Chapter 1

Introduction

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[2] **Pal, U.**, Bachmann, D., Blank, L. M., & Tiso, T. (2023). Draft Genome Sequence and Annotation of the Halotolerant Carotenoid-Producing Strain *Paracoccus bogoriensis* BOG6^T. *Microbiology resource announcements*, *12*(5), e0013323. https://doi.org/10.1128/mra.00133-23 *(Published)*

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Contributions

This chapter was written by Upasana Pal and reviewed by Lars M. Blank.

1: Introduction

1.1 Introducing the metabolically versatile genus *Paracoccus*

Motivated by the need to discover novel strains capable of utilizing cheap and renewable feedstocks, in a combination of producing industrially relevant compounds sustainably there arises a compelling impetus to delve deeper into the genus *Paracoccus* [3] (Table 1).

History of Taxonomic classification:

Table 1: Taxonomic classification of the genus *Paracoccus*

Domain:	**Bacteria**
Phylum:	Pseudomonadota
Class:	Alphaproteobacteria
Order:	Rhodobacterales
Family:	Paracoccaceae
Genus:	*Paracoccus*

The classification history of *Paracoccus* has been marked by ambiguity, beginning with isolating the first strain initially designated as *Micrococcus denitrificans* by Beijerinck et al. [4] in 1910. It wasn't until Rainey et al.'s [5] reclassification in 1999 that this strain was officially recognized as *P. denitrificans* DSM 413. Subsequent discoveries within the genus continued to be enshrouded in taxonomic uncertainty [6], exemplified by the case of two well-characterized chemolithoautotrophic sulfur bacteria, *Thiosphaera pantotropha* [7] and *Thiobacillus versutus* [8], which underwent reclassification and were later designated as *P. pantotrophus* [9] and *P. versutus* [8], respectively. Thus, what initially began as the foundation for discovering a few *Paracoccus* strains in the early 1900s has evolved into the current culmination of 1048 strains attributed to the genus *Paracoccus*

[10], comprising 116 classified species and numerous others yet to be classified. Recently, the genus underwent another taxonomic re-classification, proposed by Liang et al., 2021 [11] and Göker [12]. Historically the genus *Paracoccus* fell under the taxonomic family of Rhodobacteraceae, based on 16S rRNA gene analysis, which gave rise to taxonomic misclassifications [13]. Thereby instigating the need to perform a taxonomic re-evaluation through whole genome sequencing, followed by the construction of a phylogenetic tree using core genome sequences [14], and lastly resolving the lineages through the application of the amino acid nucleotide index score (% ANI) [15, 16]. The results of this study gave rise to several polyphyletic groups, comprising organisms with diverse evolutionary origins, lacking a common ancestor shared among its members, and commonly encompassing entities exhibiting convergent evolution. Out of these various groups, all the species of *Paracoccus* were re-classified into a new family, Paracoccaceae [11, 12].

Morphology:

Strains of the genus *Paracoccus* are gram-negative. On a morphological level, the genus demonstrates a remarkable degree of homogeneity and occurs as single cells, pairs, or clusters, and some strains such as *P. pantotrophus* DSM 2944, under nitrogen deficiency produce bioplastic polyhydroxyalkanoate (PHA) (Figure 1). The cells generally range from 0.4 to 0.9 µm in diameter or 2 µm in length [10]. Most strains of the genus are non-motile, thus energy efficient through the absence of a flagella. Exception to this is *P. carotinifaciens* [17] carrying a peritrichous flagellum [18].

Figure 1: Transmission Electron Microscope image of *P. pantotrophus* DSM 2944. The cells were grown on 40 mM of glucose as the sole carbon source under nitrogen limitation (0.2 g/L) until the cells reached the stationary phase. These nutrient-limiting conditions initiate the production of the storage molecule polyhydroxyalkanoate (PHA). The cells are embedded using a mixture of phosphate buffer and 3% glutaraldehyde and the images were procured by Dr. Miriam Buhl, Institute of Pathology, Uniklinik RWTH Aachen.

Metabolic versatility of the genus *Paracoccus*

Strains of the genus *Paracoccus* are isolated from all over the globe (Figure 2), ranging across various environmental spheres (table 2), including extremophilic habitats such as effluent wastewater treatment plants, hypersaline lakes, and terrestrial environments.

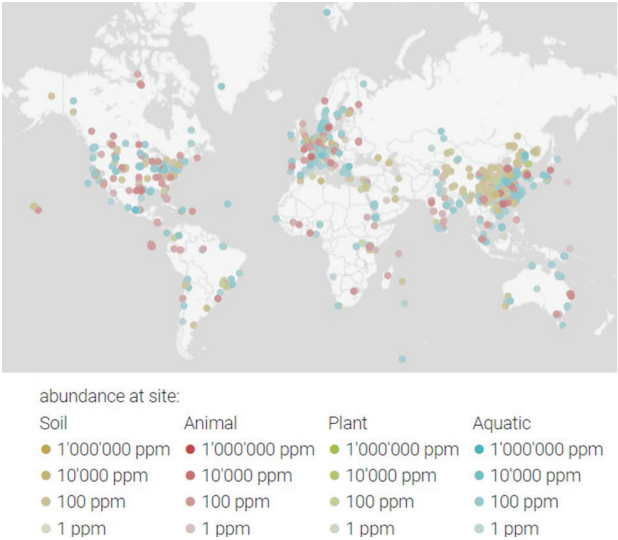

Figure 2: Global isolation habitat of *Paracoccus* species. The highest out of which are reported to be aquatic (2,441) species, followed by animal sources (1,611), soil samples (932), and lastly plant sources (205) species. The image is generated using Microbeatlas [19] and BacDrive [20].

Table 2: Strain-specific isolation habitats of genus *Paracoccus*.

Strain	**Used in this study**	**Isolated from**	**Reference**
Paracoccus acridae	Y	the insect *Acrida cinerea*, China	Zhang et al. (2016) [21]
Paracoccus aeridis		the rhizosphere of an epiphytic orchid (*Aerides maculosa*) sample collected from Amboli, Western Ghats, Karnataka, India	Rai et al. (2020) [22]
Paracoccus aerius		air at the foot of Xiangshan Mountain, Beijing, China	Xue et al. (2017) [23]
Paracoccus aestuarii	Y	tidal flat sediment in Yeosu, Republic of Korea	Roh et al. (2009) [24]
Paracoccus aestuariivivens		tidal flat sediment at Gangwha Island on the Yellow Sea, Republic of Korea	Park et al. (2016) [25]
Paracoccus alcaliphilus		the soil Niigata Factory of Mitsubishi Gas Chemical Company, Japan	Urakami et al. (1989) [26]

Paracoccus alimentarius		The fermented Walleye Pollack is a Korean foodstuff, salted pollack, Republic of Korea	Kim et al. (2018) [27]
Paracoccus alkenifer	Y	the biofilter for waste gas treatment, Germany	Lipski et al. (1998) [28]
Paracoccus aminophilus		the Niigata Factory of Mitsubishi Gas Chemical Company, Japan	Urakami et al. (1990) [29]
Paracoccus aminovorans	Y	the Niigata Factory of Mitsubishi Gas Chemical Company, Japan	Urakami et al. (1990) [29]
Paracoccus amoyensis		the seawater, Republic of Korea	Lyu et al. (2021) [30]
Paracoccus aurantiacus		the seawater in shallow-sea hydrothermal systems off Kueishantao Island, Taiwan	Ye et al. (2020) [31]
Paracoccus bengalensis		the soil adjacent to the roots of the plant *Clitoria ternatea*, a native legume of the lower-Gangetic plains of India	Liu et al. (2006) [32]
Paracoccus bogoriensis	Y	the Lake Bogoria, Kenya	Pal et al. (2023) [2]
Paracoccus caeni		the sludge at the Daejeon sewage disposal plant, Republic of Korea	Lee et al. (2011) [33]
Paracoccus cavernae		the air sample collected in Ardales Cave (Malaga, Spain)	Dominguez-Moñino et al. (2016) [34]
Paracoccus chinensis		the freshwater sediment (river, lake, pond), China	Li et al. (2009) [35]
Paracoccus communis		the sludge from saline source, Russia	Lee et al. (2024) [36]
Paracoccus contaminans	Y	the contaminant of an axenic E. coli culture in 7% NaCl, Germany	Kämpfer et al. (2016) [37]
Paracoccus denitrificans	Y	the garden soil, The Netherlands	Beijerinck et al. (1910) [4], Rainey et al. (1999) [5]
Paracoccus endophyticus		the soil, The Netherlands	Zhang et al. (2019) [38]
Paracoccus everestensis		the bioreactor, The Netherlands	Cui et al. (2022) [39]
Paracoccus fistulariae		the Bluespotted cornetfish, *Fistularia commersonii*, Republic of Korea	Kim et al. (2010) [40]
Paracoccus fontiphilus		the freshwater, Taiwan	Sheu et al. (2018) [41]
Paracoccus haematequi		the horse blood, Germany	Kämpfer et al. (2019) [42]
Paracoccus haeundaensis	Y	the seawater, Republic of Korea	Lee et al. (2004) [43]
Paracoccus halophilus		the South China Sea marine sediment, China	Liu et al. (2008) [44]

Paracoccus halotolerans		the sediment Salt Lake, China	Meng et al. (2019) [45]
Paracoccus hibisci		the soil sample from the rhizosphere of a Mugunghwa flower, Republic of Korea	Yan et al. (2017) [46]
Paracoccus hibiscisoli	Y	the rhizosphere of Mugunghwa (*Hibiscus syriacus*) in Kyung Hee University, Yongin, Republic of Korea	Lin et al. (2017) [47]
Paracoccus homiensis	Y	the sea-sand, Republic of Korea	Kim et al. (2006) [48]
Paracoccus huijuniae		the wastewater, Republic of Korea	Sun et al. (2013) [49]
Paracoccus isoporae	Y	the reef-building coral *Isopora palifera*, Taiwan	Chen et al. (2011) [50]
Paracoccus kocurii		the activated sludge for the treatment of tetramethylammonium, Japan	Ohara et al. (1990) [51]
Paracoccus kondratievae		the rhizosphere of maize, Russia	Doronina et al. (2002) [52]
Paracoccus koreensis		the wastewater treatment plant of a beer-brewing factory in Chung-Won, Republic of Korea	La et al. (2005) [53]
Paracoccus laeviglucosivorans	Y	soil, Japan	Nakamura (2015) [54]
Paracoccus liaowanqingii	Y	the Tibetan antelopes on the Qinghai-Tibet Plateau, China	Li et al. (2020) [55]
Paracoccus limosus		the activated sludge in the Mae-san sewage treatment plant, Republic of Korea	Lee et al. (2013) [56]
Paracoccus litorisediminis		the tidal flat sediment, Republic of Korea	Park et al. (2017) [57]
Paracoccus luteus		the intestinal tract of a grass carp, China	Ming et al. (2020) [58]
Paracoccus lutimaris	Y	the tidal flat sediment, Republic of Korea	Jung et al. (2014) [59]
Paracoccus mangrovi		the mangrove plant, Anping District, Taiwan	Chen et al. (2017) [60]
Paracoccus marcusii	Y	the seawater, Japan	Harker et al. (1998) [61]
Paracoccus marinaquae		coastal water of the Yellow Sea, China	Xue et al. (2023) [62]
Paracoccus marinus	Y	coastal seawater in Tokyo Bay, Japan	Khan et al. (2008) [63]
Paracoccus nototheniae		the kidney of a black rock cod (*Notothenia coriiceps)*, Chilean Antarctica	Kampfer et al. (2019) [64]
Paracoccus panacisoli		the forest soil cultivated with Vietnamese ginseng, Vietnam	Nguyen et al. (2015) [65]
Paracoccus pantotrophus DSM 2944	Y	the wastewater treatment plant, The Netherlands	Bockwoldt et al. (2020) [9]

Paracoccus pantotrophus DSM 11073	Y	the leaves of the carbon disulfide-producing Valley Oak tree (*Quercus lobata*), United Kingdom	Iordan et al. (2012) [66]
Paracoccus rhizosphaerae		the rhizosphere of the plant *Crossostephium chinense*, Taiwan	Kampfer et al. (2012) [67]
Paracoccus salipaludis		the natural saline-alkali wetland soil, China	Dong et al. (2018) [68]
Paracoccus saliphilus		the saline soil, China	Wang et al. (2009) [69]
Paracoccus sanguinis		the blood from a human patient, USA	McGinnis et al. (2015) [70]
Paracoccus sediminilitoris		the tidal flat sediment, China	Wei et al. (2019) [71]
Paracoccus sediminis	Y	the marine sediment of the East China Sea	Pan et al. (2014) [72]
Paracoccus solventivorans	Y	the soil sample of a defunct natural gas company, in Germany	Lipski et al. (1998) [28]
Paracoccus tegillarcae		gastrointestinal tract of a blood cockle, *Tegillarca granosa*, Republic of Korea	Lee et al. (2019) [73]
Paracoccus thiocyanatus	Y	the activated sludge, Japan	Katayama et al. (1995) [8]
Paracoccus tibetensis		soil, Tibet, China	Zhu et al. (2013) [74]
Paracoccus versutus	Y	urban lake in Xi'an City, Shaanxi Province, northwest China	Zhang et al. (2018) [75]
Paracoccus yeei	Y	the human abdominal dialysate, United States of America	Morinaga et al. (2020) [76]
Paracoccus zeaxanthinifaciens	Y	coast of the African Red Sea, Africa	Berry et al. (2003) [77]

The isolation habitats outlined in Table 2 and Figure 2 highlight the diverse ecological niches inhabited by *Paracoccus* species. These habitats include extreme environments, with a significant presence in activated sludge and wastewater treatment plants. For instance, strains like *P. pantotrophus* DSM 2944 exhibit denitrification and reductive capabilities alongside the presence of sulfur-oxidizing genes (SOX genes) [78, 79], enabling them to thrive in nutrient-deficient environments along with thermotolerance up to 45 ºC [80]. Coming to physiological robustness, strains such as *P. bogoriensis* BOG6, isolated from hypersaline lakes, demonstrate resilience to extreme alkaline conditions, with the ability to grow at pH levels as high as 13.5 [2] and salt tolerance of up to 6.5 % NaCl [81].

These physiological characteristics make strains from the genus *Paracoccus*, interesting candidates for industrial applications. Notably, their ability to tolerate elevated temperatures can significantly reduce production costs. Higher operating temperatures diminish the need for extensive cooling systems, thereby lowering energy consumption and operational expenses associated with maintaining lower fermentation temperatures. This thermotolerance can enhance process efficiency and cost-effectiveness in industrial fermentation processes. On the other hand, pH and salt tolerance could allow auto-sterile fermentations thus offering more economical sterilization conditions.

Paracoccus strains exhibit broad metabolic capabilities, including the utilization of diverse carbon sources, which can be obtained from lignocellulosic biomass and agricultural waste, thereby reducing dependency on traditional feedstocks like glucose and sucrose that compete with human and animal food sources. These strains also possess robust physiological traits, such as thermotolerance and osmotolerance, and produce industrially relevant compounds like carotenoids [82] and polyhydroxyalkanoates (PHAs) [1, 2, 10, 80, 83] natively. This native production eliminates the need for genetic modification, thus simplifying regulatory compliance. Consequently, the combination of feedstock versatility, physiological robustness, and production of valuable compounds makes *Paracoccus* strains highly promising for various biotechnological applications (Figure 3).