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Short Communication **Open Access**

Draft metagenome-assembled genomes of *Pseudomonas putida* isolated from human gut microbiome in Nasarawa State, Nigeria

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Abstract:

The metagenome-assembled genome (MAG) sequences of *Pseudomonas putida* PP14A and PP20A were obtained by metagenomic sequencing from the gut microbiomes of a female and a male patient both 24 years old from the same household presenting to a health outreach laboratory with complaint of headache, and occasional diarrhoea in Mararaba, Nasarawa State, Nigeria. The phylogenetic relationship observed between the two PP MAGs with other *Pseudomonas* spp MAGs from human, points to the global spread of *Pseudomonas putida* through human activity and migration.

Keywords: *Pseudomonas putida,* metagenome-assembled genome, gut microbiome, virulence, phylogeny

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Projet de génomes assemblés par métagénome de *Pseudomonas putida* isolés du microbiome intestinal humain dans l'État de Nasarawa, Nigéria

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Résumé:

Les séquences du génome assemblé par métagénome (MAG) de *Pseudomonas putida* PP14A et PP20A ont été obtenues par séquençage métagénomique à partir des microbiomes intestinaux d'une femme et d'un homme de 24 ans, tous deux âgés de 24 ans, issus du même foyer, se présentant dans un laboratoire de santé de proximité se plaignant de maux de tête et diarrhée occasionnelle à Mararaba, État de Nasarawa, Nigeria. La relation phylogénétique observée entre les deux MAG PP avec d'autres MAG de *Pseudomonas* spp provenant de l'homme indique la propagation mondiale de *Pseudomonas putida* par l'activité humaine et la migration.

Mots clés: *Pseudomonas putida*, génome assemblé par métagénome, microbiome intestinal, virulence, phylogénie

Introduction:

Pseudomonas putida is a Gram-negative bacterium which is commonly found in the soil, water and plant surfaces (1). Its ability to degrade a wide range of organic compounds and genetic adaptability has accorded it a vast use in biotechnology as a model organism (2). Here we report non-pathogenic *Pseudomonas putida* strains with foreign virulence genes derived from other bacteria in gut microbiome of human hosts.

Methodology:

Clinical presentation and phenotypic identification methods:

Two 24-years old patients (one female and one male) from the same household pre-

sented to a health outreach laboratory in Maraba, Nasarawa State, Nigeria, with complaints of headache, fever and occasional diarrhoea. The patients' symptoms were consistent with malaria and gastroenteritis, and therefore were screened for malaria parasite and their stool samples were screened for *Salmonella enterica* growth by first enriching the samples in Selenite F broth and then plating on *Salmonella-Shigella* agar. Colonies suspected to be *Salmonella* sp. were further purified on tryptone soy agar and used for conventional biochemical confirmatory tests (urease, and triple sugar iron test). Antibiotic susceptibility test (AST) was performed against ampicillin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and cefotaxime (30 µg) (Oxoid, UK) using the Kirby-Bauer diffusion method (3).

DNA extraction, library preparation and metagenomic sequencing:

The DNA was extracted using the ZYMO Research Quick-DNA Fungal/Bacterial Miniprep Kit according to the manufacturer's instructions. A sequencing library was constructed using the Nextera XT DNA Library Prep Kit and sequenced on an Illumina NovaSeq 6000 run at 250 bp paired-end with 60x coverage. The libraries were checked on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip, normalized, and pooled with the Kapa Biosystems Library Quantification Kit for Illumina. Trimmomatic v0.32 was used to trim adapter sequences as per the protocol outlined by (4).

Metagenomic sequence assembly & analysis:

A de novo assembly of the reads was performed using the St. Petersburg Genome Assembler (SPAdes) v3.15.1. (5). The reads were then remapped to the resulting contigs using BWA mem to obtain additional quality metrics. MetaBAT2 v2.15 (6) binning algorithm was used to bin the MAGs from the assembled metagenome. CheckM v1.2.2 (7) assessed the quality of the MAGs. Kraken (8) and multilocus sequence typing was applied to identify the taxonomy of the binned MAGs. FastANI v1.33 (9) was applied on sample 1 MAG and sample 2 MAG against a reference genome of *Pseudomonas putida* ATCC 12633 (GenBank ascension number GCA_024508115.1) genome to confirm their taxonomic identity as *Pseudomonas putida*, PP14A and PP20A, respectively.

The Comprehensive Antimicrobial Resistance Database (CARD) (10) resistance gene identifier was employed to identify resistance genes in PP14A and PP20A MAGs. Bakta1.8.2 (11) was used to annotate the genomes, and PathogenFinder (12) was used to investigate pathogenicity. Phigaro 1.0.1 (13) was employed to investigate prophage regions in both MAGs while the VFanalyser tool of the Virulence Factor Database (14) was utilized to identify virulence factors in PP14A and PP20A MAGs. A phylogenomic tree was inferred using IQ-TREE rapid bootstrap analysis (1000 repetitions) and concatenated alignments with all MLST genes from 46 *Pseudomonas* spp genomes, via the autoMLST interface (https://automlst.ziemertlab.com/results /9ec72497-e35d-429d-86c0-348163fd28fa/report/).

Results and Discussion:

The triple sugar iron and urease test confirmed the isolates as negative for *Salmonella enterica* growth. However, the AST of the isolate from the male patient showed antibiotic resistance to ampicillin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and cefotaxime (30 µg) while the isolate from the female patient showed sensitivity to ciprofloxacin (5 µg) and ofloxacin (5 µg), which sparked our curiosity to investigate the genome of the isolates. The PP14A and PP20A MAGs are considered high-quality metagenome-assembled genome having 98.38% completeness for both MAGs, with 0.63% (PP14A) and 0.79% (PP20A) contamination (15). PP14A and PP20A were not classified as human pathogenic strains of *Pseudomonas putida* with average nucleotide identity of 88% to *Pseudomonas putida* ATCC 12633 (Table 1). PP14A MLST sequence type was *55b3 while PP20A was *3936 sequence type.

There were no complete (100%) antibiotic resistance genes identified in both MAGs, however some incomplete genes (>70% identity) for efflux pumps (*abaQ*) were seen in both MAGs. Although, PP14A and PP20A were not classified as human pathogenic strains of *P. putida*, they both contained foreign virulence genes; *hopJ* gene for type II secretion system effector protein peculiar to *Pseudomonas syringae*; *epsE* and *epsG* for type II secretion system of *Vibrio cholerae*; and *sodCI* gene for stress adaptation in *S. enterica*, were seen in both PP14A and PP20A, while LPS-O antigen gene for *P. aeruginosa* was seen in PP20A alone. Several other virulence genes of *P. aeruginosa* LESB58 *(*GCA_000026645.1.) were also identified in both MAGs (Table 2).

Table 1: Genomic features of the two *Pseudomonas putida* (PP14A and PP20A) clinical isolates

Features	**PP14A**	**PP20A**
Genome size	5,280,125 bp	5,052,825 bp
Number of contigs	105	106
Average contig length	49812 bp	47668 bp
Largest contig length	310054bp	241253 bp
Smallest contig length	2067 bp	2294 bp
N_{50}	82,436 bp	70607 bp
G+C	62.9%	62.9%
Total number of genes	5007	4783
Number of coding regions (with protein)	4864	4651
tRNA	57	56
Non-coding RNA	41	39
Average nucleotide identity (*Pseudomonas putida* ATCC 12633)	88.0%	88.1%
Prophage regions	4	4
Horizontal gene transfer regions	168	196

RNA = ribonucleic acid

Table 2: *Pseudomonas aeruginosa* LESB58 (GCA_000026645.1.) virulence factors found in PP14A and PP20A MAGs

S/N	**Virulence Genes**	**Gene Products**
1	*fleN; fleQ; fleR* *flgC; flgD; flgE; flgF; flgG; flgH; flgI; flgJ flgK; flgL* *flhA; flhB; flhF; fliC; fliD; fliE; fliF; fliG; fliH; fliI; fliJ* *fliM; fliN; fliO; fliP; fliQ; fliR*	Flagellar synthesis regulator Flagellar basal-body rod proteins Flagellar hook-associated proteins Flagellar assembly proteins
2	*fimT, fimU, fimV,* *pilA; pilB; pilC; pilE; pilF; pilG; pilH; pilI; pilJ; pilK; pilM;* *pilN; pilO; pilP; pilQ. pilT*	Fimbrial biogenesis proteins Motility proteins
3	*chpA* *chpC*	Still frameshift probable component of chemotactic signal transduction system Probable chemotaxis protein
4	*alg44* *alg8* *algA, algQ, algR, algU, algX, algZ* *mucA; mucB; mucC*	Alginate biosynthesis protein Alginate-c5-mannuronan-epimerase GDP-mannose 6-dehydrogenase Alkaline metalloproteinase precursor
5	*fpvA* *pvdA* *pvdD, pvdE*	Ferripyoverdine receptor FpvA L-ornithine N5-oxygenase Pyoverdine synthetase
6	*clpV1, vgrG1* *hcp, icmF1*	Type VI secretion system AAA+ family ATPase Type VI secretion system substrate Hcp1

PP = *Pseudomonas putida*; MAG = Metagenome-assembled genomes

There were 168 horizontal gene transfer (HGT) regions in PP14A and 196 HGT regions from PP20A, accounting for presence of four prophage regions (from one Myoviridae and three Siphoviridae phages) (Fig 1) embedded in the bacteria genome of each of them.

The phylogenetic tree (Fig 2) shows that PP14A and PP20A shared closest clonal relationship with *Pseudomonas* spp NBRC 111 120, *Pseudomonas* spp GTC 16481, *Pseudomonas* spp NBRC 111126 and *Pseudomonas* spp NBRC 111129, all isolated from human urine from four different cities in Japan (2009-2012) (16). The closest relationship to non-human isolate was with *Pseudomonas* spp TJI-51 isolated from plant (mango orchid) collected in 2009 from Pakistan (17).

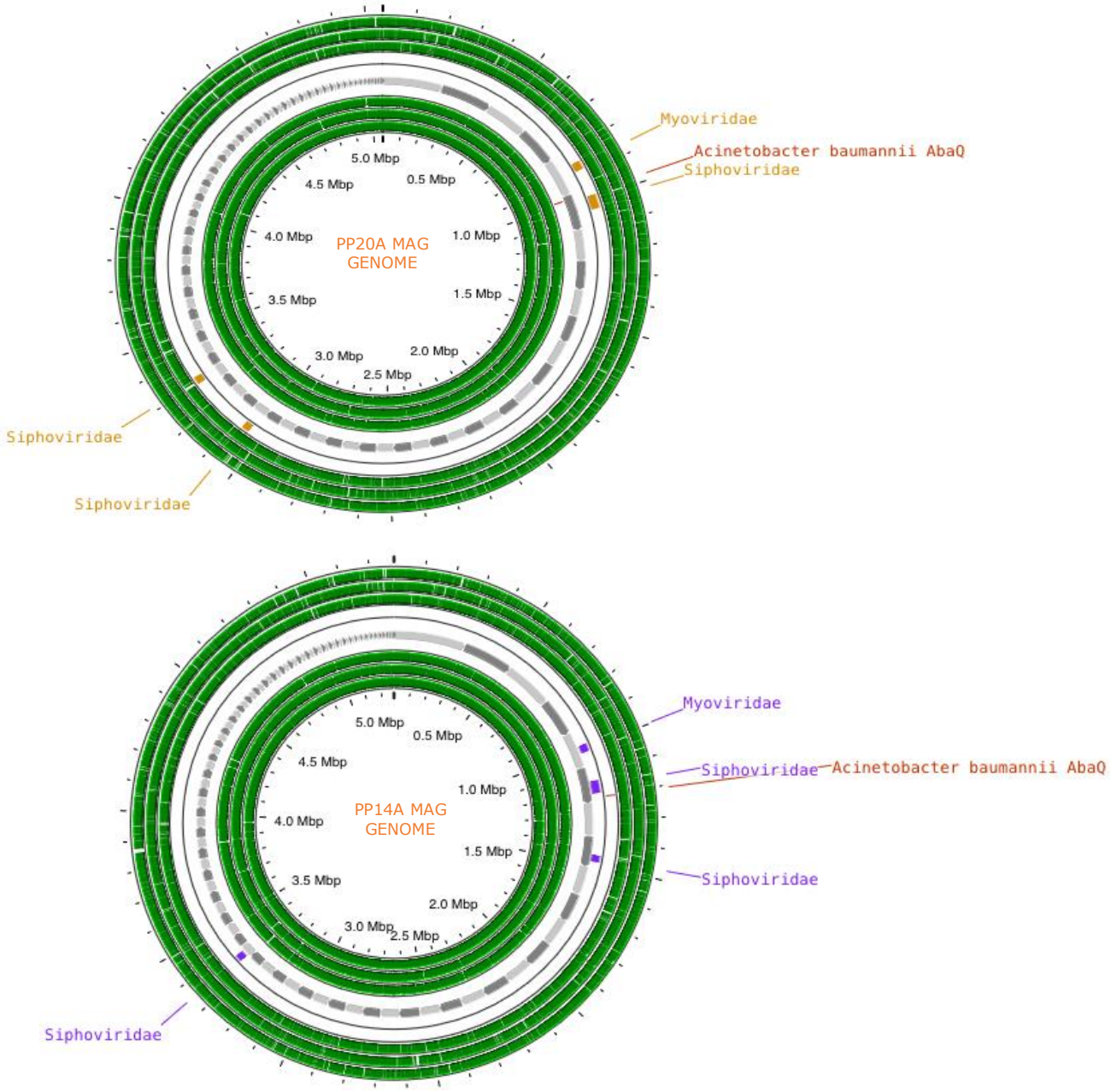

Fig 1: Circular presentation of PP14A and PP20A MAG genome highlighting prophages and antibiotic-resistant genes

Bacteria are known to exhibit plasticity and while existing in any microbiome community acquire traits that ensure its survival (18). PP14A and PP20A were isolated from gut microbiome of two different patients in the same community and although it is not yet classified as a human pathogen, its specie-inherent virulence genes as well as the foreign ones can be transferred to other pathogenic or non-pathogenic bacteria in its community promoting molecular evolution of the microbiome. The closeness of PP14A and PP20A on the phylogeny tree (Fig 1) shows that they share same ancestry and this can be seen in similar features they shared (Tables 1 and 2, Fig 2). However, the presence of *P. aeruginosa* LPS O-antigen gene in PP20A but not in PP14A may be as a result of the 12.5% strain heterogeneity in PP20A which could have been contributed by presence of *P. aeruginosa* which 16S rRNA was not appropriately captured due to limitation of the sequencing coverage (60x) in the study.

Comparing both genomes showed that although both MAGs had four prophages, and same antibiotic resistance gene (*abaQ*), they were positioned at different locations in the genome. This further proves that although the MAGs may share ancestry and were isolated from persons in the same household, they may not belong to the same generation and trans-

mission from one person to the other may have happened after a few generations. Phylogenetic relationships observed between PP14A and PP20A MAGs with other *Pseudomonas* spp (Fig 2) point to global spread of *Pseudomonas putida* through human activity and migration.

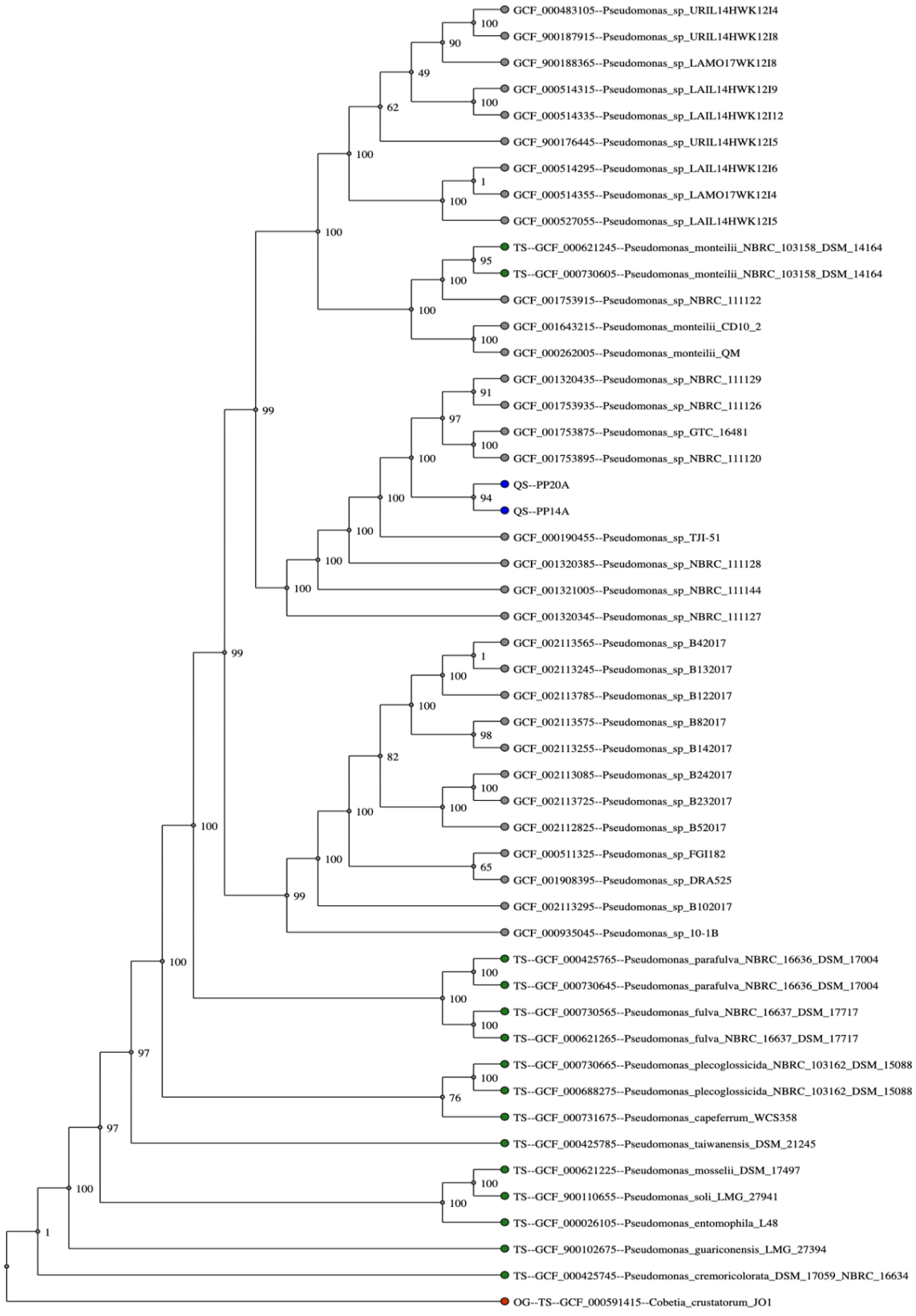

Fig 2: Phylogenetic tree of PP14A and PP20A isolates

Ethical consideration:

Ethical approval was obtained from the Nasarawa State Ministry of Health with approval number NHREC 18/06/2017 on November 16, 2020 and from the ethical review board of Faculty of Biological Science, University of Nigeria Nsukka (UNN/FBS/EC/1012). Informed consent was obtained from the study participants.

Data availability:

PP14A MAG was submitted to the NCBI database under Biosample SAMN41785133 and SRR 28820204. PP20A MAG was submitted to the NCBI database under Biosample SAMN4180 4069. Both PP14A and PP20A sequencing projects are registered on the NCBI under Project number PRJNA1075677.

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Contributions of authors:

The author conceptualized the study, analysed and interpreted the data and wrote the manuscript.

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Conflict of interest:

Author declares no conflict of interest.

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