Proteus rupertus sp. nov., isolated from used kitchen sponges

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ABSTRACT

A study was performed to test if there are any foodborne bacterial pathogens in kitchen sponges, also to test if any novel bacteria species were present in used kitchen sponges. Several kitchen sponges were collected from a neighbourhood in Pretoria. Six bacterial colonies (*Eschirichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Salmonella enterica*, *Pseudomonas aeruginosa* and an unknown *Proteus* sp.) were isolated from the water expelled from the sponge and grown on LB media. The bacteria were then put through Gram-stain tests and their flagella were counted. The unknown bacterium was identified as an undescribed *Proteus* species. Common antibiotics were used on the bacteria and interesting enough, none of the antibiotics has any effect on *Proteus* sp. The chemotaxonomic characteristics of the bacteria were tested using an API kit. Genotypic characterisation was then performed on the *Proteus* sp. By sequencing the 16S rRNA gene of the unknown bacterium and comparing it to 16S rRNA sequences available on NCBI, it was found that it has a 95% similarity to an undisclosed *Proteus* sp. Housekeeping genes were sequenced as well and a concatenated phylogenetic tree was drawn. The unknown isolate's genome was then sequenced by use of the C+G%, average nucleotide index (ANI) and digital DNA-DNA hybridization (dDDH). The C+G% were determined as 56%, the dDDH as 80.8% and ANI as 23.5%, enough for specie delineation, thus we propose *Proteus rupertus* sp. nov. as a novel species.

INTRODUCTION

Kitchen sponges are used for a variety of reasons in the kitchen, the most notable, cleaning dishes and cutlery. Other uses could include but are not limited to cleaning sinks and counters in the kitchen. During these cleaning procedures, food particles adhere to the sponges [1]. This, along with the moisture caught in the sponge and a mild to hot temperature allows for the perfect breeding ground for bacteria and more especially, foodborne pathogens.

Globally, foodborne bacteria result in thousands of deaths each day. Undeveloped countries are at the forefront of lives lost due to foodborne pathogens. Foodborne diseases (FBD) create global diseases which cost the global economy billions of dollars each year in medical cost and lost productivity. There is no simple solution to this dilemma, since food in the modern world we are living in, reaches the consumer through long chains of industrial production, creating the opportunity for contamination along the way [2]. However, with basic hygiene practices and strict regulations for the food sector about how the products are managed, the world will save millions of dollars as well as countless lives.

The genus *Proteus* is a member of the *Morganellaceae* family. There still is not much clarity on the Proteus genus, but it is published under the ICNP as having 15 species. Nine of which are validly published and correctly named, *P. alimentorum*, *P. cibi*, *P. columbae*, *P. faecis*, *P. hauseri*, *P. mirabilis*, *P. penneri*, *P. terrae* and *P. vulgaris* [3].

ISOLATION AND ECOLOGY

During the study to determine the foodborne pathogens active in kitchen sponges, ten kitchen sponges were collected from kitchens located in the Brooklyn neighbourhood (GPS location: 25°45'42.7" S, 28°14'03.0"E) in Pretoria. The used sponges were placed in 100 ml of sterile water for five minutes and then by using sterile forceps, were squeezed dry. A dilution series was then performed on the bacterial suspension, expelled by the sponges. Each sponge was diluted to a final dilution factor of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, and the diluted suspension were spread on to Petri dishes containing LB media and incubated at 35°C and results were recorded at 24h, 48h and 7d [4].

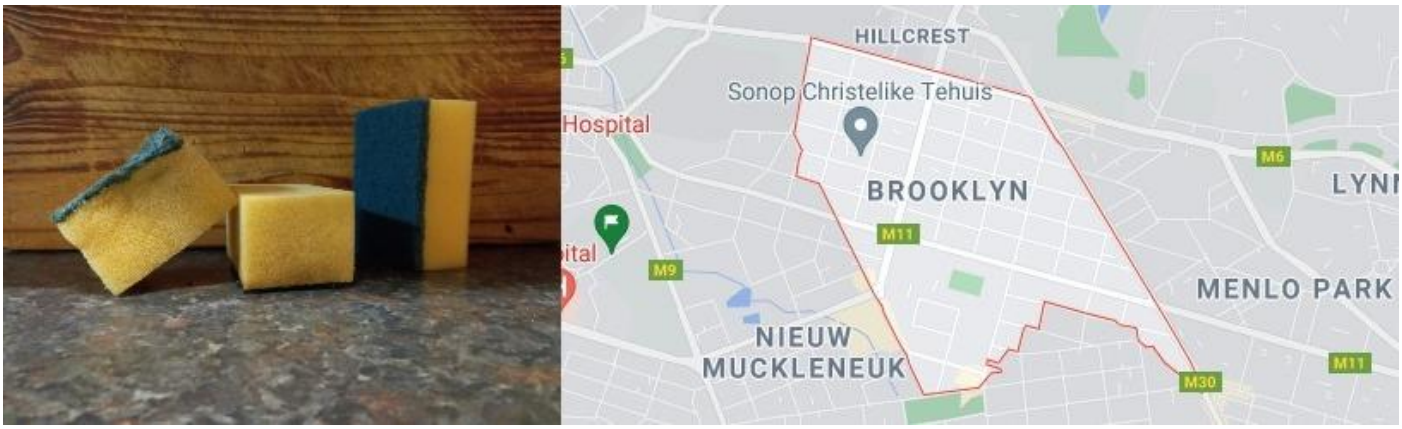

Figure 1. The sponges from which the bacteria were isolated (left) and the neighbourhood where the bacteria was isolated (right).

16S rRNA PHYLOGENY

The 16S rRNA gene sequences of strain ATCC 51471^T as well as the rest of the type strains of all the Proteus species were analysed to determine the taxonomic position of strain ATCC 51471^T. The sequence information was obtained from the GenBank database. The sequenced length of the 16S rRNA gene was 830bp for strain ATCC 51471^T [5].

Through the 16S rRNA sequence analysis it was determined that strain ATCC 51471^T belongs to the family *Morganellaceae*, order *Enterobacterales*, class *Gammaproteobacteria*. Sequence similarity calculations revealed that *P. rupertus* sp. nov. had a high sequence similarity to other species of the genus *Proteus*. The comparisons between strain ATCC 51471^T and P. vulgaris (a confirmed member of the genus *Proteus*) returned a 95% similarity between the two species. The cut-off for a genus is less than 94%, thus, it can be concluded that *P. rupertus* sp. nov. should be regarded as a species of the genus *Proteus*.

GENOME FEATURES

A phylogenetic tree based on the five individual HKGs (*dnaJ, mdh, pyrC, recA, rpoD*) were constructed, to reveal exactly where the species falls into the genus *Proteus*. MLSA techniques were used to delineate genetic similarities among *Proteus* species [6]. MLSA could be regarded as a powerful tool for discrimination, classification and phylogenetic analysis. The MLSA of the five HKGs divided the *Proteus* strains into eleven clusters, representing thirteen species (Figure 2).

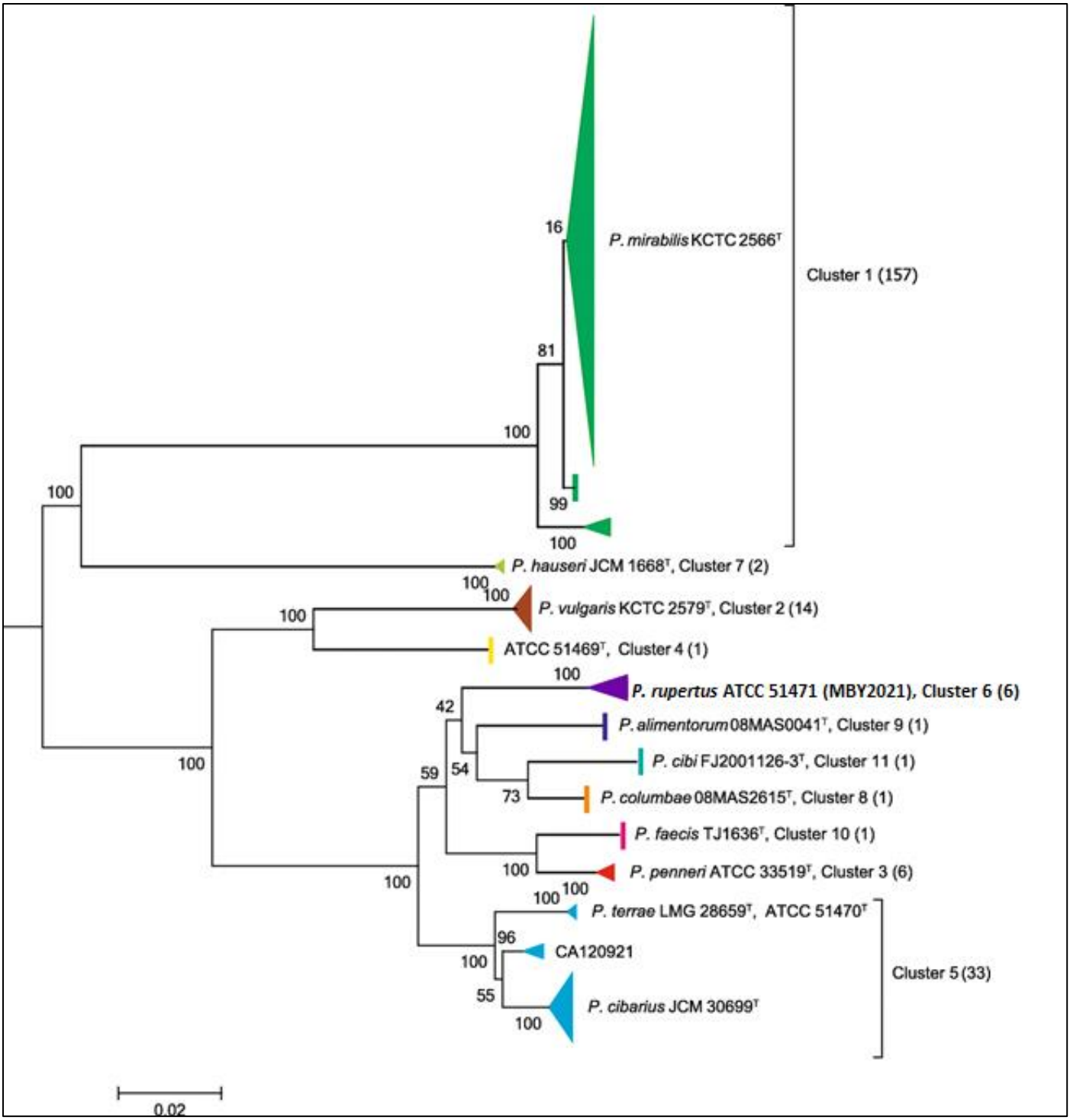

Figure 2. Phylogenetic reconstruction of the strains of *Proteus* based on concatenated HKG sequences. The scale bar indicates substitutions per site. The type strains are indicated in each cluster, if included. The strain number of the species are in the parentheses. Numbers at nodes are bootstrap percentages.

For further classification of *P. rupertus* sp. nov. ATCC 51471^T as a member of the genus *Proteus*, we have gathered the *Proteus* isolate's genome sequence data from the NCBI. The estimated genome size was 4.04Mb with an average of 56% G+C content. Furthermore, by studying the NCBI's entry for *Proteus* geno-species 6, we found that the genome consisted out of 3,673 protein-encoding genes, 9 rRNA and 76 tRNA genes.

Average nucleotide identity (ANI) calculations were performed in our study, we compared the species in question with the other major species of the *Proteus* genus, and recorded it as having 82.4 – 94.4%, all the results of the ANI calculations supported the claim for listing strain ATCC 51471^T as a separate and legit species of the genus *Proteus*, since the cut-off for species delineation is 95% [6].

Web-based DNA-DNA hybridization, dDNA, was also performed, where we compared *P. rupertus* sp. nov. against the other species of the genus *Proteus*, just like with the ANI calculations. Once again, the results supported our claim, with dDDH values of 25.0 – 52.4% all less than the accepted cut-off level for species delineation [6] (Table 1).

Table 1. Comparison of *Proteus* genospecies 6 with the other species of the genus *Proteus*. In bold are the values that warrant the assignment of Proteus genospecies 6 (ATCC 51471T) to a novel species (<95% ANI, <70% dDDH).

***P. rupertus* sp. nov. MBY2021**		
	ANI (%)	**dDDH(%)**
P. mirabilis	**82.4**	**25.0**
P. hauseri	**83.4**	**26.1**
P. vulgaris	**89.0**	**36.0**
P. alimentorum	**93.7**	**52.4**
P. cibi	**92.0**	**49.2**
P. columbae	**94.4**	**57.1**
P. faecis	**92.2**	**47.1**
P. penneri	**91.7**	**44.8**
P. terrae	**92.4**	**47.3**
P. cibarius	**92.5**	**47.1**
Proteus genospecies 4	**89.5**	**37.8**
Proteus genospecies 5	**92.3**	**47.3**

PHYSIOLOGY AND CHEMOTAXONOMY

P. rupertus sp. nov. was grown on LB media at 37°C, it had an irregular shape, between 1 and 2mm in diameter and an effuse elevation. The translucent structure's surface glistened, with a greyish-white colour. To view the cellular morphology of single cells, the bacterium was observed using transmission electron microscopy (TEM). It was observed as rod-shaped and 2µm in length, with four to ten peritrichous flagella.

The strain was shown to be Gram-negative by performing a Gram-stain test. It is motile and swarms in periodic cycles when grown on a LB media containing 1.5% agar at 37°C for 24 hours. Also, the strain is characterized as being a facultative anaerobe and can survive in the presence or absence of oxygen. *P. rupertus* sp. nov is oxidase-negative and catalase positive.

To test the chemotaxonomic characteristics of the bacterium, it was put to test by using an API 20E kit. The test was carried out according to the manufacturer's directions. The results were recorded as follows, strain ATCC 51471^T tested positive for arginine dihydrolase, citrate utilisation, hydrogen sulfide production, urease activity, tryptophan deaminase as well gelatin liquification. It also showed a weak tendency to ferment gluten (Table 2).

Table 2. Biochemical markers of *P. rupertus* sp. nov. Data for the chemotaxonomic tests were obtained using an API 20E test.

P. rupertus MBY2021 ATCC 514741^T	
ONPG	**-**
ADH	**+**
LDC	**-**
ODC	**+**
CIT	**+**
H2S	**+**
URE	**+**
TDA	**+**
IND	**-**
VP	**-**
GEL	**+**
GLU	**-***

MAN	-
INO	-
SOR	-
RHA	-
SAC	-
MEL	-
AMY	-
ARA	-

Abbreviations: ONPG, β-galactosidase; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; CIT, citrate utilisation; H2S, H_2S production; URE, urease activity; TDA, tryptophan deaminase; IND, indole production; VP, acetoin production, GEL, gelatin liquification; GLU, glucose fermentation; MAN, mannitol fermentation; INO, inositol fermentation; SOR, sorbitol fermentation; RHA, rhamnose fermentation; SAC, saccharose fermentation; MEL, melibiose fermentation; AMY, amygdalin fermentation; ARA, arabinose fermentation.
* Shows a weakened reaction, not negative, but also not an overwhelmingly positive reaction.

DESCRIPTION OF *PROTEUS RUPERTUS* SP. NOV.

Proteus rupertus (ru'per.tus N.L. gen. n. *rupertus* named in honour of Dr Anton Rupert, South African billionaire, former chancellor of the University of Pretoria (1987-1992) and Tukkie of the Century).

Cells are Gram-stain-negative, facultative anaerobic, motile, rod-shaped and approximately 2 µm in length. Colonies of the bacterium are irregular in shape, have an effuse elevation, a greyish-white colour and are glistening and translucent with a diameter of between 1 and 2mm when grown overnight at 37°C on LB media. In API20E tests, positive for arginine dihydrolase, citrate utilisation, hydrogen sulfide production, urease activity, tryptophan deaminase as well gelatin liquification. It also showed a weak tendency to ferment gluten the rest tests negative.

The type strain, ATCC 51471^T, was isolated from used kitchen sponges from kitchens in Brooklyn, Pretoria. It has a genome size of 4.04Mb and a G+C content of 56% with 3,673 protein-encoding genes.

AUTHOR STATEMENTS

Conflicts of interest
The author declares that there are no conflicts of interest.

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ABBREVIATIONS

1. ANI – average nucleotide identity
2. dDDH – digital DNA-DNA hybridization
3. API – analytical profile index
4. FBD – foodborne diseases
5. MLSA – multilocus sequence analysis
6. HKG – housekeeping gene
7. LB media – Luria-Bertani media

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