




# Characterisation of hydrocarbon degradation, biosurfactant production, and biofilm formation in *Serratia* sp. Tan611: a new strain isolated from industrially contaminated environment in Algeria

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**Abstract** A novel bacterial strain was isolated from industrially contaminated waste water. In the presence of crude oil, this strain was shown to reduce the rate of total petroleum hydrocarbons (TPH) up to 97.10% in 24 h. This bacterium was subsequently identified by 16S rRNA gene sequence analysis and affiliated to the *Serratia* genus by the RDP classifier. Its genome was sequenced and annotated, and genes coding for catechol 1,2 dioxygenase and naphthalene 1,2-dioxygenase system involved in aromatic hydrocarbon catabolism, and LadA-type monooxygenases involved

in alkane degradation, were identified. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of crude oil after biological treatment showed that *Serratia* sp. Tan611 strain was able to degrade n-alkanes (from C<sub>13</sub> to C<sub>25</sub>). This bacterium was also shown to produce a biosurfactant, the emulsification index (E24) reaching 43.47% and 65.22%, against vegetable and crude oil, respectively. Finally, the formation of a biofilm was increased in the presence of crude oil. These observations make *Serratia* sp. Tan611 a good candidate for hydrocarbon bioremediation.

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## Introduction

The exploitation of petroleum resources from offshore platforms and the increase in maritime transport are regularly the source of significant pollution by hydrocarbons (Ławniczak et al. 2020), and different techniques have then to be implemented for the treatment of spilled oil. These include physical removal of the pollutants, conventional skimming methods, mechanical restoration using oil absorbents, and biological methods, i.e. the use of hydrocarbonoclastic bacteria

capable of degrading hydrocarbons (Marzan et al. 2017; Morales-Guzmán et al. 2017).

Bacteria work as primary degraders of spilled oils in the environment, and several species of the *Oleispira*, *Oleiphilus*, *Thalassolituus* and *Alcanivorax* genera are even known to feed exclusively on hydrocarbons (Brooijmans et al. 2009). Microbial degradation of crude oil often occurs by the attack of alkanes or light aromatic fractions, while the high molecular weight hydrocarbons, aromatic, resins, and asphaltenes are considered recalcitrant (Kanaly and Harayama 2000; Koshlaf and Ball 2017). The saturated components of crude oil (alkanes and cycloalkanes), particularly the n-alkanes with intermediated lengths (C<sub>10</sub>–C<sub>20</sub>), are the first degraded compounds. Although the degradation of both types of hydrocarbons, i.e. saturated hydrocarbons and aromatic compounds, requires different metabolic pathways, the difficulty to isolate bacterial strains owning such a double capacity is probably due to other factors such as the abundance of naturally occurring hydrocarbons, nutrient limitation, the formation of aerobic and anaerobic conditions, and the role of surfactants in biodegradation (Ławniczak et al. 2020). The determination of 16S rRNA gene sequences of oil-utilising bacteria has revealed that they are predominantly affiliated to Gammaproteobacteria and Actinobacteria (Isaac et al. 2015). Some Enterobacteriaceae, including *Serratia* sp. isolated from a petroleum-contaminated soil, were also found to be potential degraders of crude oil hydrocarbons (Borah et al. 2019).

Several bacteria are also known to produce surfactants, i.e. amphiphilic molecules that can be used as dispersing agents of crude oil, and microbial bioremediation is therefore one of the most broadly accepted methods to fight oil contamination because of its eco-friendly nature. Biosurfactants produced by petroleum-degrading microorganisms have an important role in the emulsification of hydrocarbons to reduce them, make them more soluble and less toxic, so that they can be used in their metabolism (Ron and Rosenberg 2002; Mahanty et al. 2006; Deepak and Jayapradha 2015; Borah et al. 2019). In this respect, petroleum-contaminated sites may serve as a potential source of novel indigenous hydrocarbon-degrading microorganisms (Chaudhary and Kim 2017; Méndez et al. 2017; Hu et al. 2018). The adoption of nucleic acid based technologies makes it possible to assess

their microbial biodegradation potential by detecting the presence of catabolic genes.

We report here the characterisation of *Serratia* sp. Tan611, a novel strain isolated from an industrial site located in Algeria. This bacterial strain displays outstanding degradation capacities against petroleum hydrocarbons and an ability to emulsify crude oil and form a biofilm, improving its degrading power. The analysis of its genome provides a comprehensive understanding of this strain, opening up perspectives for its application in bioremediation.

## Materials and methods

### Bacterial isolation and growth conditions

A bacterium, designated as ‘Tan611’, was isolated from a tannery effluent in the western region of Algeria. The strain was isolated on MSM minimal medium plates containing per liter: 1.2 g NH<sub>4</sub>Cl; 1.6 g K<sub>2</sub>HPO<sub>4</sub>; 0.4 g KH<sub>2</sub>PO<sub>4</sub>; 0.1 g NaCl; 1 g KNO<sub>3</sub>; 20 g MgSO<sub>4</sub> 7H<sub>2</sub>O; 10 g CaCl<sub>2</sub> 2H<sub>2</sub>O; 0.05 g FeCl<sub>3</sub>; pH 7.1 (Sidkey et al. 2016). A solution of trace elements (1 ml) was added separately under sterile conditions. Crude oil (1% v/v) was used as a carbon source to enrich the growth medium, and the plates were incubated at 30 °C. The strain was further purified on glycerol nutrient broth (1:1, v/v), and kept at – 20 °C. The bacterial strain was characterised for different phenotypic traits, including motility on tryptone swarm plates containing 1% Bacto-Tryptone, 0.5% NaCl, and 0.3% Bacto-Agar (Bertin et al. 1994). Swarm plates were inoculated with a 1:100 diluted sample and incubated at 30 °C for 15–24 h. Qualitative assay for the production of different enzymes was performed from a liquid bacterial culture using the API-ZYM-20 test kit (Biomérieux, USA). The strips were inoculated according to the manufacturer’s instructions and incubated for 4 h – 4 h 30 at 37 °C.

### Crude oil biodegradability

To determine bacterial crude oil degradation, the total petroleum hydrocarbon (TPH) of residual crude oil was quantified after growth in triplicates using 250 ml flasks containing 100 ml of mineral salt medium MSM with 20 ppm of crude oil as a carbon source. Crude oil was provided by Sonatrach Company (National

Society for Petroleum Research, Production, Transport, the Transformation and the Commercialization of Hydrocarbons). Tan611 strain was incubated for 48 h while uninoculated medium served as a control. The TPH degradation level was determined on an Oil Content Analyzer OCMA-310 equipment of Sonatrach Company (NF T90-203 standard) using HORIBA's special S-316 Solvent to extract the oil components from oily water samples, soil samples, or product surfaces. The extract is measured using IR absorbance, a non-dispersive infrared spectrophotometric technique which is specific to hydrocarbons such as oil. The OCMA-310 measures absorption in the 3.4–3.5  $\mu\text{m}$  range in which all hydrocarbons, including oils, absorb infrared radiation. As a result, the unit measures any hydrocarbons in the extraction solvent quickly and accurately, with no distortion of values due to the presence of the solvent (NF T90-203 standard). The amount of crude oil to be added in ppm was calculated according to the refinery's treatment plant protocol, as follows (SIEMENS Omniflo manual):

$$(\text{ml}) = \frac{V \text{ treated water} \cdot [C] \text{ ppm}}{\rho(\text{slop}) \cdot 1000}$$

#### Molecular characterisation of *Serratia* sp. Tan611

The molecular identification of the strain was carried out by 16S rRNA sequence analysis following standard procedures. Briefly, genomic DNA was extracted from a liquid culture according to the manufacturer's instructions (Wizard Genomic DNA Purification Kit, Promega, USA). Amplification of 16S rRNA gene from genomic DNA was performed using universal forward and reverse primers, fD1 (5'-AGAGTTT-GATCCTGGCTCAG-3') and rD1 (5'-AAGCT-TAAGGAGGTGATCCAGCC-3') respectively, in DNA Mastercycler Nexus (Eppendorf AG, Hamburg, Germany) with the following cycling parameters: 94 °C for 5 min; 35 cycles of 94 °C, 56 °C and 72 °C for 1.30 min each, and final extension at 72 °C for 3 min. The amplification PCR products were kept at 4 °C, purified and sequenced by Eurofins Genomics (Ebersberg, Germany). The sequence was compared with National Center for Biotechnology Information (NCBI) GenBank entries using the BLAST algorithm

and with RDP Naive Bayesian rDNA Classifier Version 2.11.

#### DNA library preparation and genome sequencing

In order to sequence the Tan611 genome, the strain was grown in LB liquid medium for 72 h at 30 °C and DNA was extracted using the MasterPure™ Complete DNA & RNA Purification Kit (Epicentre, Madison, Wisconsin) according to the manufacturer's instructions. ONT Library preparation and sequencing were performed according to the manufacturer's instructions "1D Native barcoding genomic DNA" (EXP-NBD103 and SQKLSK109)". At each step, DNA was quantified using the Qubit dsDNA HS Assay Kit (Life Technologies). DNA purity was tested using the nanodrop (ThermoFisher) and size distribution and degradation assessed using the Fragment analyser (AATI) High Sensitivity DNA Fragment Analysis Kit. Purification steps were performed using AMPure XP beads (Beckman Coulter). For 1 Flowcell, 5  $\mu\text{g}$  of each DNA (10 samples) were purified then sheared at 20 kb using the megaruptor system (diagenode). A one step DNA damage repair + END-repair + dA tail was performed on 700 ng of each sample. Then specific indexes were ligated to each sample. The library was generated by an equimolar pooling of these barcodes samples. Then adapters were ligated to the library. Library was loaded on a R9.4.1 revD flowcell and sequenced on GridION instrument at 0.03 pmol within 48 h. Illumina DNA-seq libraries were prepared according to Illumina's protocols using the Illumina TruSeq Nano DNA HT Library Prep Kit. Briefly, DNA was fragmented by sonication, size selection was performed using SPB beads (kit beads) and adaptators were ligated to be sequenced. Library quality was assessed using a Advanced Analytical Fragment Analyser and libraries were quantified by QPCR using the Kapa Library Quantification Kit. DNA-seq experiments were performed on an Illumina MiSeq using a paired-end read length of  $2 \times 150$  pb with the Illumina MiSeq Reagent Kits V2.

Short reads were filtered with the BBDuk program from JGI's BBTools (<https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide>) to trim adapters and extremities with a quality value per base lower than 20. Read pairs having an average quality less than 30 after the trimming step were discarded. Adapters were trimmed off long reads using the qcat program

developed by Oxford Nanopore Technologies (<https://github.com/nanoporetech/qcat>). Long reads were subsequently filtered with Nanofilt version 2.5.0 (De Coster et al. 2018) to keep only reads with an average quality of 9. Genome assembly was performed with the Unicycler hybrid assembly pipeline (Wick et al. 2017) using both short and long filtered reads, yielding circular replicon of 4,963,609 bp. The quality assessment of the assembly by Quast version 5.0.2 (Gurevich et al. 2013) could be considered as good with 99.91% of reads mapping back on the assembly with an average coverage depth of 168x. According to the CheckM version 1.0.11 (Parks et al. 2015) analysis, the genome assembly was 99.88% complete and showed insignificant contamination (0.51%) and no strain heterogeneity. The annotation of the genome was done using the MicroScope platform (Médigue et al. 2019). Ribosomal RNA genes were predicted with barnap (<https://github.com/tseemann/barnap>). The complete sequences of the *Serratia* Tan611 genome have been deposited in DDBJ/EMBL/GenBank under BioProject PRJEB40361 and assembly accession number GCA\_905188235.

#### Identification of LadA-type alkane monooxygenase sequences

The protein sequences of *Geobacillus thermoleovorans* B23 LadA $\alpha$  (BAM76377.1), LadA $\beta$  (BAM76372.1) and LadB (BAM76371.1) alkane monooxygenases (Boonmak et al. 2014) were used as BlastP (Camacho et al. 2009) queries to search the Tan611 proteome on the MicroScope platform. Reference LadA protein sequences were retrieved from UniprotKB database (UniProt Consortium 2019) by keyword search on gene names. Since only fragments were returned with an equivalent search for LadB, the sequence of BAM76371.1 was used to search UniprotKB with BlastP. The first 10 sequences with different genera were retrieved and used as LadB-like reference sequences. The 3 potential LadA-like protein sequences found in Tan611 (SERRA\_v1\_1764, SERRA\_v1\_2493, SERRA\_v1\_3086) were aligned with muscle 3.8.425 (Edgar 2004) to the 12 LadA, the 11 LadB-like reference sequences, and *Pseudomonas oleovorans* AlkB as an outgroup. A phylogenetic tree was computed from the resulting alignment with FastTree 2.1.11 (Price et al. 2010) using default parameters and Le Gascuel model (-lg option).

#### GC–MS analysis of oil fractions

Oil fractions (abiotic control medium and medium after treatment with Tan611 strain) were analysed using an experimental protocol directly adapted from those used in petroleum geochemistry (Charrié-Duhaut et al. 2000). Briefly, an organic extract was fractionated by polarity classes, which simplifies the molecular mixtures and thus the structural identification. The less polar fraction containing among others saturated and aromatic hydrocarbons, which are the main constituents of oils, was analyzed by gas chromatography coupled with mass spectrometry (GC–MS) in order to obtain the precise molecular composition of each sample, as previously described (Brito et al. 2006).

All solvents used were HPLC grade (Sigma Aldrich, purity: 99.9%) and CH<sub>2</sub>Cl<sub>2</sub> cleaned glassware was used. 2 ml of MSM medium supplemented with 1% crude oil after incubation at 30 °C for 72 h in the presence of Tan611 strain and taken after manual homogenization of the medium, was extracted completely twice with dichloromethane. The same procedure was applied to the control medium supplemented with 1% crude oil but without inoculation. The organic extracts were then evaporated under gentle nitrogen flow.

The organic extract dissolved in 50  $\mu$ l of CH<sub>2</sub>Cl<sub>2</sub> was fractionated using a column chromatography on silica gel 60 Å FLUKA 35–75  $\mu$ m granulometry and the less polar fraction was recovered by elution with 1.5 ml of cyclohexane. These fractions, which includes saturated and aromatic hydrocarbons, were evaporated under gentle nitrogen flow prior to GC–MS analysis.

GC–MS analyses were performed on a Focus GC gas chromatograph interfaced with an ISQ mass detector ThermoScientific (splitless mode injector, HP-5 MS column, 30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness, temperature program: 40 °C (1 min), 40–100 °C (10 °C/min), 100–320 °C (4 °C/min), isothermal 320 °C (30 min), carrier gas: Helium). Mass spectra were produced by electron impact at 70 eV in full detection mode over 40–800 amu. Peak assignment was based on the interpretation of mass spectra and comparison with spectra available in literature and NIST library 2.0 and by consideration of the retention time.

The biodegradation rate of selected n-alkanes, measured as a percentage, was calculated based on

the difference between the area of the corresponding peaks in the incubated sample ( $A_S$ ) and in the control ( $A_C$ ) for the same supplementation in crude oil, using the following formula:  $P (\%) = 100 - (A_S \times 100 / A_C)$ . The total degradation of the n-alkanes ranging from  $C_{13}$  to  $C_{25}$  was calculated by using the total area of the peaks from  $C_{13}$  to  $C_{25}$  in the incubated sample ( $TAs (C_{13}-C_{25})$ ) and in the control ( $TA_C (C_{13}-C_{25})$ ) by the same formula (Abena et al. 2020).

#### Oil displacement activity (ODA)

The determination of oil displacement activity was performed as previously described (Huang et al. 2020). Briefly, crude oil or vegetable oil (100  $\mu$ l) was placed on a surface of 40 ml distilled water Petri dish. Cell-free supernatant (10  $\mu$ l) of Tan611 strain was applied onto the oil drop and the diameter of the clearance zone was observed for 30 s. Water and Triton X-100, were used as negative and positive controls, respectively.

#### Emulsifying potential against crude and vegetable oils

The emulsifying ability of Tan611 strain against crude oil such as petrol and vegetable oil such as sunflower was determined from the emulsification index ( $E_{24}$ ) using pre-cultures in MSM broth. 3 ml of oil were added to 3 ml of cell-free broth in a test tube, which was vortexed vigorously at high speed for 2 min and allowed to stand for 24 h (Gaur et al. 2019). The emulsification index value ( $E_{24}$ ) was calculated using the following equation (Sumiardi et al. 2018):

$$E_{24} = \frac{\text{Height to emulsion formed} * 100}{\text{Total height to solution}}$$

All experiments were performed in triplicate and in the absence of any oil source as a control.

#### Biofilm formation

To evaluate the biofilm forming ability of the Tan611 strain, overnight cultures in the absence or the presence of 1% crude oil were diluted 1:100 in Luria–Bertani medium (LB, MP Biomedicals, USA) and added to sterile 24-well polystyrene plates. Following a further 48 h aerobic incubation at 37 °C, unattached bacterial cells were removed by the aspiration of culture medium

and biofilms were washed twice with phosphate-buffered saline solution (PBS; pH 7.2), as previously described (Andres et al. 2013). Biofilm biomass was then heat-fixed by exposing them to hot air at 55 °C for 25 min, and next stained with 1 ml crystal violet 0.1% (w/v) for 30 min. The dye was discarded and wells were washed three times with PBS and dried. Biofilms were resolubilised with 1 ml 95% ethanol, and optical density (OD) of each well was measured at 595 nm using BIO-RAD reader (SmartspectPlus spectrophotometer). Negative control wells contained uninoculated broth medium. The strain was then categorised into one of the 4 following categories (Zhuo et al. 2014; Hemati et al. 2020): no biofilm producer ( $OD \leq OD_c$ ); weak biofilm producer ( $OD_c < OD \leq 2xOD_c$ ); moderate biofilm producer ( $2xOD_c < OD \leq 4xOD_c$ ); and strong biofilm producer ( $4xOD_c < OD$ ), where  $OD_c = \text{average OD of negative control} + (3 \times \text{standard deviation (SD) of the negative control})$ .

## Results

### Phenotypic characterisation

The Tan611 strain isolated from the tannery effluents produced round colonies without pigmentation on nutrient rich medium, and was motile on swarm plates. Its optimal growth was observed between 28 and 35 °C and in pH values ranging from 5 to 9 (data not shown). On API-ZYM-20 strips, the strain was shown to produce eight extracellular hydrolytic enzymes (Table 1). The strain harbored alkaline phosphatase, esterase lipase (C8), leucine arylamidase activity, valine arylamidase activity, cystine arylamidase, trypsin,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities but no esterase (C4), lipase (C14),  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -mannosidase or  $\alpha$ -fucosidase activity. The Tan611 strain was also capable of utilizing vegetable oil and crude oil as energy source.

To determine its crude oil degradation activity, the strain was grown in 50 ml mineral medium containing 20 ppm crude oil as a unique carbon and energy source. After 48 h incubation at 30 °C, analysis of TPH degradation with OCMA-310 equipment indicates a significant difference in absorption (according to the bands corresponding to the valence vibrations of

**Table 1** Enzyme activity profiles of *Serratia* sp. Tan611 strain as determined by the API-ZYM system. The strain was shown to produce eight extracellular hydrolytic enzymes: +, positive reaction; −, negative reaction

Enzyme	Substrate	Results
Alkaline phosphatase	2-naphtyl phosphate	+
Esterase (C4)	2-naphtyl butyrate	−
Esterase lipase (C8)	2-naphtyl caprylate	+
Lipase (C14)	2-naphtyl myristate	−
Leucine arylamidase	L-leucyl-2-naphtylamide	+
Valine arylamidase	L-valyl-2-naphtylamide	+
Cystine arylamidase	L-cystyl-2-naphtylamide	+
Trypsin	N-benzoyl-DL-arginine-2-naphtylamide	+
α-chymotrypsine	N-glutaryl-phenylalanine-2-naphtylamide	−
Acid phosphatase	2-naphtyl phosphate	+
Naphtol-AS-BI-phosphohydrolase	Naphtol-AS-BI-phosphate	+
α-galactosidase	6-Br-2-naphtyl-αD-galactopyranoside	−
β-galactosidase	2-naphtyl-βD-galactopyranoside	+
β-glucuronidase	Naphtol-AS-BI-βD-glucuronide	−
α-glucosidase	2-naphtyl-αD-glucopyranoside	+
β-glucosidase	6-Br-2-naphtyl-βD-glucopyranoside	+
N-acetyl-β-glucosaminidase	1-naphtyl-N-acetyl-βD-glucosaminide	+
α-mannosidase	6-Br-2-naphtyl-αD-mannopyranoside	−
α-fucosidase	2-naphtyl-αL-fucopyranoside	−

the CH<sub>2</sub>–CH<sub>3</sub> groups) between the control and the Tan611 strain with 20 ppm and 0.58 ppm respectively. This corresponds to a degradation rate of 97.10%, providing evidence that this strain possess a high degradation ability.

#### Genotypic and genomic characterisation of Tan611 strain

The 16S-based taxonomic prediction by the RDP classifier server (Wang et al. 2007) identified the Tan611 strain as *Serratia* sp. Its genome was sequenced and the assembly produced a single circular chromosome with 4,963,609 bp, and a GC content of 57.96%. The annotation with the MicroScope platform (Médigue et al. 2019) predicted the existence of 5018 genes, of which 4784 were coding sequences (CDS), 85 were tRNA genes, and 22 were rRNA genes. To define more accurately the taxonomic position of the strain, the complete genomes of 26 strains (10 representative genomes + 16 other strains) of the genus *Serratia* were obtained from the NCBI RefSeq library. Analysis of the Tan611 genome and the 26 *Serratia* genomes with GToTree v1.5.38 using a set of 172 single-copy genes in Gammaproteobacteria indicated that the closest match for Tan611 is *Serratia* sp. MYb239. The Tan611 strain

can be considered to be close to MYb239, KUDC3025, *rubidea* and FGI94. The tree was drawn with iTol online (Interactive Tree of Life (iTOL) v4) (Fig. 1).

We investigated the metabolic network of hydrocarbon degradation in *Serratia* sp. Tan611 strain, in particular the n-alkane and PAH degradation pathways previously identified in other strains belonging to the same genus (Wang et al. 2008; Lai et al. 2012). The genome of *Serratia* sp. Tan611 contains genes coding for LadA-type monooxygenases (Boonmak et al. 2014) (Fig. 2). In addition, several genes associated with the metabolism of aromatic hydrocarbons and PAH degradation were identified in the genome. Two colocalised genes and a third one, *cbdA*, *benB* and *bedA*, respectively, encode the large and β-subunits of dioxygenases involved in benzene degradation. Two genes, i.e. *catA* and *ndoA*, that encode dioxygenase and the ferredoxin component of the naphthalene 1,2-dioxygenase (NDO) system, respectively, were also identified in *Serratia* sp. Tan611 strain.

#### Crude oil fraction analysis

To further investigate the high-degradation capacity of *Serratia* sp. Tan611 strain, we performed a GC–MS analysis on the fraction containing saturated and



◀ **Fig. 1** Unrooted Maximum Likelihood phylogenetic tree of Tan611 and the 26 publicly available *Serratia* complete genomes. The tree was obtained with the GToTree phylogenomic workflow using the Gammaproteobacteria predefined gene set of 172 single-copy genes and default parameters (Lee MD. GToTree., 2019). The *Serratia* sp. Tan6.1 closest relatives were *Serratia* sp. MYb239, KUUC3025, *rubidea* and FGI94. The RefSeq accession numbers of the 26 complete genomes were: *Serratia proteamaculans* 568 (GCF 000,018,085.1), *Serratia* sp AS12 (GCF 000,214,195.1), *Serratia plymuthica* AS9 (GCF 000,214,235.1), *Serratia* sp AS13 (GCF 000,214,805.1), *Serratia* sp FGI94 (GCF 000,330,865.1), *Serratia liquefaciens* ATCC 27,592 (GCF 000,422,085.1), *Serratia* sp FS14 (GCF 000,695,995.1), *Serratia* sp SCBI (GCF 000,747,565.1), *Serratia rubidaea* 1122 (GCF 001,572,725.1), *Serratia surfactantfaciens* YD25 (GCF 001,642,805.2), *Serratia fonticola* FDAARGOS 411 (GCF 002,588,845.1), *Serratia* sp SSNIH1 (GCF 002,935,055.1), *Serratia* sp MYb239 (GCF 002,966,855.1), *Serratia* sp 1D1416 (GCF 003,641,105.1), *Serratia* sp 3ACOL1 (GCF 003,668,775.1), *Serratia* sp P2ACOL2 (GCF 003,691,565.1), *Serratia* sp LS-1 (GCF 003,719,595.1), *Serratia* sp FDAARGOS 506 (GCF 003,812,745.1), *Serratia nematodiphila* DHS01 (GCF 004,768,745.1), *Serratia proteamaculans* 336X (GCF 009,660,185.1), *Serratia* sp KUUC3025 (GCF 009,817,885.1), *Serratia marcescens* BP2 (GCF 011,602,465.1), *Serratia ureilytica* CC119 (GCF 014,304,635.1), *Serratia ficaria* NCTC12148 (GCF 900,187,015.1), *Serratia odorifera* NCTC11214 (GCF 900,635,445.1), *Serratia quinivorans* NCTC13188 (GCF 900,638,135.1)

aromatic hydrocarbons. After 72 h of growth, the gas chromatogram of the medium was compared to that of an abiotic control sample (Fig. 3). The fraction of the abiotic control included a classic distribution of linear saturated hydrocarbons (n-alkanes) ranging from C<sub>13</sub> to C<sub>30</sub> without even or odd predominance (Fig. 3, Table 2). The amount of material present was much less in the incubated medium: the intensities of all peaks in the less polar fraction were significantly decreased and n-alkanes were present only in trace amounts, unlike the control sample where they were always present in large quantities. As an example, *Serratia* sp. has the capacity to degrade 73.77% of the crude oil n-alkanes ranging from C<sub>13</sub> to C<sub>25</sub> in only 72 h of incubation (Table 2).

In addition, the degree of degradation of short-chain n-alkanes (C<sub>13</sub> to C<sub>16</sub>) was higher than that of long-chain n-alkanes with more than 90% degradation for C<sub>13</sub> and C<sub>14</sub> (Table 2). These results further support the existence of hydrocarbon degradation mechanisms in *Serratia* sp. Tan611 strain. The bacterial growth was

also accompanied by a visual change in the appearance of oil as compared to an abiotic medium used as a control. This modification of the culture medium (emulsification) was detectable on the separation chromatography column, possibly resulting from the presence of biosurfactants produced by the strain.

#### Emulsifying potential against crude oil and vegetable oil

Using an oil spreading technique, the displacement of the clear zone demonstrated a biosurfactant production by *Serratia* sp. Tan611 strain (data not shown). To further study the production of bioemulsifier by *Serratia* sp. Tan611 strain, the biosurfactant activity was evaluated by emulsification (E<sub>24</sub>) assay (Bonilla et al. 2005). The index value obtained was higher for crude oil as compared to the emulsification with vegetable oil (E<sub>24</sub> = 65.2 ± 0.5 and E<sub>24</sub> = 43.5 ± 0.1, respectively) after 24 h incubation at 30 °C. These differences may be explained by the emulsion forming and stabilising capacity of biomolecules being specific for certain hydrophobic compounds present in crude oil (Freitas et al. 2009; Maalej et al. 2016). Finally, the emulsions observed in the presence of crude oil were found to be stable after 7 days (Fig. 4), which further supports the important emulsifying capacities of *Serratia* sp. Tan611 strain.

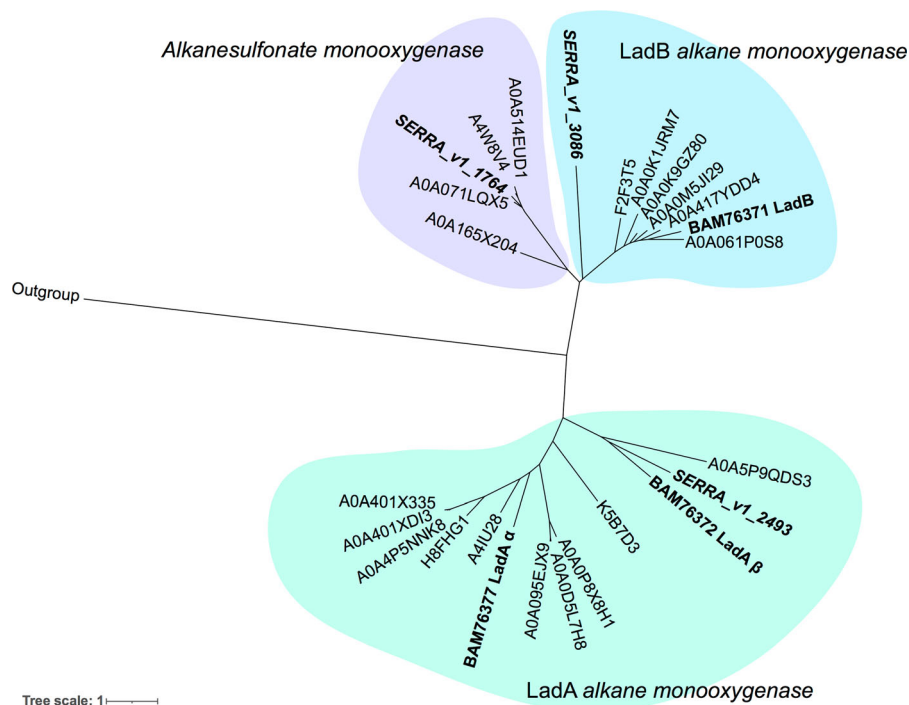
#### Biofilm formation

To determine the possible impact of crude oil on the biofilm formation by *Serratia* sp. Tan611, bacterial cells were grown in microtiter plates and stained with crystal violet. The results showed that biofilms produced by *Serratia* sp. Tan611 were thicker in the presence of 1% crude oil than those formed in its absence, with OD<sub>595</sub> (means ± SD) of 3.2 ± 0.5 and 2.5 ± 0.7, respectively (Fig. 5). According to the OD values, *Serratia* sp. Tan611 was categorised as a strong producer.

#### Discussion

The most successful process for the removal or the elimination of hydrocarbons from the environment is their microbial transformation and biodegradation. The present study aimed at exploring the indigenous





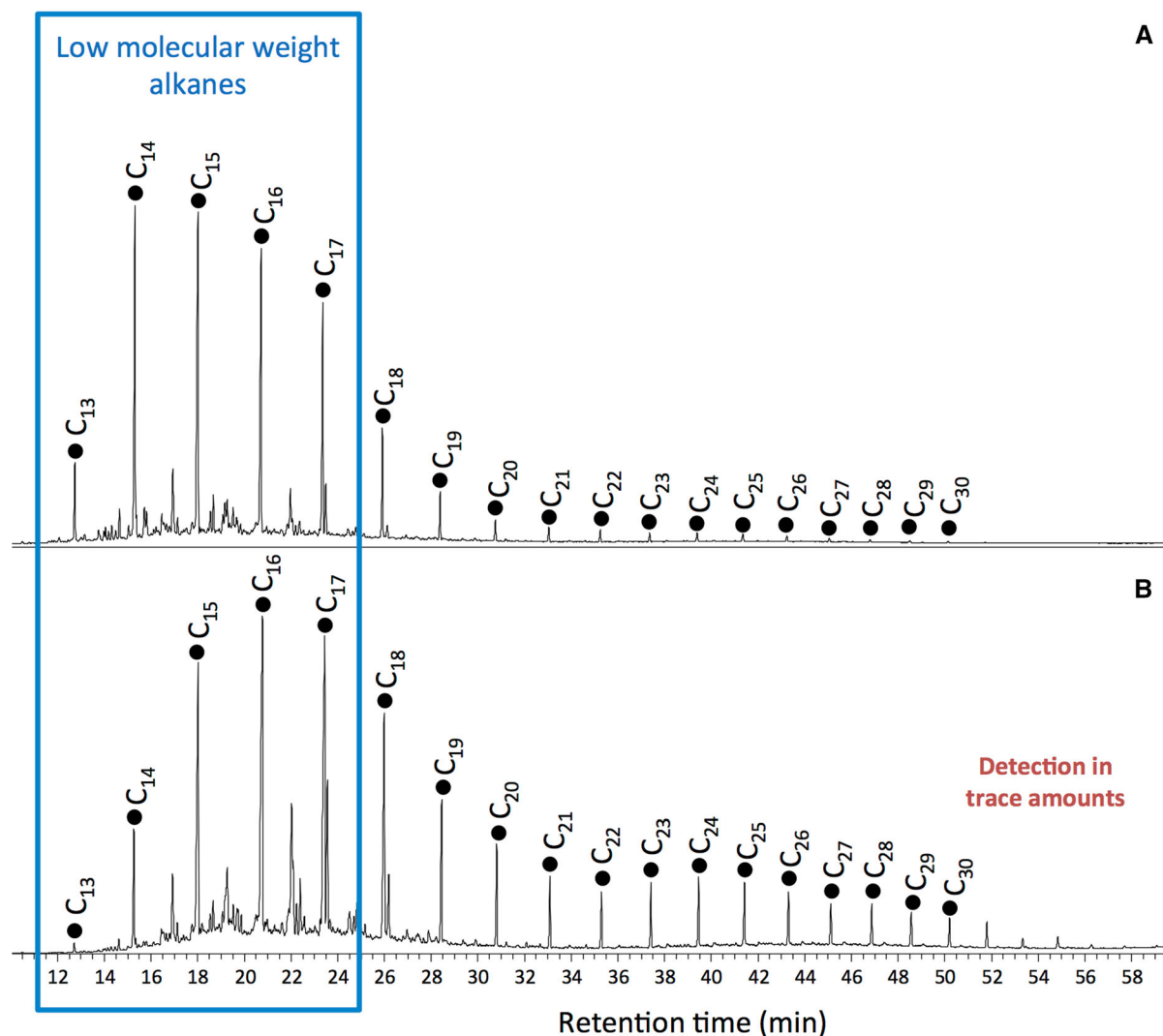
**Fig. 2** Phylogenetic tree of LadA-type alkane monoxygenase sequences. The tree was drawn with iTOL online (Interactive Tree of Life (iTOL) v4) showing the positions of *Serratia* sp. Tan611 sequences SERRA\_v1\_1764, SERRA\_v1\_2493 and SERRA\_v1\_3086 relative to other LadA-like proteins. SERRA\_v1\_2493 is grouped with LadA alkane monoxygenases whereas SERRA\_v1\_3086 is closer to the LadB type. SERRA\_v1\_1764, however is grouped with the alkanesulfonate monoxygenases, which were among the LadB-like reference sequences identified by BlastP in UniprotKB. Lad A: BAM76377, *Geobacillus thermoleovorans* B23 LadA $\alpha$ ; BAM76372, *Geobacillus thermoleovorans* B23 LadA $\beta$ ; A0A5P9QDS3, *Luteimicrobium xylanilyticum*; K5B7D3, *Mycolicibacterium hassiacum*; A0A0P8X8H1, *Pseudomonas fluorescens*; A0A0D5L7H8, *Burkholderia* sp. 2002721687;

microflora of an Algerian industrial site and investigating its hydrocarbon degradation and biosurfactant production potential. The initial characterisation of different isolated strains revealed multiple cellular morphologies and a predominance of Gram-negative bacteria belonging to the Enterobacteriaceae. One strain was further characterised and shown by 16 rRNA sequencing analysis to belong to the *Serratia* genus. The genome of this bacterial strain, designated as *Serratia* sp. Tan611, was sequenced and annotated to identify metabolic pathways possibly involved in hydrocarbon degradation. The analysis of the whole genome revealed the presence in *Serratia* sp. Tan611

A0A095EJX9 *Burkholderia cepacia*; A4IU28 *Geobacillus thermodenitrificans* NG80-2; H8FHG1 *Xanthomonas citri* pv. *mangiferaeindicae* LMG 941; A0A401X335 *Acetobacter pasteurianus* NBRC 3278; A0A401XDI3 *Acetobacter pasteurianus* NBRC 3299; A0A4P5NNK8 *Komagataeibacter diospyri*; Lad B: BAM7631.1 *Geobacillus thermoleovorans* B23 LadB; F2F3T5 *Solibacillus silvestris* StLB046; A0A061P0S8 *Geomicrobium* sp. JCM 19038 alkanesulfonate monoxygenase; A0A0K1JRM7 *Paenibacillus* sp. 320-W; A0A417YDD4 *Oceanobacillus profundus*; A0A0K9GZ80 *Peribacillus loiseleuriae*; A0A0M5JI29 *Bacillus gobiensis*; SsuD: A0A165X204 *Aeribacillus pallidus*; A0A071LQX5 *Mangrovibacter* sp. MFB070; A4W8V4 *Enterobacter* sp. 638; A0A514EUD1 *Raoultella electrica*

strain of various genes involved in hydrocarbon degradation such as the *ladA*-type genes in alkane degradation (Boonmak et al. 2014), the *catA*, *ndoA* genes in aromatic hydrocarbon catabolism (Dong et al. 2017) and *cbdA*, *benB*, and *bedA*, in benzene degradation (Xu et al. 2017).

GC–MS analysis of the less polar fraction containing saturated and aromatic hydrocarbons, which are the main constituents of oils, confirmed the hydrocarbon degradation capacities of *Serratia* sp. Tan611 strain. Such an approach provides access to the molecular composition of the effluents and thus makes it possible to selectively monitor the degradation of the



**Fig. 3** GC-MS total ion chromatograms (TIC) of the less polar fractions. The results were obtained from A, the abiotic control sample; B, the sample after 72h of treatment with *Serratia* sp.

different families of compounds. In addition, a possible interpretation of the apparently complete degradation of the ( $C_{13}$ – $C_{15}$ ) group is that this group of alkanes is less recalcitrant than the other hydrocarbons present and was biodegraded before the limitations of N and P occurred (Coulon et al. 2005). In addition, GC/MS analysis showed that the petroleum hydrocarbons were almost completely degraded by *Serratia* sp. Tan611 strain after 72 h of total continuous feed. These results demonstrated the performance of this bacterium in the biodegradation of petroleum hydrocarbons for a wide range of n-alkanes. Overall, short-

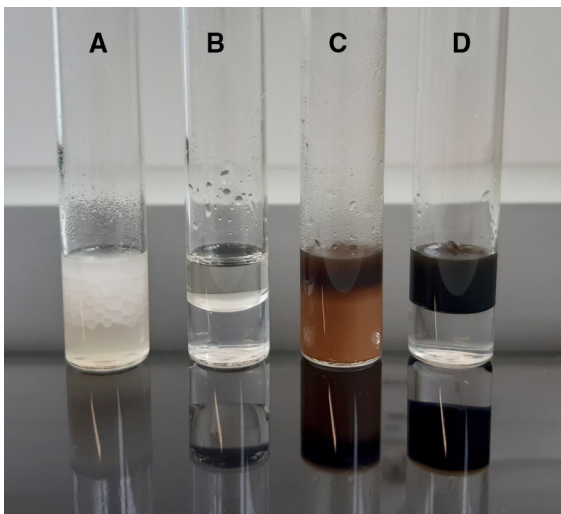
Tan611 strain.  $C_x$ : notation for a chain with x carbon atoms. The blue box highlights the shortest alkane chains which were shown to be strongly degraded by the *Serratia* sp. Tan611 treatment

and medium-chain alkanes were degraded more rapidly than long-chain alkanes. Alkanes, especially those with relatively short chains ( $< C_{12}$ ), are generally readily degraded due to their lower molecular weight and water solubility. Medium-length ( $C_{12}$ – $C_{16}$ ) and higher molecular weight long-chain ( $> C_{16}$ ) alkanes are generally degraded at a relatively slow rate due to their hydrophobic nature (Sathishkumar et al. 2008). In addition, the sensitivity of crude oil to microbial degradation depends on the intrinsic ability of the microorganisms to degrade the crude oil and activate the key enzymes of the first phase of

**Table 2** Percentage degradation of components of 1% crude oil by *Serratia* sp. Tan611 strain after 72 h of treatment. The distribution of the selected components is presented on the GC–MS chromatogram (Fig. 3). The degradation percentage of a specific component was calculated based on the difference between the area of the corresponding peaks in the incubated sample ( $A_S$ ) and in the control ( $A_C$ ) for the same supplementation in crude oil, using the following formula:  $P (\%) = 100 - (A_S \times 100/A_C)$ . The total degradation of the n-alkanes ranging from  $C_{13}$  to  $C_{25}$  was calculated by using the total area of the peaks from  $C_{13}$  to  $C_{25}$  in the incubated sample ( $TAs (C_{13}-C_{25})$ ) and in the control ( $TA_C (C_{13}-C_{25})$ ) by the same formula

Retention time (min)	Components of crude oil	Degradation percentage (%)
12.7	n-tridecane ( $C_{13}$ )	97.42
15.3	n-tetradecane ( $C_{14}$ )	93.90
18	n-pentadecane ( $C_{15}$ )	83.91
20.7	n-hexadecane ( $C_{16}$ )	71.65
23.4	n-heptadecane ( $C_{17}$ )	62.25
26	n-octadecane ( $C_{18}$ )	43.78
28.4	n-nonadecane ( $C_{19}$ )	33.56
30.8	n-eicosane ( $C_{20}$ )	17.04
33	n-heineicosane ( $C_{21}$ )	26.88
35.3	n-docosane ( $C_{22}$ )	22.33
37.4	n-tricosane ( $C_{23}$ )	5.02
39.4	n-tetracosane ( $C_{24}$ )	24.10
41.4	n-pentacosane ( $C_{25}$ )	26.83
	Total degradation	73.77

biodegradation. The crude oil used in this study consisted mainly of medium- and long-chain alkanes. Therefore, key enzymes that could have been involved in the biodegradation process were more likely

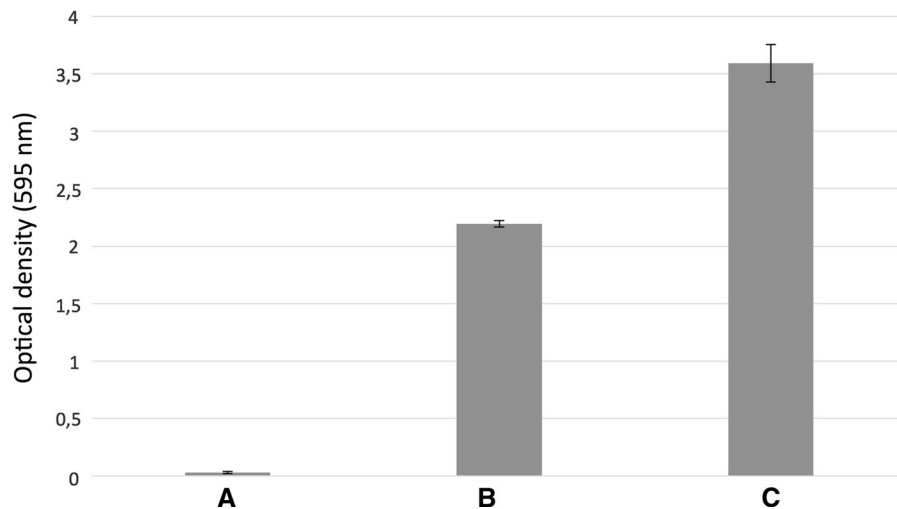


**Fig. 4** Emulsification potential of *Serratia* sp. Tan611 strain with vegetable or crude oil. The emulsification assay was performed in minimal MSM medium with A, vegetable oil; B, crude oil (1% w/v for 24h). B and D are negative control of MSM medium without bacterial inoculum

enzymes for the degradation of medium ( $C_8-C_{16}$ ) and long chain alkanes, such as LadA-type alkane monooxygenases (Boonmak et al. 2014; Chen et al. 2017).

Biomolecules preferentially partition at the interface between polar and apolar molecules (e.g. hydrocarbons and water) to produce microemulsions that often enhance the bioavailability and degradation of hydrocarbons (Niknezhad et al. 2018). The emulsification assay was performed using 2 oils, i.e. crude oil and vegetable oil, the best production being observed with crude oil. The biosurfactant produced by *Serratia* sp. Tan611 was capable of forming an emulsion stable for more than two weeks at room temperature. Oil degradation is possible if the substrate, generally poorly soluble, is emulsified beforehand by bacterial biosurfactants (Sumiardi et al. 2018). Indeed, biosurfactants reduce the surface and interface tension between liquid and solid substances, leading to the formation of emulsions in liquids. This can facilitate intracellular uptake and degradation of hydrocarbons by microorganisms, which should improve the biological treatment used in oil refinery processing plants.

Finally, the biofilm way of life represents a natural bacterial strategy to survive and optimise living



**Fig. 5** Biofilm formation by *Serratia* sp. Tan611 strain with or without 1% crude oil. A, negative control consisting of non-inoculated Luria-Bertani medium; B, *Serratia* sp. Tan611 biofilm in Luria-Bertani medium without crude oil; C, *Serratia*

sp. Tan611 biofilm in Luria-Bertani medium with 1% crude oil. The biofilm formation was determined by the optical density measurement of crystal violet at 595 nm. Error bars represent standard deviations

conditions, in particular in contaminated sites (Isaac et al. 2017). The microbial nearness in biofilm structures is beneficial for cellular interactions, which improve the degradation processes (Edwards and Kjellerup 2013). The potential of biofilm formation by *Serratia* sp. Tan611 was shown to be more than 60% higher in the presence of crude oil. Taken together, our results suggests that *Serratia* sp. Tan611 strain can represent a good candidate, alone or in consortium, for bioremediation and wastewater treatment in bioreactors. The specific metabolic pathway of hydrocarbons is still unclear for many bacterial species, including *Serratia* sp. Therefore, further research is still needed to understand the mechanism of hydrocarbon degradation in strain Tan611 and to identify the intermediate metabolites that are produced during the biodegradation process. Similarly, the potential of these strains still needs to be investigated in soils contaminated with crude oil.

## Conclusion

In this study, we isolated *Serratia* sp. Tan611 strain, an hydrocarbon-degrading microorganism, by enrichments obtained from a TPH and industrial pollutants contaminated site. This novel strain showed a percentage reduction of the oil content up to 97.10% from

an initial content of 20 ppm of crude oil. By sequencing and annotation of its genome, we identified the presence of *ladA*-type genes involved in alkane degradation and dioxygenase genes such as *catA*, and *ndoA* genes, and *cbdA*, *benB*, *bedA* genes involved in the catabolism of aromatic hydrocarbons. The formation of emulsifiers and biofilm further supports the possible use of *Serratia* sp. Tan611 strain in bioremediation.

**Author's contribution** Annela Semai performed research and wrote the paper; Frédéric Plewniak performed research and wrote the paper, Armelle Charrié-Duhaut performed research and wrote the paper, Amalia Sayeh and Lisa Gil performed research, Céline Vandecasteele contributed new methods, Céline Lopez-Roques wrote the paper, Emmanuelle Leize-Wagner analyzed data, Farid Bensalah analyzed data, Philippe N. Bertin conceived study, analyzed data and wrote the paper.

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**Data availability** The complete sequences of the *Serratia* Tan611 genome have been deposited in DDBJ/EMBL/GenBank under BioProject PRJEB40361 and assembly accession number GCA\_905188235.

**Code availability** Not applicable.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Not applicable.

**Consent to participate** All authors gave their consent to participate in this study.

**Consent for publication** All authors gave their consent to publish results from this study and to be listed as a co-author.

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