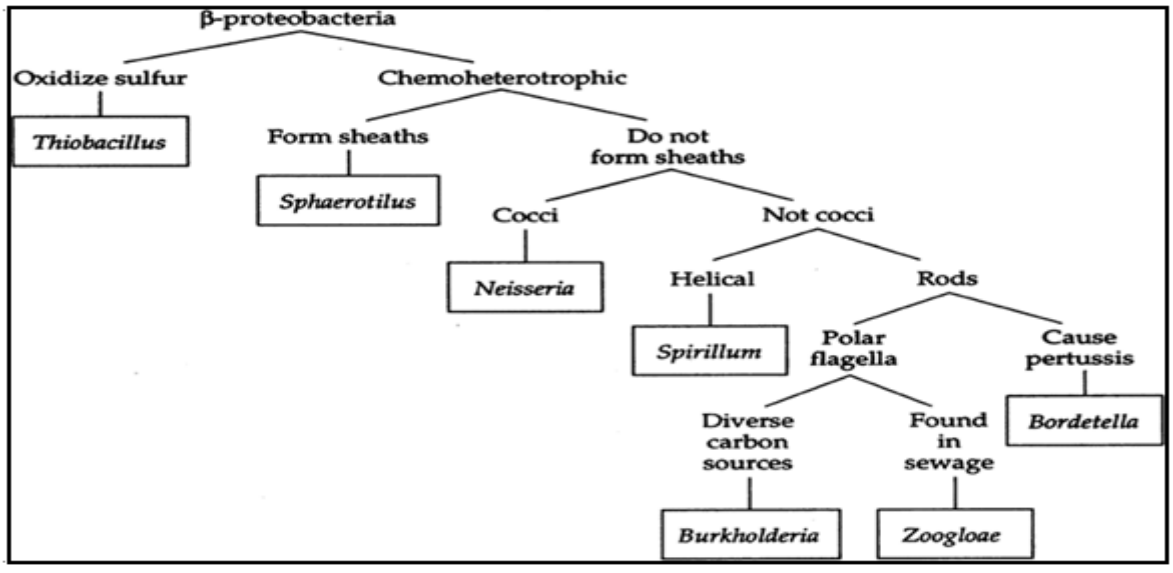

Figure 1: Classification of beta-proteobacteria

2. Habitat of Burkholderia

Burkholderia species are associated with plants, forming symbiotic or pathogenic relationships with monocot and dicot plants. These interactions define the species, with some species solely existing in plants. B. glumae causes bacterial rice grain rot, leading to significant losses in rice crops in Japan and Korea. The B. cepacia complex, consisting of at least 18 closely related species, has emerged as pathogens in plants, animals, and humans. (Choi et al., 2021).

Burkholderia species are versatile in their habitats, thriving in natural and artificial environments. They are significant for bioremediation due to their ability to grow on different substrates. Research has focused on their role in degrading polychlorinated biphenyls, toxic man-made chemicals. (Morya et al., 2020).

2.1. Natural environments

Both Burkholderia species and plants live there, albeit the Burkholderia species can be found in various environments, ranging from soil to water or even the atmosphere. B. pseudomallei is often discovered near rice fields and in the humid soils and waters and can be linked to various agricultural activities. In addition to it, the bacteria can also be observed in the roots of the palm, cysts, and in the cases of osteomyelitis.(Jayasinghearachchi et al., 2023). Burkholderia species comprise the largest pangenomes, which are based on the interconnected nature of these microorganisms. They are also highly versatile microorganisms that utilize the different nutrient sources and various environments at the same time.(Lee et al., 2021)

2.2. Association with plants

Burkholderia species can be found in vastly diverse ecological niches that include soil, water, and even microbial communities that live on or in tissues of plants or animals. There are some strains emerging as pathogens to animal and plant life, but the majority are apparently incapable of damaging us (Choi et al., 2021). A bacteria called Burkholderia has been characterized as having benefits for crops and having bioremediation activity (Romero-Gutiérrez et al., 2020). Establishing collaborative partnerships with plants is also one of their strengths because they promote plant growth with the NPK (nitrogen, phosphorus and potassium) nutrients (Hamidizade et al., 2024). Burkholderia species also have a role as endosymbionts on the plant roots as they promote the growth of the plant. They are omnivores that have got the capacity to relate to a variety of host species. Through gene alterations they can be friends or enemies (Choi et al., 2021). There are some reports on Burkholderia isolates that gain virulence genes from the pathogens causing severe infections in animals or humans through horizontal gene transfer. This diversity of niches and hosts allows Burkholderia to evolve a broader spectrum of pathogenic and plant-beneficial traits (Romero-Gutiérrez et al., 2020).

3. Identification of Burkholderia

Phenotypic methods and molecular techniques are widely used in identification and classification of micro-organisms (Prommachote et al., 2022). Recently, these methods were employed for the characterization and identification of B. pseudomallei, B. cepacia, and B. mallei. Growth on Ashdown's agar becomes the important primary identification test for differentiation of B. pseudomallei and B. cepacia from other Burkholderia species. B. pseudomallei will grow as blue colonies within 24 h, unlike other Burkholderia species (Orababa et al., 2023). Colonies of B. cepacia are brown pigment producing but can be distinguished from B. pseudomallei by the absence of a zone of clearing around the colony on Ashdown's agar; they will often grow but without giving definitive results (Prommachote et al., 2022). B. mallei will grow white colonies on Ashdown's agar. Ashdown's agar is a semi-selective agar used for isolation of Burkholderia species from other BCC and Pseudomonas species. B. mallei, B. pseudomallei, and Burkholderia species are oxidase positive, an important diagnostic test as other non-glucose fermenting bacilli are oxidase negative. Glucose

non-fermenting isolates should be confirmed by BCC-specific PCR. API 20NE is a useful identification strip for Burkholderia species which are oxidase and glucose non-fermenting (Purushotham et al., 2021). It can differentiate between some species and now with the use of API web is becoming better at identifying new species by obtaining extra information from strip codes. B. pseudomallei and B. cepacia will grow typical of Pseudomonas-like colonies on trypticase soy agar at 42°C and are catalase positive (Orababa et al., 2023). This is useful for identification of strains isolated from patients where mislabelling of Pseudomonas species may occur. B. pseudomallei is latex agglutination test positive for detection of its capsular polysaccharide (Prommachote et al., 2022). This test is currently available only in Thailand but has potential as a rapid diagnostic test to differentiate from other glucose non-fermenting bacilli. Phage typing is an old technique and has limited application for Burkholderia identification but has been used for B. pseudomallei. Randomly amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE) have been used for many bacterial identification and epidemiological studies. Primers directly specific for B. pseudomallei are now available which can be used to produce more definitive results from RAPD. It has been used successfully for differentiating B. mallei and B. pseudomallei strains in Malaysia and Thailand. It is important that these techniques are also used for typing new Burkholderia species to prevent misidentification. (Mohanty et al., 2023)

3.1. Phenotypic methods

When PCR tests were done on B. pseudomallei strains, it was difficult to differentiate them from B. thailandensis due to their high similarity. Phenotypic tests were not effective in distinguishing between the two. Some B. pseudomallei isolates tested positive for oxidase, arabinose, and negative for mannitol, but these tests also gave positive results for B. thailandensis (Merritt & Inglis, 2024). The only test that was 100% specific to B. pseudomallei was the assimilation of L-arabinose. Therefore, more emphasis should be placed on testing metabolic pathways to find unique tests for B. pseudomallei. In a separate study, MAC and Ashdown's agar were found to yield non-burkholderia isolates that resembled B. pseudomallei. Differentiating between burkholderia and non-burkholderia isolates was challenging. Both media showed high lactose fermentation rates in non-burkholderia isolates (Suvanasuthi et al., 2023). Although differences in colony appearances between B. pseudomallei and B. thailandensis were observed on Ashdown's agar, there was no direct comparison of the two species on the same media batch. Further studies comparing selective and differential media for isolating B. pseudomallei and other Burkholderia species should be conducted. (Syed, 2022)

3.2. Molecular techniques

Non-radioactive chemiluminescent labeling of PCR products specific for B. cepacia can detect its presence in clinical or environmental samples. Southern blotting tests using DNA probes are valuable in determining strain similarity. Digital image analysis of PCR and hybridization products can differentiate Burkholderia species. DNA sequencing is the ultimate means of identifying Burkholderia. (Janesomboon et al., 2021)(Fu et al., 2022)

Molecular techniques have improved Burkholderia detection through PCR, which has been modified for rapid identification. Primers can be based on 16S rRNA gene or other genes specific to Burkholderia. However, 16S rRNA gene sequences do not provide enough resolution for species or strain identification. RAPD has been used to identify unique DNA sequences for B. cepacia, but reproducing fingerprints can be challenging. (Fu et al., 2022)(Aung et al., 2023)

4. Pathogenicity of Burkholderia in humans

The clinical manifestations of Burkholderia infections in humans vary depending on the species of the infecting organism. B. mallei and B. pseudomallei are the most severe and well-documented infections in humans. B. mallei primarily affects solipeds and humans in the tropics and subtropics (Nasiri et al., 2023). The

disease glanders, caused by B. mallei, is still prevalent in certain regions. Human transmission occurs through skin abrasions or inhalation. There are three forms of B. mallei infection, with 10-15% of exposed individuals developing clinical disease. Localized infections produce cutaneous or nasal lesions, while disseminated infection can result in acute or chronic systemic disease (Santos et al., 2020). If left untreated, the fatality rate for B. mallei infections is 95%. B. mallei has been used as a biological weapon in the past, raising concerns about its potential re-emergence. (Taitt et al., 2024)

4.1. Clinical manifestations

Some strains of Burkholderia, particularly the B. cepacia complex (BCC), are pathogenic to humans, particularly those with cystic fibrosis (CF). The BCC can infect the lungs and cause a rapid decline in health. In non-CF patients, BCC infections are similar to those caused by Pseudomonas aeruginosa (Tavares et al., 2020). Other Burkholderia species like B. mallei and B. pseudomallei cause infections in animals and humans, leading to glanders and melioidosis, respectively. B. mallei was eradicated from the US but weapons-grade B. mallei is now a concern. Melioidosis is low risk but endemic in tropical regions. Due to the severity and prevalence of these diseases, the pathogenesis of Burkholderia species has been heavily studied. (De et al., 2021)

Burkholderia sp. can thrive in low-nutrient environments, growing in soil and within hosts. They inhabit the rhizosphere of plants, acting as saprophytes or parasites. Burkholderia have developed catabolic activities, enabling them to exploit different niches (Romero-Gutiérrez et al., 2020). This ability is partly attributed to the large B. cepacia genome, encoding various metabolic pathways. (Ghazali et al., 2023)

4.2. Mechanisms of infection:

Type III and Type VI involvement is one of the significant factors in B. cepacia for external invasion and overall survival among those tested. Whilst a functional Type III system and Bsa T3SS is important for invasion of both red line respiratory and macrophage cells by B.pseudomallei and B.mallei , the precise role of these systems in disease pathogenesis is still unknown. In addition to that, T3SS Bsa contains in intercellular spreading and helps expanding the lesions of infection from agnosida and glanders (Bzdyl et al., 2022). Furthermore, the envelope built by type VI system protects Burkholderia bacteria and lets them live inside the cell as active agents controlling the response to the stress and affect cell structure and function. It is also now acknowledged that this resistive mechanism is indeed one of the key factors in manifestation of virulence.(Choh et al., 2021)

5. Pathogenicity of Burkholderia in animals:

In mammalian species, especially in rodents, bacterial multiplication and the spread of infections have been observed, specifically with B. pseudomallei and B. mallei. B. mallei has caused significant infections in horses in the past, primarily due to contaminated feed and water (Shanmugasundaram et al., 2022). Research is needed to understand the pathogenicity characteristics affecting both humans and animals, such as isolation, characterization, and comparison of B. pseudomallei or B. mallei isolates. The disease is currently being tested for vaccine candidates worldwide. (Narayanan, 2022)

High levels of Burkholderia sp. in soil can contaminate water, especially in wet regions, spreading diseases among animals. Prior cases in Australia and Malaysia were linked to animals infected from contaminated water or soil. (Jayasinghearachchi et al., 2023)

5.1. Animal hosts:

Animal hosts primarily include humans, horses, cows, and rats. Majority of knowledge comes from studying B. mallei and B. pseudomallei. These bacteria are host-restricted and most animal infections occur from environmental exposure. B. mallei causes glanders in horses and humans; it has never been isolated from hosts other than horses or humans (Desoutter et al., 2024). B. mallei and B. pseudomallei have a close genetic relationship, suggesting B. pseudomallei is the progenitor of B. mallei. Glanders is nearly eradicated in Western Europe and North America

but remains endemic in the Middle East, Africa, Asia, and Central/South America. B. mallei infection in humans is usually incidental, manifesting with similar symptoms as in horses. Clinical symptoms vary and include skin lesions, ulcers, nasal discharge, and pulmonary involvement. There are sub-acute and chronic forms, and infection can lead to carrier state. Disease mortality is generally low. (Wang et al., 2021)

5.2. Disease outcomes:

B. pseudomallei causes various clinical manifestations, mimicking chronic diseases and leading to misdiagnosis. (Purushotham et al., 2021). Acute disease may occur following respiratory infection with B. pseudomallei, characterized by severe pneumonia and high fatality rate (Chang and Lee2023). Glanders and B. mallei infection also have similar mortalities, manifesting as fever, septicemia, pneumonia, pleuritis, and lung abscesses. B. cepacia is commonly found in cystic fibrosis patients, leading to declining respiratory function and death. (He et al., 2023). Many pathogenic bacteria can cause severe clinical symptoms by evading the immune system through various mechanisms. Burkholderia infections can result in either acute or chronic disease that lasts for a long time. (Meumann et al., 2024)

6. Epidemiology of Burkholderia:

B. cepacia was recognized as a human pathogen in 1977 during an epidemic of "cepacia syndrome" bronchopneumonia and septicemia in Cleveland, USA. It later reoccurred in Toronto in 1986. Since then, it has been a concern in CF patient populations globally (Behroozian et al., 2023). In 1994, outbreak strains and chronic infections were linked to poor prognosis and increased mortality rates in CF populations in the USA and Europe. B. cepacia was called the "most dangerous of all CF pathogens" in 1995 (Luk et al., 2022). However, the epidemiology of B. cepacia infection in CF has changed, with localized infections in non-CF patients and an increased range of host infections. It is now classified as a Biosafety Level 2 organism. (Luk et al., 2022)

Burkholderia cepacia and B. pseudomallei, traditionally soil organisms, have been found in various environments causing diseases. B. gladioli and B. plantari are implicated in plant diseases but show little evidence of infecting humans (Parfitt, 2022). B. mallei is a host-restricted pathogen, while B. pseudomallei is only found in specific regions. Hence, B. cepacia is the only species that has truly emerged, impacting cystic fibrosis patients and beyond. (LaBonte, 2022)

6.1. Global distribution:

The genus Burkholderia includes over 20 environmental bacterial species with diverse habitats. B. pseudomallei is found in endemic areas around the equator, while B. mallei caused glanders in horses worldwide. B. vietnamiensis and B. cepacia contaminate pharmaceuticals and disinfectants, while B. dolosa and B. cenocepacia infect cystic fibrosis patients (Loaiza et al., 2021). Burkholderia species cause infections and diseases in humans and animals due to their wide environmental distribution. (Bzdyl, 2021)

6.2. Outbreaks and transmission:

The largest outbreak of B. pseudomallei in Uganda in 2007 led to 411 cases, including 61 melioidosis cases with 39 deaths. All cases were linked to heavy rain in Gulu, causing contamination of the water supply (Green, 2022). Investigations led to reporting to the U.K. government and funding for surveys in Uganda to identify B. pseudomallei hotspots and assess risk factors. This research outcome is highly anticipated in the melioidosis community. (536 characters) (Topluoglu et al., 2023)

7. Virulence factors of Burkholderia:

Bacteria express virulence factors to promote disease. B. pseudomallei LPS is a potent inducer of TNF-α, comparable to E. coli LPS. Other toxins and factors associated with B. pseudomallei include metalloprotease, haemolysin, phospholipase C, chemotactic factor, and a type III secretion system (TTSS). TTSS is linked to invasion and survival within host cells. Utaisincharoen et al. (2001) showed the role of TTSS in the escape and survival of B. pseudomallei. (Bzdyl et al., 2022)

7.1. Toxins and secreted proteins:

Two protein groups crucial for B. pseudomallei and B. mallei pathogenesis have been identified: proteins that damage or kill host cells, and those that facilitate intracellular survival and replication (Khan et al., 2022). The functions of these protein sets are intertwined, suggesting some proteins may have multiple roles during infection. The main cytotoxin studied in B. pseudomallei is a 111 kDa haemolysin with similarity to other pore-forming toxins (Baker et al., 2021). Mutants lacking this protein showed reduced cytotoxicity but no loss of virulence in a mouse model, indicating the presence of alternative cytotoxins or redundant cell damage pathways. B. thailandensis has a similar haemolysin and may be used as a model for studying Burkholderia pathogenesis. (Baker et al., 2021)

7.2. Adhesion and invasion mechanisms:

Bloodworth and Holman (1992) found that B. pseudomallei binds more strongly to human epithelial cells than non-phagocytic cells like mouse fibroblasts. A study showed that a 67kDa protein mediates both bacterial invasion and adhesion to human epithelial cells. In addition, this protein also fuels the bacterium's agglutinin, which facilitates adhesion between B. pseudomallei and erythrocytes. Agglutinin protein, found in B. pseudomallei, ensures higher attachment of this bacterium with alveolar type 2 cell. However, mycobacterial surface antigen, when bound to this cell, decreased B. pseudomallei presence in the alveolar type 2 cells (Choh et al., 2021). Members of the type 2 imine are taken into cells by actin-dependent phagocytosis. Here, there is an escape of the particular member from the first phagosome to proliferate in the cytoplasm; (Bzdyl et al., 2022). Most Burkholderia virulence factor research focuses on B. pseudomallei, making it unclear if factors are similar to those of B. cepacia or B. mallei. Adherence to endothelial cells and invasion of type 2 cells require specific virulence factors not found in environmental Burkholderia (Choh et al., 2021). Burkholderia vietnamiensis, an opportunistic pathogen in CF patients, upregulates a novel pili related to its ability to utilize inorganic phosphate. This contrasts with B. mallei, a horse pathogen that loses this capacity. The pili plays a crucial role in biofilm formation, highlighting B. vietnamiensis' distinct virulence factors. (Bzdyl et al., 2022).

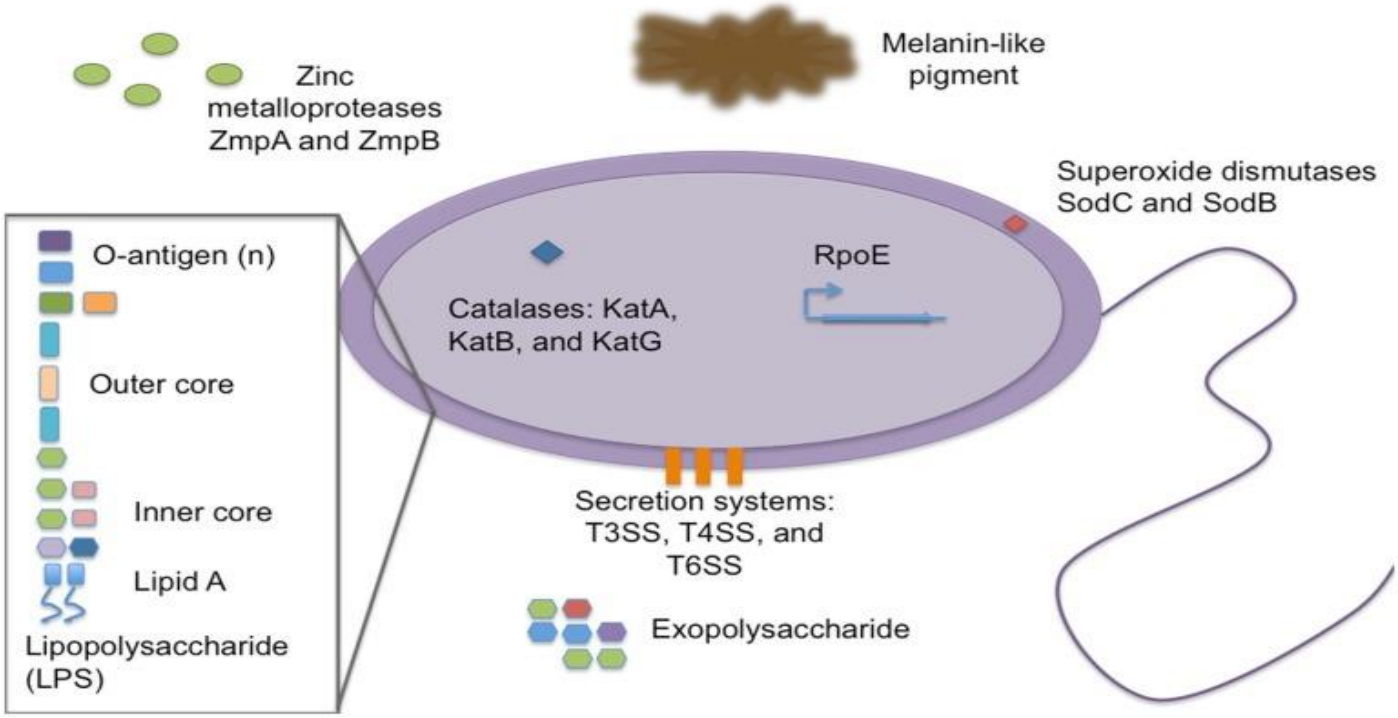

Figure 2: The virulence factors of Burkholderia cepacian (Porter & Goldberg, 2011)

8. Antibiotic resistance in Burkholderia:

Antibiotic resistance is a growing concern in healthcare. Bacteria like E. coli can quickly develop resistance, making antibiotics ineffective. Increasing the extent to which these bacteria can remain resistant to many antibiotics is particularly concerning with Bcc, which can be naturally resistant to numerous antibiotics. Bcc is tolerant to various stress conditions, and there is a probability to contaminate pharmaceutical products (Beca and others, 2023). They have proven to be the nosocomial pathogens, responsible for the often-severe infections in the patients with cystic fibrosis and chronic granulomatous disease, as well as melioidosis, a fatal disease in Southeast Asia and Northern Australia. Bacterial resistance to antibiotics is the main reason of their ineffectiveness which causes irremediable trouble. The research should cover this issue as well as suggest an effective strategy. The mechanisms of resistance may be a consequence of inherent or acquired factors. Extensively, inherent resistance arises when bacterial cells abstain from the target site or withdraw the drug from itself. Resistance that is acquired is obtained by the transforming into another form by bacteria mutations or gain of genetic material like plasmids and transposons. (Häfliger et al., 2020)

8.1. Mechanisms of resistance:

A particular study revealed that clinical isolates of B. cepacia complex were more resistant to the majority of antibiotics when compared to environmental isolates. Various genes were detected in B.cenocepaciathat contributes towards the AMR. Knocking-out the antibiotics genes shorten the course of disease (Hrenovic et al., 2022). On the other hand, antibiotic resistance might drive the surviving bacteria with more strength to live. Identification of the resistance genes would contribute to the notion that some specific antibiotics may cause the formation of drug-resistant species of Burkholderia. Herein lies an important aspect of antibiotic studies for Burkholderia treatment, which is optimal choice.(Okomo et al., 2020). Burkholderia has demonstrated cross-resistance with penicillin, chloramphenicol, erythromycin, cycletracycline, gentamicin, polymyxin B, trimethoprim, and cotrimoxazole. It remains unclear exactly what leads to the resistance but the development of the resistance apparently occurs as a result of exposure to antibiotics during treatment.(Kavanaugh et al., 2021)

8.2. Impact on treatment options:

High discontinuation rates in the trial using combination therapy to eradicate Bcc from CF patients' respiratory tracts led to limited benefit in long-term suppression of Bcc. This raises doubts about the effectiveness of new antibiotics for treating Burkholderia infections in immunocompromised individuals. (Lord et al., 2020).

The presence of multidrug resistance in Burkholderia species limits therapy choices. Combination antibiotic regimens are used to increase bacterial clearance, using antibiotics of different classes to exploit diverse mechanisms. Ceftazidime and sulfamethoxazole-trimethoprim are used together for Bcc infections, with improved outcomes. However, if the strain is resistant to one or more antibiotics, combination regimens may be compromised. This may result in continued use of combination therapy or increased dosage of the remaining effective drug, with cost and toxicity implications. Failure to resolve Burkholderia infections is problematic for individuals with CGD or cystic fibrosis. (Gutiérrez Santana & Coria Jiménez, 2024).

Burkholderia species' intrinsic resistance requires consideration of species and their sensitivities when choosing antibiotics. Bcc, linked to nosocomial infections, is highly resistant, impacting treatment decisions and patient outcomes. (Kwayess et al., 2022).

9. Phylogeny of Burkholderia:

Yabushita discovered a new rRNA superbranch in 1992. 16S rRNA gene sequencing is commonly used for species identification and phylogenetic relationships in Burkholderia. The 16S-23S rRNA gene ITS provides higher taxonomic resolution. DNA-DNA hybridization is time-consuming and impractical for distinguishing species (Jin et al., 2020). Multilocus sequence analysis is proposed

as a simpler method for Burkholderia taxonomy. Typing methods are important for studying bacterial pathogens. The taxonomy of Burkholderia is poorly defined, leading to inaccuracies in species identification. A consensus approach and phylogenetic framework should be used for species identification. (Fu et al., 2022)

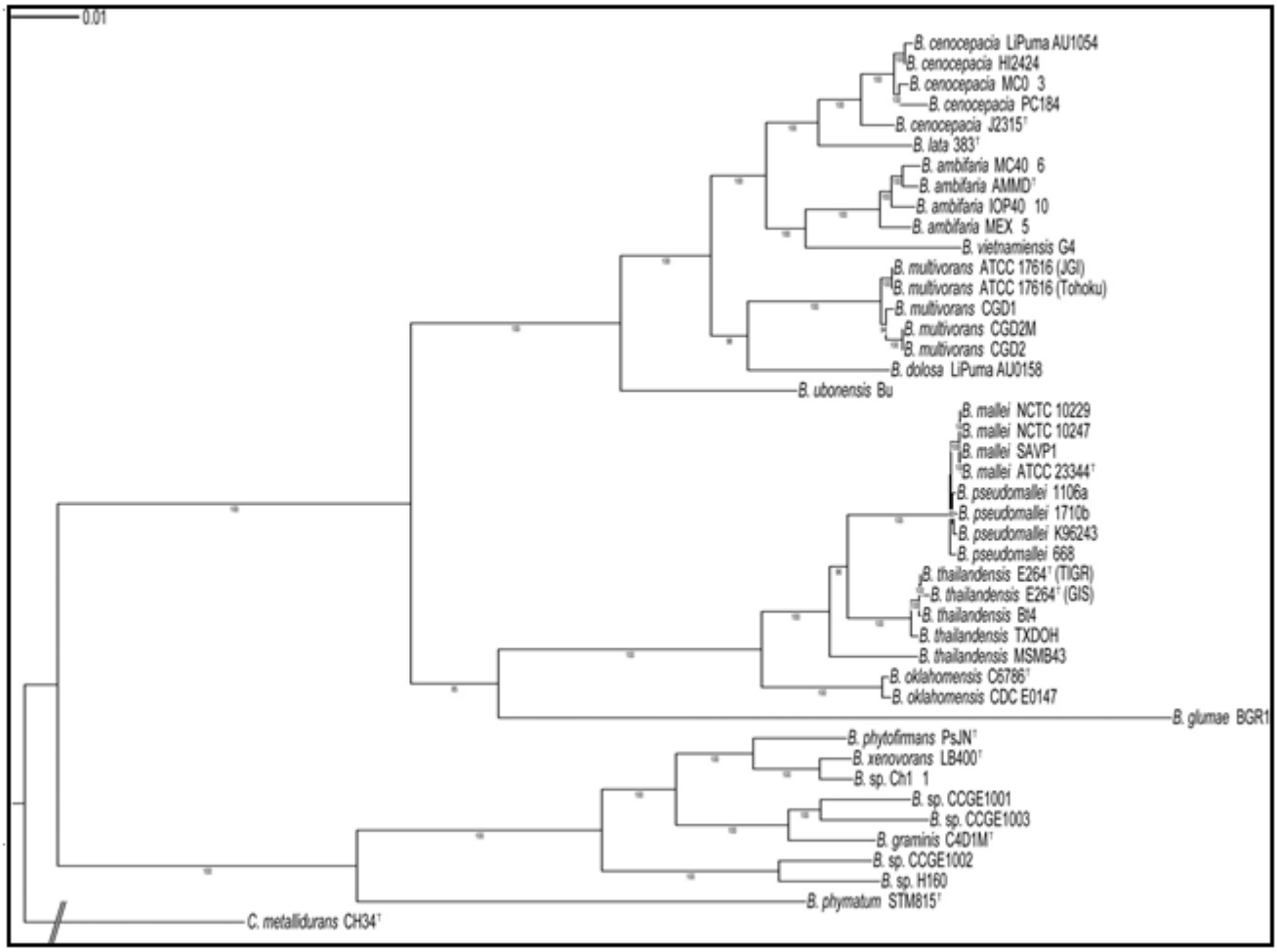

Figure 3: Phylogenetic tree of Burkholderia species with C. metallidurans CH34^T (Vandamme & Dawyndt, 2011)

9.1. Evolutionary relationships:

The evolutionary relationships between strains have implications for taxonomic systems. Changes in the taxonomy of the B. cepacia complex have been driven by phenotypic and molecular studies. DNA hybridization, fatty acid analysis, and sequencing techniques have identified nine genomovar species. Furthermore, molecular methods throw light on the evolutionary studies by showing the correct evolutionary relationships between complex species and strains (Lood et al., 2021). The clonal character of the different B.c consultants complex suggests a shift from non-human to human subjects who happen to be susceptible to contamination. Furthermore, the rising of some several genomovars in addition to nomenclature also make it hard, especially for healthcare workers who deal with immune-compromised patients, especially the ones of the other sector of the economy. Other names were invented which made the way much simpler.(Grund et al., 2021)

9.2. Genetic diversity:

Genetic diversity in Burkholderia has been studied through various methods: DNA-DNA matching, AFLPs and 16S rRNA gene sequencing, First of all, a burkholderia and ralstonia have come up with the idea of merging them together, but later research supported its distinction (Jin et al., 2020). Phylogenetic approaches according to

various approaches have put a spotlight on the evolution of Burkholderia. Using 16S rDNA sequencing and DNA-DNA hybridization rates, several recent Burkholderia spp. have been revealed. Among them, Burkholderia rhizoxinica was the first species (Depoorter et al. (2020)). 16S rRNA gene sequences have to be utilized as a tool in finding out taxonomy and phylogeny for the genus of Burkholderia. Genomic technologies such as whole genome sequencing and next-generation sequencing have ultimately offered newer routes to investigate the diversity of Burkholderia in more depth (Depoorter et al., 2020). These tools were then used to develop a tool to identify specific clones of clinical isolates and interpret the population structure of this species. With the present technological advancement, the understanding of genomic variation of Burkholderia and its evolutionary descendants would further increase by applying the technology.(Bach et al., 2023)

10. Species diversity of Burkholderia:

Genus has more than forty species, but not all of them have been officially named. Some varieties are still waiting to be discovered. The pathogenicity of the B. cepacia complex (Bcc) lies in a clade of one of the Bcc species, but the possibility that there are many species within the one complex is still to be fully elucidated. Since B. pseudomallei is uniquely present to some part of Southeast Asia and Northern Australia, the bacteria's effect is usually in these parts (Hamidizade et al., 2024). Understanding evolution and species definition is important to comprehend disease epidemiology and biology. B. gladioli is unique in its heterogeneity and wide range of habitats as a pathogen. Identification of B. cepacia ssp. is significant for its association with cystic fibrosis infection. These organisms have specific genetic traits that determine their ability to cause infections. The diversity and global distribution of Burkholderia species pose challenges for taxonomy and misidentification may occur. (Jia & Lu, 2024)

10.1. Known species:

As of November 2006, there are 14 validly named genomic species. 11 of them have not yet received formal latinizing and article names, except for B. phymatum (Bach et al., 2022). The known plant pathogen species are B. gladioli, B. planticola, B. caryophilli, B. andropogonis, B. glabae (a complex of four related species pathogenic on rice), B. oklahomensis (causes onion yellowing), and B. cepacia (a complex of at least nine species, some distantly related to others) (Depoorter et al., 2020). B. mallei and B. pseudomallei are pathogenic to humans. B. xenovorans and B. ambifaria are environmentally important, and finally, there is B. vietnamiensis whose taxonomy and relation to other species is not fully understood. (Jin et al., 2020)

The genus Burkholderia consists of species that are pathogens to humans, animals, and plants or important saprophytes. They have diverse metabolisms and can degrade organic compounds. Taxonomic revisions have resulted in new species being given Latinized vernacular names, such as B. mallei and B. pseudomallei causing glanders disease and melioidosys, respectively (Espinosa-Victoria et al., 2020). The former B. caryophilli is now B. gladioli and B. alliicola. Burkholderia is constantly evolving and expanding, with many unclassified strains and incomplete knowledge of species diversity. (Elshafie & Camele, 2021)

10.2. Novel species discovery:

The discovery that the named Burkholderia species may be a minority is supported by Coenye et al.'s work in 2001. They found that many isolates did not belong to named species (Morales-Ruíz et al., 2022). Similar findings were reported by the same group in 2004 using gene sequence analysis. Gillings et al. also isolated Burkholderia species from soil and developed PCR tests for further discovery. However, whether these should be classified as new species or not depends on taxonomic changes.(Tsuji & Kadota, 2020)

Currently, there are 14 recognized Burkholderia species strains, including B. cepacia, B. mallei, B. pseudomallei, B. thailandensis, and two unnamed species isolated from cystic fibrosis patients

(Burtnick et al., 2024). These 14 species are an anomaly given the wide range of habitats Burkholderia species can inhabit. B. mallei and B. pseudomallei, the etiological agents of glanders and melioidosis, will not be discussed here. (Meumann et al., 2024).

Conclusion:

There are many bacteria in Burkholderia genus that have high differentiation levels and seem to have nature conservation, agriculture, and bioremediation aspects that are essential with for mankind. The composition of taxonomy and classification of this organism is determined from its genetic diversity and the ability to thrive in different environments and hosts with ease. Pathogenic variants of Burkholderia species, particularly B. cepacia complex, B. pseudomallei, and B. mallei, are tough to tackle since their virulence makes them agentive, antibiotic resistance deprives us of many drugs, and their affect on already weak and vulnerable people is very devastating and difficult to fight. Our analysis demonstrates that in order to combat the Burkholderia infections, new diagnostic techniques are required, a better insight into the pathogenic mechanisms of this microbe should be taken, and new treatment methods should be developed. Furthermore, the ecological functions of Burkholderia in bioremediation and plant growth support suggest that it may itself balanced to pose problems. Researchers need to investigate the detailed genetic roots that enable the multi-purpose nature of Burkholderia. They can enable these applications to treat infections and to be used to better the man-made environment.

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