### Title

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# Whole genome sequence of *Serratia marcescens* 39_H1, a potential hydrolytic and acidogenic strain

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ABSTRACT

Here, we report a high quality annotated draft genome of *Serratia marcescens* 39_H1, a Gram-negative facultative anaerobe that was isolated from an anaerobic digester. The strain exhibited hydrolytic/acidogenic properties by significantly improving methane production when used as a single isolate inoculum during anaerobic digestion of water hyacinth and cow dung. The total genome size of the isolate was 5,106,712 bp which corresponds to an N50 of 267,528 and G + C content of 59.7 %. Genome annotation with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) predicted a total of 4,908 genes of which 4,755 were protein coding genes; there were no plasmids detected. A number of genes associated with hydrolytic/acidogenic activities as well as other metabolic activities were identified and discussed.



## 1. Introduction

Biogas production from substrates such as water hyacinth and cow dung is an effective and environmentally friendly means of managing these waste streams. Water hyacinth is a complex lignocellulosic biomass that is rich in cellulose and hemicellulose and is therefore a potential substrate for biogas production [1]. However, the recalcitrant properties of the water hyacinth is an important challenge during the hydrolytic phase of anaerobic digestion (AD) [2]. Hydrolysis is the rate-limiting phase of AD of lignocellulosic substrates as insolubility of cellulose is the basis of its recalcitrance. This is as a result of the crystalline structure of cellulose fibres that are bound by β-1,4-glycosidic linkages [3]. The β-1,4-glycosidic linkages limit or inhibit the depolymerisation of cellulose hence the need for enhanced hydrolysis and subsequent acidogenic, acetogenic and methanogenic reactions to produce biogas.

The need to facilitate the process and improve biomethane production through an existing effective and eco-friendly technology known as bioaugmentation with bacteria is imperative [4]. Bioaugmentation will assist in eliminating some expensive and not so eco-friendly pretreatment processes. Bioaugmentation is the addition of specialised actively growing pure or mixed cultures to the indigenous microbial community of anaerobic digesters in order to enhance microbial activity and improve degradation of organic wastes [5–7]. Bioaugmentation with hydrolytic bacteria seeks to boost the digestion of lignocellulosic substrates through the activities of inoculated bacteria. These bacteria possess active enzymes such as cellulases, β-glucosidases, acetate kinases and lactate dehydrogenases that are encoded by specific genes. Such enzymes are associated with depolymerisation of cellulose (etc.) via hydrolysis and acidogenesis [8]. Both pure and mixed cultures have been employed in bioaugmentation studies [9,10]. Suitable bioaugmentation with pure hydrolytic/acidogenic bacteria could mitigate the challenges of recalcitrant nature of lignocellulosic biomass through facilitation of hydrolysis and subsequent acidogenesis. Improved hydrolytic/acidogenic rate can increase the production of methane from water hyacinth during AD. In this study, a high quality annotated draft genome sequence of *Serratia marcescens* 39_H1 is reported and observed hydrolytic/acidogenic phenotypic characteristics of the isolate was correlated to its genotype. Moreover, the potential application of the strain to promote plant growth was explored from the genotypic angle. Similarly, the potential dangers the strain may pose were also discussed as the *Serratia species* are known to be opportunistic pathogens.

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## 2. Materials and methods

### 2.1. Isolation and characterization of the bacterial isolate 39_H1

Bacterial strain 39_H1 was isolated from previously running digesters of Nkuna et al. [11] that had water hyacinth and cow dung as feedstocks. One milliliter of digestate sample was serially diluted under aseptic conditions up to $10^{-6}$ dilution and 0.1 ml of each dilution was spread plated on nutrient agar and incubated for 24 h at 28 ± 2 °C. Different bacterial colonies were obtained and classified based on their phenotypic traits, pure bacterial isolate was obtained after several sub-culturing on nutrient agar and stored at 4 °C on an agar slant for further downstream applications [12]. Preliminary identification of the bacterial strain was based on growth evaluation of the strain at a temperature of 25 °C on cultivation on nutrient agar for 48 h [13]. Characterization of strain 39_H1 for its ability to solubilize calcium phosphate was ascertained by cultivation on Pikovskaya's agar for 6 days at 28 °C and the solubilisation index (SI) was determined with the formula below [14,15]:

$$SI = \frac{Halozone\ \ diameter + colony\ \ diameter}{colony\ \ diameter}$$

Pikovskaya's broth with pH 7 was inoculated with 5 % (v/v) of 48 h old bacterial strain 39_H1. Culture was incubated for 6 days at 28 °C under rotatory conditions (120 rpm). pH of the culture was ascertained at the end of the incubation period. Production of acid by the strain was determined via titratable acidity where the supernatant (8000 rpm for 10 min) was titrated with 0.1 M NaOH. Two drops of phenolphthalein indicator was used to visually indicate the equivalence point during titration [16,17].

Five microliters of overnight grown culture of strain 39_H1 was spot plated on carboxymethyl cellulose (CMC) agar plates for characterization cellulose degradability of the isolate. The CMC comprised of 0.1 % $K_2HPO_4$; 0.05 % $MgSO_4$; 0.05 % $CaCl_2$; 0.2 % CMC; 0.02 % bacteriological peptone; 0.2 % $NaNO_3$; 1.5 % bacteriological agar, 1 l of distilled water and at pH 7 [18,19]. Inoculated plates were incubated at 37 °C for 72 h and flooded with Gram's iodine (2 g KCl; 1 g Iodine in 0.3 l of distilled water) for 3-5 min after incubation. Qualitative estimation of cellulolytic index (hydrolysis capacity) was performed according to [19]. The metabolic potential of the bacterial strain 39_H1 was further ascertained via a rapid, effective and precise assessment technique, Biolog EcoPlate technique. The technique establishes the results of redox reaction through measurement of the concentration of the colour change of the reducing pigment, tetrazolium violet [20]. The 31 carbon sources on the Biolog EcoPlate was grouped into five biochemical classes (amines, amino acids, carbohydrates, carboxylic acids and polymers) inclusive of cellobiose, glucose phosphates, glycerorol phosphate etc. Forty-eight hour old pure bacterial cells were harvested, washed and suspended in 0.85 % saline and ideal concentration of the culture was adjusted to 0.7 at $OD_{595}$ [21]. Biolog EcoPlates were inoculated with 120 µl of the bacterial suspension (Supplementary material Fig. 5a) and incubated at 28 °C and inoculated plates were read in the Synergy HTX multi-mode microplate reader (Bio Tek Instruments, Inc. Vermont USA) at a wavelength of 590 nm immediately after inoculation. Microplate reading was subsequently conducted after 2 h of incubation and after 24 h of incubation.

Bacterial strain was identified based on the partial sequence of 16S rRNA gene via colony polymerase chain reaction (colony PCR) using the primers 27 F and 1492R [22]. One percent gel electrophoresis was used to evaluate the quality of the amplicons. Amplicons were sequenced in both directions at Inqaba Biotechnical Industries (Pty) Ltd South Africa. Sequences were aligned with Bioedit and CLUSTALW software and the BLAST algorithm was used to identify the bacterial strain based on Genbank database.

### 2.2. Inoculation experiment to enhance biogas production

Water hyacinth (WH) was sampled from the Hartbeespoort dam (25°44′51′′S 27°52′1′′E) in the North West province of South Africa, transported to the laboratories of Agricultural Research Council – Soil Climate and Water, Pretoria, Gauteng, South Africa and pretreated by size reduction. Cow dung (CD) was collected from the dairy parlor of the Agricultural Research Council –Animal Production, Irene Gauteng South Africa. WH and CD were digested anaerobically in 500 ml Schott batch bottles in the ratio of 2:1, respectively using 2 % total solids. Five percent (v/v) bacterial culture was cultivated on Luria-Bertani growth medium for 24 h at 30 °C in Erlenmeyer flasks and uninoculated blank was used as control. Bacterial cells were harvested by centrifugation of culture at $OD_{600}$ = 1.5 using a Spectrophotometer (DR 5000 Hach, Canada) [23]. Enumerated bacterial cells of $10^9$ cfu/ml was used to inoculate the 500 ml Schott batch bottles and incubated at 30 °C for a period of 35 days during which methane and carbon dioxide production were carefully monitored using a Gas Chromatograph (SRI 8610C, CHROMSPEC Canada). Treatments were conducted in triplicate including an appropriate control treatment without inoculum.

### 2.3. Whole genome sequence analysis

Genomic DNA of the bacterial strain (inoculum) was isolated with DNeasy PowerSoil kit (Whitehead Scientific (Pty) Ltd), in conformity with the manufacturer's instructions. Illumina paired-end libraries were generated and whole genome shotgun sequencing of the genomic DNA was executed with Illumina Miseq platform with 250-bp paired-end reads. Quality of raw reads were evaluated with FastQC [24] and trimmed with Trimmomatic (v.0.39). Low quality nucleotides and sequences that were less than 50 bp were clipped [25]. *De novo* assembly of sequences was executed with SPAdes (v.3.12.0) [26] and obtained scaffolds were compared to reference genome of *Serratia marcescens subsp. Marcescens Db11* (GenBank: HG326223), henceforth referred to as SmarDb11, to summarize the completeness of the genome assembly. This analysis was accomplished using the Quality assessment tool for genome assembly, QUAST (v.5.0.2) program with "split scaffolds' option and minimum 200bp long contigs [27]. Obtained contigs were rearranged to correspond to the order in the reference genome, SmarDb11 in Mauve (v.2.4.0) command line (progressive Mauve) and visualized [28]. Genome annotation was completed with NCBI prokaryotic genome annotation pipeline (PGAP) [29] while functional annotation and subsystem classification was conducted with Rapid annotation subsystem technology (RAST) [30]. Gene predictions with BLAST (v.2.7.0) [31] and average nucleotide identity (ANI) was determined with OrthoANI (v.1.4) for EZ Biocloud [32]. FastTree 2.1 was used to infer approximately-maximum-likelihood phylogram from alignment of the genomes. All software used were set at default unless stated otherwise.

## 3. Results and discussion

### 3.1. Isolation and characterisation of the bacterial isolate 39_H1

Bacterial strain 39_H1 was selected based on its distinct characteristics. The bacterial strain showed the production of the pigment, prodigiosin, when cultivated at 25 °C (Fig. 1a) and this initially identified it as *Serratia sp*. Based on the partial sequence analysis of the 16S rRNA genes, the bacterial strain was identified to be 99 % homologous to *Serratia marcescens* and was deposited at the Genbank under the accession number MK104517.

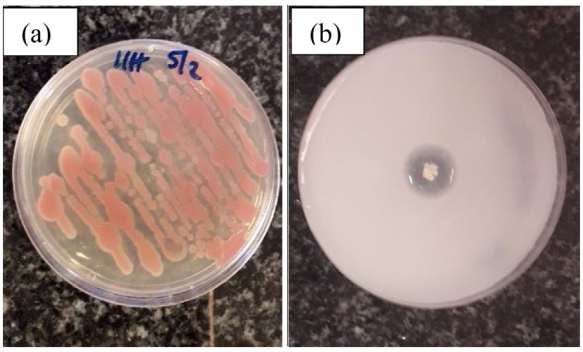


**Fig. 1.** (a) Growth evaluation of *Serratia marcescens* 39_H1 at 25 °C; (b) Phosphate solubilisation assay of *Serratia marcescens* 39_H1.

The strain also showed zone of clearance after 7 days of incubation on Pikovskaya's agar which signifies its ability to solubilise phosphate (Fig.1b). Quantification of the strain's ability to solubilise phosphate showed an SI of 3.1. The pH of the Pikovskaya's broth decreased to 5.1 after 6 days of incubation and the titratable acidity was recorded as 0.392 %. The predominant acid produced during phosphate solubilisation, gluconic acid was employed in the calculation of the titratable acidity. Changes in pH and titratable acidity has been attributed to phosphate solubilisation potential of the strain as titratable acidity measured the concentration of acid produced by the bacterial strain 39_H1 [17].

Estimation of the cellulolytic index exhibited the cellulolytic ability of strain 39_H1. The cellulolytic potential was estimated as 1.952. The colour change on the Biolog EcoPlates is as a result of reduction of tetrazolium violet to formazan. This colour intensity and increase in absorbance signified the potential of the bacterial strain 39_H1 to utilise the carbon sources on the Biolog EcoPlates and metabolise the carbon compounds. The molecules of cellobiose are linked by β-1,4-glycosidic bonds and can be hydrolysed to glucose by the actions of cellobiase (G4234_02595). Metabolism of the organic substrates, cellobiose by the bacterial strain was indicated by the quick colour change after 24 h of incubation (Supplementary material Fig. 5b) and as well as increase in absorbance value (Supplementary material Fig. 6).

### 3.2. Inoculation experiment to enhance biogas production

The inoculated strain proved to enhance biomethane production when used as a single isolate inoculum for bioaugmentation of biogas production (Fig. 2). Methane produced in both treatments

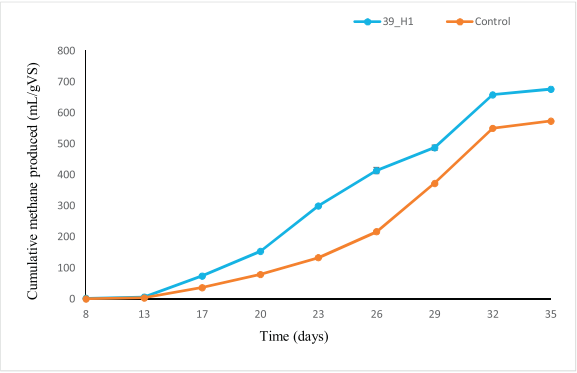


**Fig. 2.** Cumulative methane produced from anaerobic digestion of water hyacinth and cow dung in the presence and absence of bioaugmentation with *Serratia marcescens*. Error bars represent standard deviation (n = 3). Analysis of variance (ANOVA) for methane production revealed a significant difference between the treatments, $P < 0.05$.

were similar in the first week of AD but later increased in both treatments but at different rates. Identified effects of bioaugmentation specifies the enhancement of hydrolysis of lignocellulosic substrates by *Serratia marcescens* 39_H1 and improvement of the subsequent phases of AD.

### 3.3. Whole genome sequence analysis

Genomic features of *Serratia marcescens* 39_H1 are presented in (Table 1). The genome of 39_H1 has an N50 of 267,528 and 55 contigs were generated.

According to the phylogram (Fig. 3), the strain 39_H1 aligned with *Serratia marcescens* KHCo-24B (GCF_002592035.1) to establish a clade with 99.8 % homology when compared with different strains of *Serratia marcescens* of clinical and environmental (plant growth promoting) source. All strains compared have ANI values of >95 % with *Serratia marcescens*. Strain KHCo-24B (GCF_00292035.1) has been previously presented as a plant growth promoting rhizobacteria with abilities to control phytopathogens [33].

A metabolic pathway was structured based on the NCBI prokaryotic genome annotation. Straight arrows denote identified genes (with their NCBI PGAP locus tag) that are encoded by the genome. Broken arrows denote recognised pathways of which their associated genes were not identified in *Serratia marcescens* 39_H1 genome.

Inclusion of the bacterial strain, *Serratia marcescens* 39_H1 as a single isolate inoculum for bioaugmentation accelerated the production of biomethane. The genes that encode the cellulase and β-glucosidase enzymes were identified in the genome of *Serratia marcescens* 39_H1 (Fig. 4). A progressive correlation was established in this study between the genotypic and phenotypic characteristics of 39_H1 when it comes to hydrolytic and acidogenic phases of AD. Hydrolysis was linked to cellulase activity while acidogenesis was associated with phosphate solubilisation capacity of the strain. Cellulase (G4234_20980) catalyses the hydrolysis of cellulose to cellobiose while β-glucosidase (G4234_02595), the rate-limiting enzyme of hydrolysis of cellulose, converts cellobiose to fermentable sugars such as glucose [34]. Identification of some other hydrolytic genes that hydrolyse the glycosidic linkages of polymeric galactan by β-galactosidase (G4234_12230) also confirmed the capability of this strain to participate in the rate-limiting phase of AD (hydrolysis) of lignocellulosic substrates. The key mechanism of phosphate solubilisation is the secretion of organic acids such as gluconic acid, citric acid, propanoic acid, acetic acid and butyric acid of which acetic, butyric and propanoic acids are volatile fatty acids and products of acidogenesis [14]. Acidogenic abilities of this strain were ascertained via the direct oxidation pathway of β-D-glucose to gluconic acid [35]. β-D-glucose was oxidised by a periplasmic glucose-1-dehydrogenase (G4234_24170) that involves pyrroquinoline quinone as a coenzyme. The metabolite, D-glucono-1,5-

**Table 1**
Genomic features of *Serratia marcescens* 39_H1.

| Features | Value |
|---|---|
| Genome size | 5,106,712bp |
| Genome coverage | 34.2x |
| G + C content | 59.7 % |
| Genes | 4,908 |
| CDs | 4,785 |
| CDs (with protein) | 4,755 |
| RNA | 123 |
| rRNA | 25 |
| tRNA | 82 |
| Pseudo genes | 30 |

CDs, coding sequences.

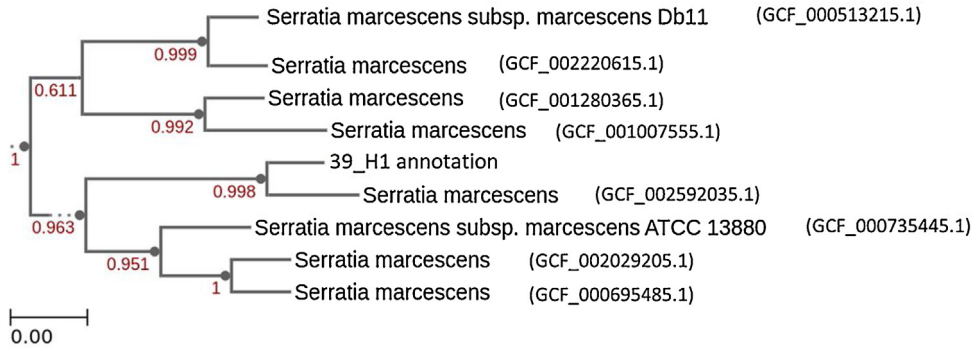


**Fig. 3.** Unrooted maximum likelihood phylogram showing closely related type strains of *Serratia marcescens*. The phylogram was constructed with FastTree 2.1 with 100 bootstrap repetitions and the RefSeq assembly accession numbers are in parenthesis.

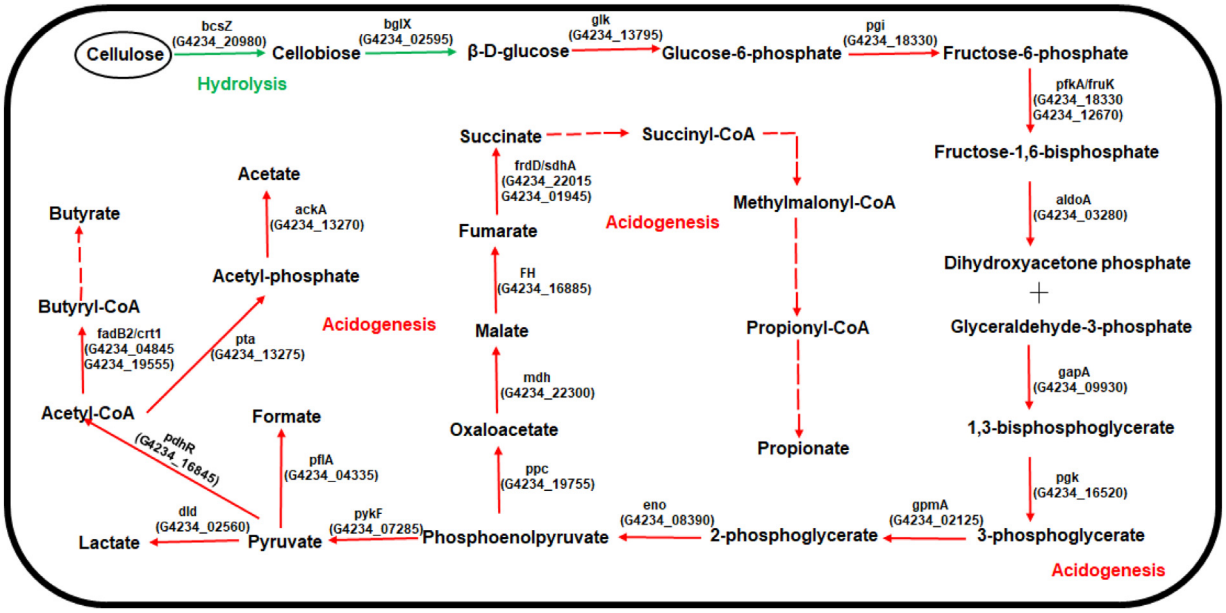


**Fig. 4.** Metabolic pathways of hydrolysis and acidogenesis in *Serratia marcescens* 39_H1.

lactone was oxidised to D-gluconic acid by gluconolactonase (G4234_06610), further oxidation to 6-phosphogluconate was catalysed by gluconokinase (G4234_10115) and to 2-keto-3-deoxy-6-phosphogluconate by 6-phosphogluconate dehydratase (G4234_18415). 2-keto-3-deoxy-6-phosphogluconate was then oxidised to pyruvate and glyceraldehyde-3-phosphate by 2-keto-3-deoxy-6-phosphogluconate aldolase (G4234_10110). In the course of the phosphate solubilising assay, the strain possibly secreted an organic acid that initiated the formation of clearance zone around it (Fig. 1b), suggesting its ability as phosphate solubiliser and an acidogenic bacterial strain.

A closely related strain of 39_H1, KHCo-24B has been previously characterised as a non-clinical strain of *Serratia marcescens* but as an environmental/plant growth promoting strain when categorised according to the core genome multilocus sequence (cgMLST) and whole genome multilocus sequence typing (wgMLST) [33,36]. Based on the 21 recognised virulence genes associated with *Serratia marcescens* strains of both clinical and environmental and plant growth promoting (PGP) derivation, KHCo-24B which formed a clade with 39_H1, amassed a greater quantity of genes associated with plant growth promotion [33]. However, genes associated with virulence and antibiotic resistance were predicted in the genome of 39_H1 but some of these genes are beneficial and could correspondingly function as plant growth promoting agents. These include regulatory genes, *phoPQ* (G4234_06440; G4234_06435) and *rcsA* (G4234_11510) which regulates genes for extracellular biosynthesis of polysaccharides. Biofilm formation by bacteria support their survival when exposed to abiotic stress conditions and these genes were identified in 39_H1 as biofilm formation regulator genes (G4234_05420; G4234_20990) [37,38]. Chitinases (G4234_01750; G4234_14125) control phytopathogens that affect plant health [39]. The above mentioned attributes confirms the relevance of strain 39_H1 in agriculture. Moreover, 39_H1 possessed several cold-tolerance genes including *CspE* (G4234_01545), *CspC* (G4234_10320), *CspD* (G4234_04250), *hupB* (G4234_01000), gyrA (G4234_13035), *aceE* (G4234_16840) and *aceF* (G4234_16835) indicating its potential to be used as an inoculum to augment psychrophilic AD in low-cost, non-heated rural anaerobic digesters [40–42].

*Serratia marcescens* strain 39_H1 has been proven to be a hydrolytic/acidogenic bacteria due to the possession of the relevant genes. Its involvement in hydrolysis and acidogenesis is vital to the improvement of the quantity of biogas generated during anaerobic digestion of lignocellulosic substrates. Different strains of *Serratia marcescens* have also been linked to the promotion of plant growth and development through the exhibition of some plant growth promoting traits such as production of indole acetic acid [43], enhancing the accessibility

of phosphate to plants through phosphate solubilisation [44]; this study]; improving plants' resistance to abiotic stress such as heavy metal toxicity [45]. To the best of our knowledge, this is the first study to report on the hydrolytic and acidogenic attributes of *Serratia marcescens* with regard to single bacterial isolate bioaugmentation for optimal production of biogas.

## Data accessibility

Genome sequences of *Serratia marcescens* 39_H1 have been deposited with the Genbank under the accession number JAAIKV000000000. The version described in this paper is version JAAIKV010000000. Related BioProject and Biosample accession number is PRJNA603151and SAMN13921475 respectively. The raw sequence data can be obtained from the Sequence Read Archive (SRA) under the accession number SRR11109663.

## CRediT authorship contribution statement

**Linda U. Obi:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing. **Memory Tekere:** Writing - review & editing, Supervision. **Ashira Roopnarain:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Funding acquisition. **Tomasz Sanko:** Software, Formal analysis, Data curation. **Tawanda E. Maguvu:** Software, Formal analysis, Data curation. **Cornelius C. Bezuidenhout:** Software, Formal analysis, Data curation. **Rasheed A. Adeleke:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors have no competing interests to declare.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00542.

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