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Draft genome sequence of *Stenotrophomonas maltophilia* strain P13 gives insight into its protease production and assessment of sulfur and nitrogen metabolism

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ABSTRACT

Stenotrophomonas maltophilia strain P13 was isolated from the Kanchengayao glacier's accumulation zone. A comprehensive study was done on this strain as it produced protease enzyme and thus having industrial potential. The whole genome sequence, FAME, morphological and biochemical characteristics of the *S. maltophilia* strain P13 is being presented. The genome of P13 strain possesses 2,689,565 total reads, with an average $G+C$ content of 69.9%. The whole-genome assembly is having 548 contigs (with PEGs). The genome contains 2,985 coding sequences with 55 rRNA predicted genes. More than 88% of the total pre-processed reads from samples were mapped. The identified coding proteins were classified into 27 functional categories based on COG classification. The genome was found to possess genes for ammonium assimilation, galactosylceramide and sulfatide metabolism. The major enzymes present are beta-galactosidase, thiol peroxidase, thiolredoxin reductase, glutamate synthase, and glutamate-ammonia-ligase adenyltransferase. The genome information of *Stenotrophomonas maltophilia* P13 provides the basis for understanding the functional properties and abilities to act as a potential cold-active enzyme producer and nitrogen-fixing bacteria.

1. Introduction

Stenotrophomonas maltophilia is one of the most commonly found bacterium in earth's biosphere (Dunne et al., 1997). Taxonomical lineage for this non-fermentative Gram-negative bacterium had long deliberations and was earlier referred as *Pseudomonas maltophilia* syn. *Xanthomonas maltophilia*. But recent emended nomenclature states it as *Stenotrophomonas maltophilia* (Adegoke et al., 2017). The proposal for the genus *Stenotrophomonas* was suggested by Palleroni and Bradbury (1993), after the amended classification of *Xanthomonas maltophila* to *Stenotrophomonas maltophila*. This genus consists of four valid species - *Stenotrophomonas maltophila* (Palleroni and Bradbury, 1993), *Stenotrophomonas nitritireducens* (Finkmann et al., 2000), *Stenotrophomonas acidaminiphila* (Assih et al., 2002) and *Stenotrophomonas rhizophila* (Wolf et al., 2002).

S. maltophilia is referred to as one of the most common opportunistic pathogen for humans as it can infect immunocompromised patients (Crossman et al., 2008). Various strains of this bacterium provides immunity to plants and also promote their growth by producing certain proteins (Jakobi et al., 1996) and disease management. Non-pathogenic *S. maltophilia* strains were discovered from the plant roots by Zhu et al. (2011). Some strains of *S. maltophilia* produces antifungal volatile organic compounds having high hydrolytic activities. The biocontrol activity of *S. maltophilia* is attributed to its proteolytic and chitinolytic properties (Zhang and Yuen, 1999; Zhang et al., 2001).

With the advent of whole-genome sequencing, the field of life sciences has changed drastically which can provide invaluable information related to the identification of gene functions and thus it can have several applications in biotechnological applications. It can provide information related to bacterial metabolism, functional genes, and cryostability inducing proteins, factors and enzymes. A novel bacterial strain was isolated from the accumulation zone of Kanchengayao glacier ice core of North Sikkim district, Sikkim. We performed whole genome sequencing, fatty acid methyl ester analysis (FAME), morphological and biochemical characterization, and scanning electron microscopy (SEM) of *Stenotrophomonas maltophilia* strain P13.

2. Materials and methods

2.1. Site description

Kanchengayao glacier has originated from the southern slope of Mt. Kanchengayao peak of North Sikkim, India. It is a north-south facing glacier having latitude of 27°59′57.872′N and longitude 88°37′8.785′E

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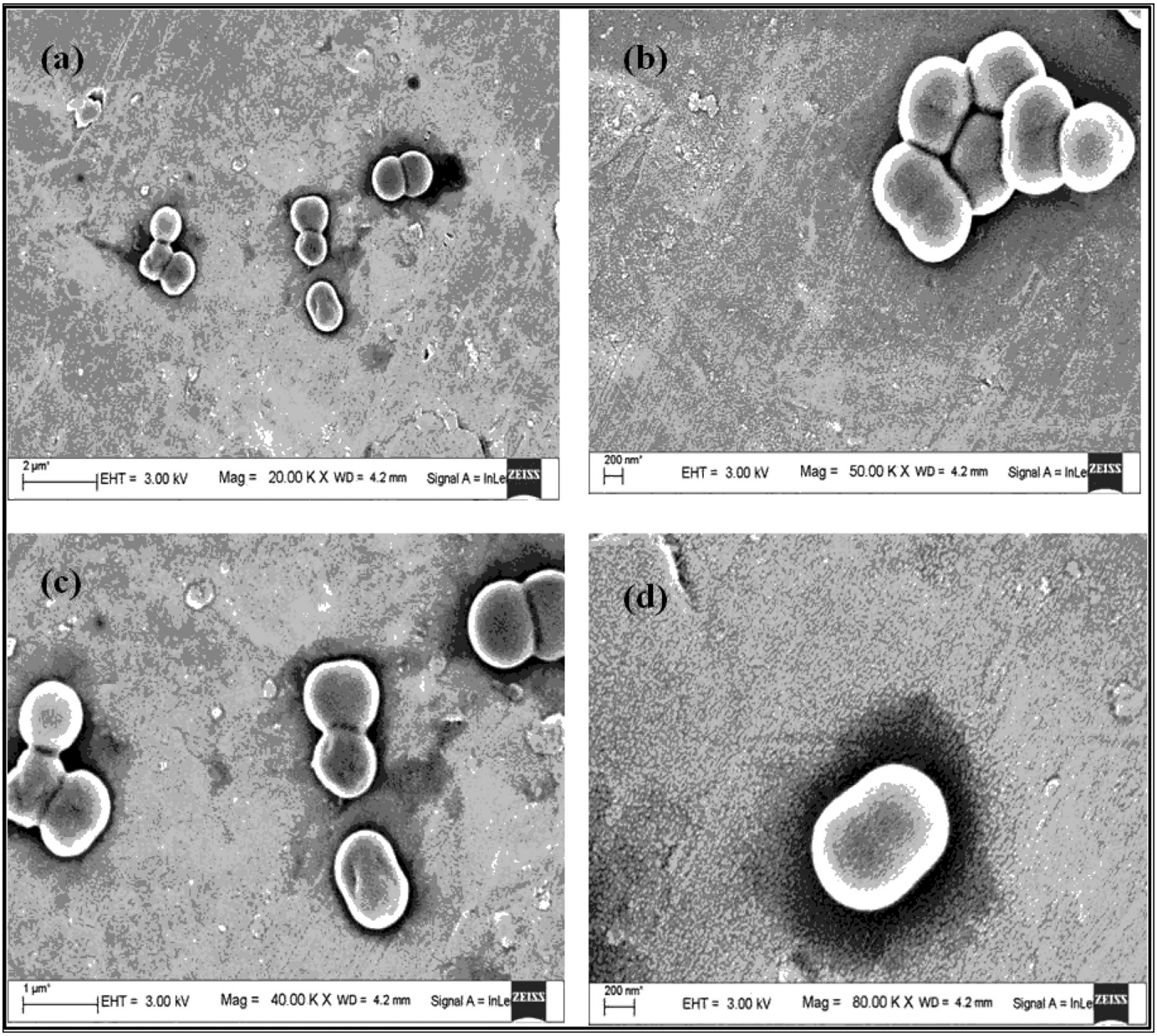

Fig. 1. Scanning Electron Microscope images of *Stenotrophomonas maltophilia* strain P13.

and an altitude of 1393 m (Sherpa et al., 2018, 2019). The coordinates of Kanchengayao glacier accumulation zone sampling sites were measured by GPSMAP 78S (Garmin, India), and analyzed through the Google Earth software.

2.2. Glacier ice core sampling and isolation of bacteria

The glacier ice core sample was taken by drilling the surface of the glacier ice core from the accumulation zone of Kanchengayao glacier, North Sikkim, and the sample was immediately transported to the laboratory. Then the samples were mechanically processed by slicing 10 mm from the surface layer of the ice core with sterilized saw-tooth knife. The samples were cut into many sections of smaller size (approx. 5inches) for better handling purposes and also were carefully preserved at −20 °C. The inner ice core section was aseptically rinsed with chilled ethanol (95%) and was cut around 5 mm annulus with the sterile saw-tooth knife and it was discarded. Then the internal core was again rinsed carefully with chilled ethanol (95%), followed by cold autoclaved water. It was melt by maintaining the temperature of cold incubator at 4 °C in sterile glass beaker. All the handling was conducted under sterile conditions, maintaining temperature <20 °C using positive pressure laminar flow hood; sterile gloves (Xiang et al., 2004; Zhang, 2003). 200 µL meltwater obtained from thawing the glacial ice core was spread plated in Antarctic Bacterial Media (ABM), and incubated at 15 °C for 3 weeks. Another, 200 µL meltwater was enriched in ABM broth and grown at 15 °C for 2 weeks in shaking conditions (120 rpm). Post-enrichment period, the broth was spread plated on ABM agar and cultivated for 3 weeks at 15 °C until the colonies became visible. Among, the many colonies obtained, strain P13 was isolated from one of them and pure culture of it was obtained through quadrant streaking and the monoculture was cryopreserved at −80 °C in 40% glycerol (Sherpa et al., 2019).

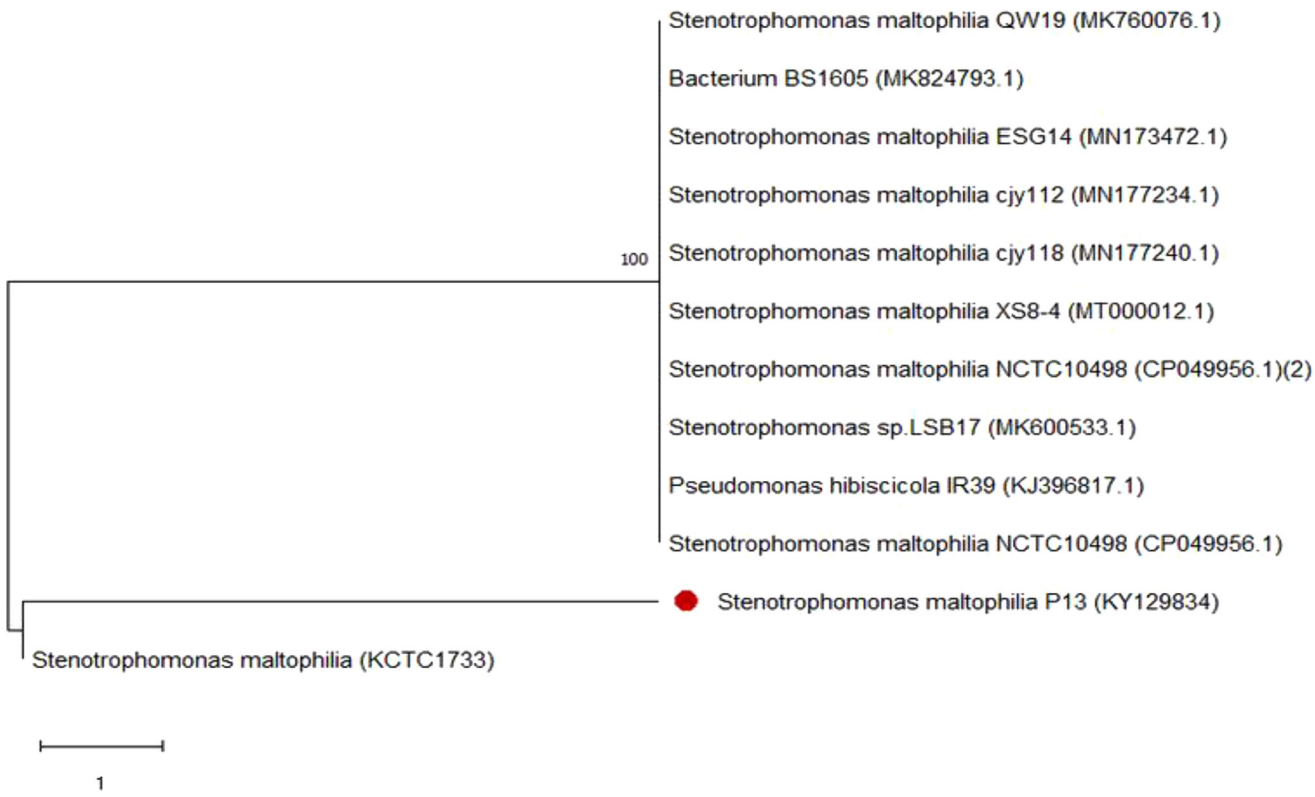

Fig. 2. Phylogenetic tree showing strain P13 among *Stenotrophomonas* species and related taxa. The evolutionary history was inferred using the maximum likelihood method (Tamura et al., 2007). The evolutionary distances were computed using Tamura three-parameter method and are in units of the number of base substitutions per site. This analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1335 positions in the final dataset. Evolutionary analyses were conducted in MEGA 10 software (Tamura et al., 2007).

2.3. Morphological classification

A morphological characteristic such as colony color, form, margins elevation, density, motility, and staining were done (Reddy et al., 2010).

2.4. Scanning electron microscope (SEM)

SEM was done to measure the size and shape of the novel isolate as per Golding et al. (2016) using Scanning Electron Microscope- JCM-5700 (JEOL USA, Peabody, MA, USA).

2.5. Growth profile at various physical parameters

The growth at diverse physical parameters were checked such as temperature from (5 °C-40 °C), pH (4–10), and NaCl (1–10%) was verified as per the given procedures (Reddy et al., 2010).

2.6. Biochemical characterization

Various biochemical parameters were tested such as carbohydrate fermentation, enzymatic tests such as amylase, catalase, lipase, oxidase, protease, nitrate reductase, methyl red test were performed as per the given procedures (Reddy et al., 2010). All of the biochemical tests were also confirmed by BIOLOG system as per the (Liu et al., 2020) methods.

2.7. Fatty acid methyl ester (FAME) analysis

FAME analysis of the *Stenotrophomonas maltophilia* P13 was performed at 20 °C. The fatty acids were extracted and analysed by following the manufacturer's protocol by MIDI (Microbial Identification System) (Kunitsky et al., 2006). The RTSBA6 system was used and the results were investigated by Sherlock Version 6.2.

2.8. Molecular identification and phylogeny

The bacterial genomic DNA was extracted with the help of HiPurA^{TM} kit (Himedia, India). The bacterial 16S rRNA genes were amplified by PCR using two universal primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′), and 1492R (5′-CGGTTAC CTTGTTACGACTT-3′) in 50μL PCR tube using 4μL each dNTP, 2μL $MgCl_2$, 2μL template DNA, 1μL each primer (forward and reverse), 1μL Taq DNA polymerase, and 33μL nuclease-free water (Himedia, India). Reactions were performed in the MasterCycler gradient (Eppendorf, India) with the following reaction conditions; 94 °C (5 min) for early denaturation steps followed by 30 cycles of 94 °C (30 s), 55 °C (1 min), 72 °C (1 min), and extension at 72 °C (10 min). The purification of the PCR products were done by using HiPurA^{TM} PCR clean up system kit (Himedia, India) and the sequencing was done through Applied Biosystems ABI (3500 Genetic Analyzer, Japan) using each universal primer (27F and 1492R) (Sherpa et al., 2018). The sequences were assembled and aligned with the aid of the Codon-Code Aligner software. The sequences were identified using NCBI BLASTn and the phylogenetic tree was created by using the neighbor-joining method using MEGA v.10 (Erickson, 2010; Saitou and Nei, 1987). Gene bank accession was obtained post Bankit submission.

2.9. Genomic DNA preparation and sequencing

The genomic DNA was extracted using Qiagen QIAamp DNA Mini Kit (Qiagen, India) from the isolate. The quality of DNA was checked on 1% agarose gel electrophoresed and quality of DNA was checked using Nanodrop 2000. Purified DNA (approx. 300 ng) was sent to AgriGenome (Kochi, India) for whole genome sequencing (WGS).

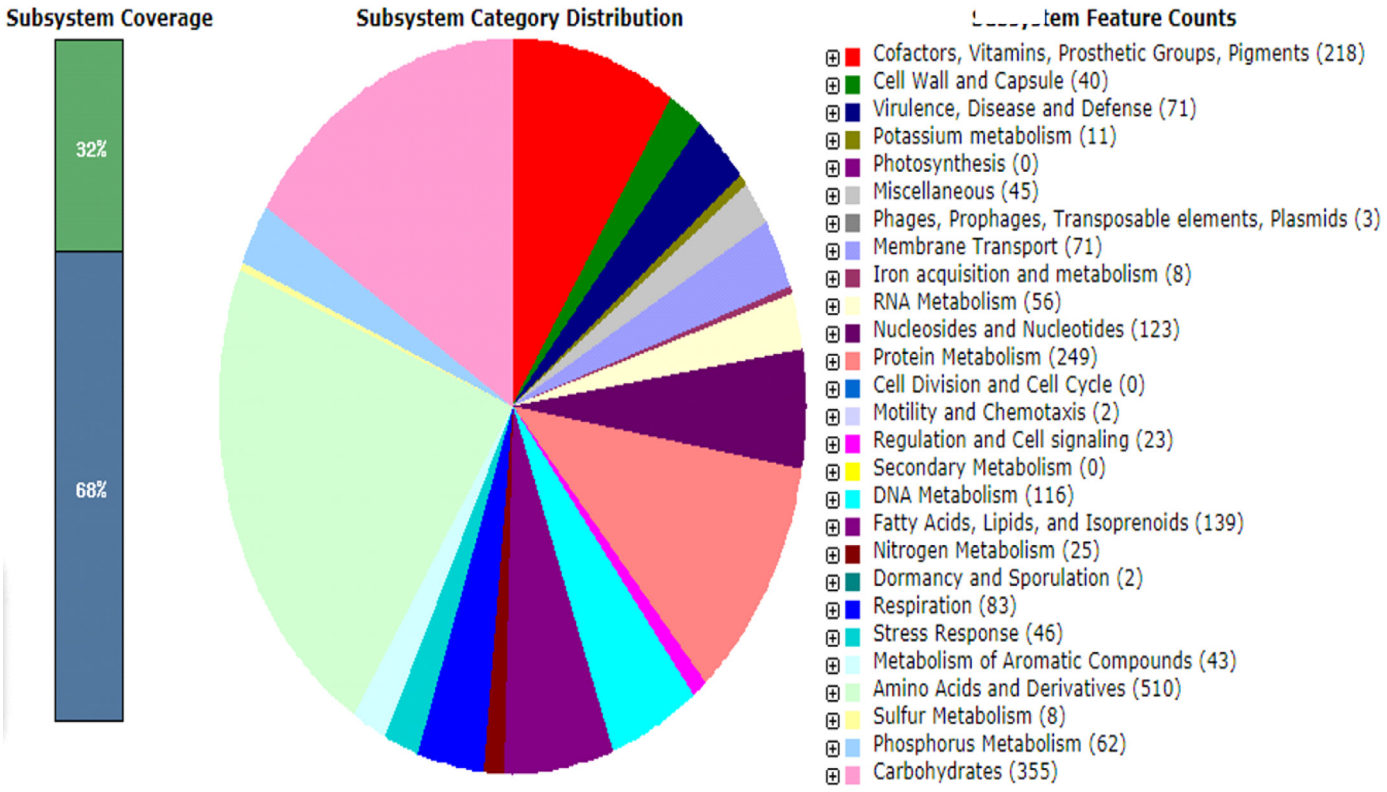

Fig. 3. Subsystem category distribution of major protein coding genes of *Stenotrophomonas maltophilia* strain P13 as annotated by the RAST annotation server. The bar chart shows the subsystem coverage in percentage (blue bar corresponds to percentage of proteins included). The pie-chart shows the percentage distribution of the 27 most rich subsystem categories. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1
Comparative analysis of different phenotypic characteristics of P13 and other related strains: 1= *Stenotrophomonas maltophilia* strain P13; 2 = *P. hibiscicola*; 3= *Pseudomonas geniculata* LMG 2195^T (data in the column 2 and 3) were taken from Heylen et al. (2007); 4 = *S. acidaminiphila*, data from Assih et al. (2002); 5 = *S. rhizophila* data from Wolf et al. (2002). Symbols used: "+" positive reaction; "-" negative reaction; "v" variable reaction; "w" weakly positive; ND: not detected.

Characteristics	1	2	3	4	5
Oxidase	+	–	–	+	+
Growth at					
4 °C	+	–	–	–	+
40 °C	+	+	+	+	–
Growth in the presence of 5% (w/v) NaCl	+	–	+	–	–
Hydrolysis of:					
Aesculin	–	+	+	+	+
Gelatin	+	+	+	–	+
Tween 80	+	ND	ND	+	+
Assimilation of:					
D-Arabinose	–	–	–	–	–
D-Mannose	–	w	w	+	–
D-Xylose	–	–	–	–	–
Cellobiose	w	–	w	–	+
Gentiobiose	w	–	–	–	+
D-Glucose	+	w	w	+	+
Lactose	w	+	–	–	+
D-Mannose	–	w	w	+	+
Maltose	w	+	w	+	+
Melibiose	w	–	–	–	+
Sucrose	+	–	–	–	+
Turanose	w	–	–	–	+
Salicin	+	w	w	–	+

Table 2
Cellular fatty acid profiles of *Stenotrophomonas maltophilia* strain P13 and its closest phylogenetic neighbours strains: 1= *Stenotrophomonas maltophilia* strain P13; 2= *Pseudomonas hibiscicola* LMG 980^T (data were taken from Heylen et al. (2007)); 3 = *S. daejeonensis* MJ03T; 4 = *S. acidaminiphila* KACC 11356^T; 5 = *S. humi* DSM 18929^T; 6 = *S. nitritireducens* KACC 10891^T; 7 = *S. maltophilia* KCTC 1773^T.

Fatty acid	1	2	3	4	5	6	7
Saturated							
C10:0	0.50	–	1.6	–	1.1	–	2.7
C14:0	0.63	3.7	1.6	–	1.1	2.3	1.6
C15:0	–	–	2.5	3.1	4.0	5.1	6.1
C16:0	7.72	–	1.9	1.9	1.8	–	–
isoC10:0	–	–	10.1	11.5	7.0	12.0	7.2
isoC11:0	0.12	–	9.6	5.0	16.3	6.6	0.6
isoC14:0	0.29	0.7	33.7	26.8	14.6	27.7	39.9
isoC15:0	2.40		2.7	3.4	16.4	4.0	2.7
isoC16:0	8.43	44.4	4.2	8.6	5.4	8.4	10.1
anteisoC15:0	30.26	14.0					
Unsaturated			4.6	4.1	1.8	5.1	2.1
Iso-C 15:1 F	0.06	–	7.3	7.9	6.3	9.6	4.9
Iso-C 17:1 w 9c	–	–	–	–	–	–	
Hydroxy	–	–	–	2.5	2.5	2.9	2.7
C12:0 3-OH	2.32	–	–	1.9	–	–	–
C13:0 2-OH	–	–	6.4	7.6	2.2	5.9	4.3
isoC11:0 3-OH	0.13	–	4.1	5.1	8.5	5.1	3.1
isoC12:0 3-OH	–	–	6.1	5.6	1.7	5.3	6.2
isoC13:0 3-OH	–	–	1.5	3.0	7.3	–	3.0

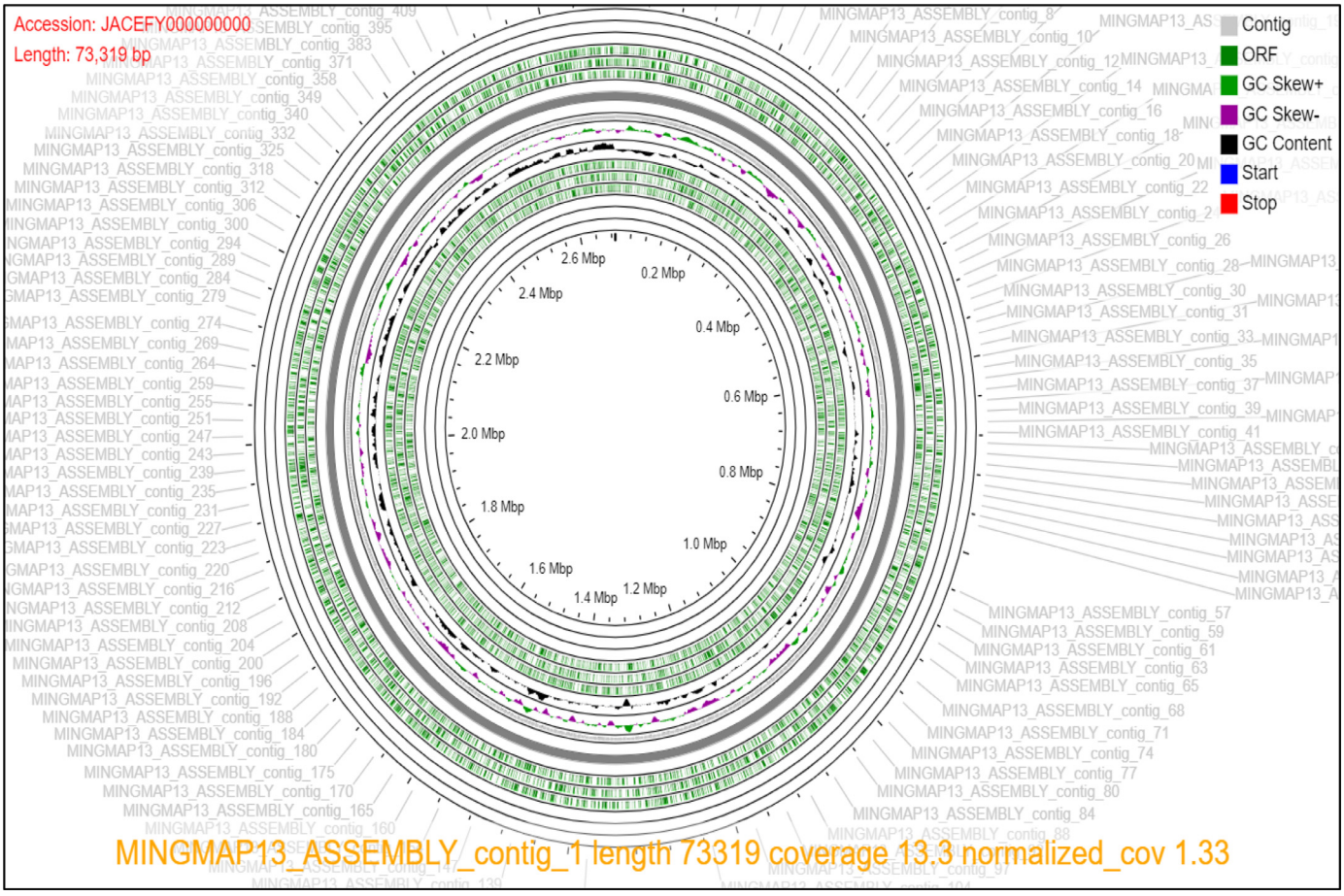

Fig. 4. Circular representation of complete genome *Stenotrophomonas maltophilia* strain P13 labeling from the outside to inside circle: ORF (green), stop codon (red), and start codon (blue). $(G+C)$ content (peaks out/inside the circle indicate values higher or lower than average $(G+C)$ content, respectively, colored black),GC skew (calculated as (G-C)/$(G+C)$, green/purple peaks out/inside the circle indicates values higher or lower than 1, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

2.9.1. Genome assembly and gene prediction

The WGS was administered on a sequencing platform Illumina HiSeq 1500 with a paired-end read length of 150 bp. Data filtration was done using Next generation Sequencing Quality Control (NGSQC) Tool kit and SQit to obtain high quality data and genome assembly (Najar et al., 2018; Patel and Jain, 2012). The primary genome assembly was done by using Velvet (V1.2.10) (Zerbino and Birney, 2008) and its scaffolding was performed by SSPACE (V 3.0) (Boetzer et al., 2011). De-novo genome sequencing validation and quality control was done by Bowtie (V 2.2.2) (Laslett and Canback, 2004) and RNAmmer 1.2 server (Lagesen et al., 2007). Quality control of assembled genome based on non-core genomic elements was also done. Non-core genomic elements such as plasmids were screened by plasmid Finder (V 1.3) (Carattoli et al., 2014). The genome assembly of the raw sequences was performed with the PATRIC assembler (Wattam et al., 2017). The Rapid annotation using Subsystems Technology (RAST) web server (htpps://rast.nmpdr.org) with the default parameters was used to catalog all the predicted genes into subsystems according to the functional categories (Aziz et al., 2008). CGView was used to produce the map of circular genomes with gene features information (Stothard and Wishart, 2005).

2.9.2. Nucleotide sequence accession numbers

The draft genome of *Stenotrophomonas maltophilia* P13 strain was submitted to the NCBI GeneBank and the accession number JACEFY000000000, and with Bio-sample and Bio-project numbers as SAMN15589928 and PRJNA647540 were obtained for it respectively (Supplementary Table 3).

3. Results and discussion

3.1. Biochemical and morphological features support the assignation of P13 strain to the Stenotrophomonas maltophilia

S. maltophilia strain P13 is a rod-shaped pigmented Gram-negative (1 μm diameter; Fig. 1) bacterium of the order *Xanthomonadales*, belonging to *Gammaproteobacteria* class. The cells are aerobic and motile. It is catalase, oxidase, and protease positive and with respect to carbon source utilization, P13 strain was able to assimilate the sugar like *α*-D-glucose, sucrose, D-salicin and ferment sugar for example dextrose, fructose and sucrose. Other sugars like cellobiose, gentiobiose, lactose, maltose, melibiose, and turanose were weakly positive whereas lipase, nitrate, and amylase utilization were shown negative by strain P13 strain, and non-sporulation (Table 1; Supplementary Table 1). P13 strain can grow between 5 °C-40 °C in media, like Luria Bertani Agar, and Antarctic Bacterial Medium and its optimum growth temperature was 30 °C. After 48 h on Luria Bertani agar (LBA) plates, P13 strain stain colonies are white elevated and circular form. Besides, this strain can grow in a wide range of pH i.e. from 2.0 to 10.0 and NaCl ranging from 1% to 10% where its optimum growth is at pH 6 and 1% NaCl concentration (Supplementary Fig 1, 2 and 3). It was found that strain P13 was resistant to Vancomycin, Tetracycline and Ampicillin whereas it was sensitive to Streptomycin (Supplementary Table 2). Further, strain P13 was protease positive as it grew on skimmed milk agar and produced holo-zone around colonies (Supplementary Fig 4).

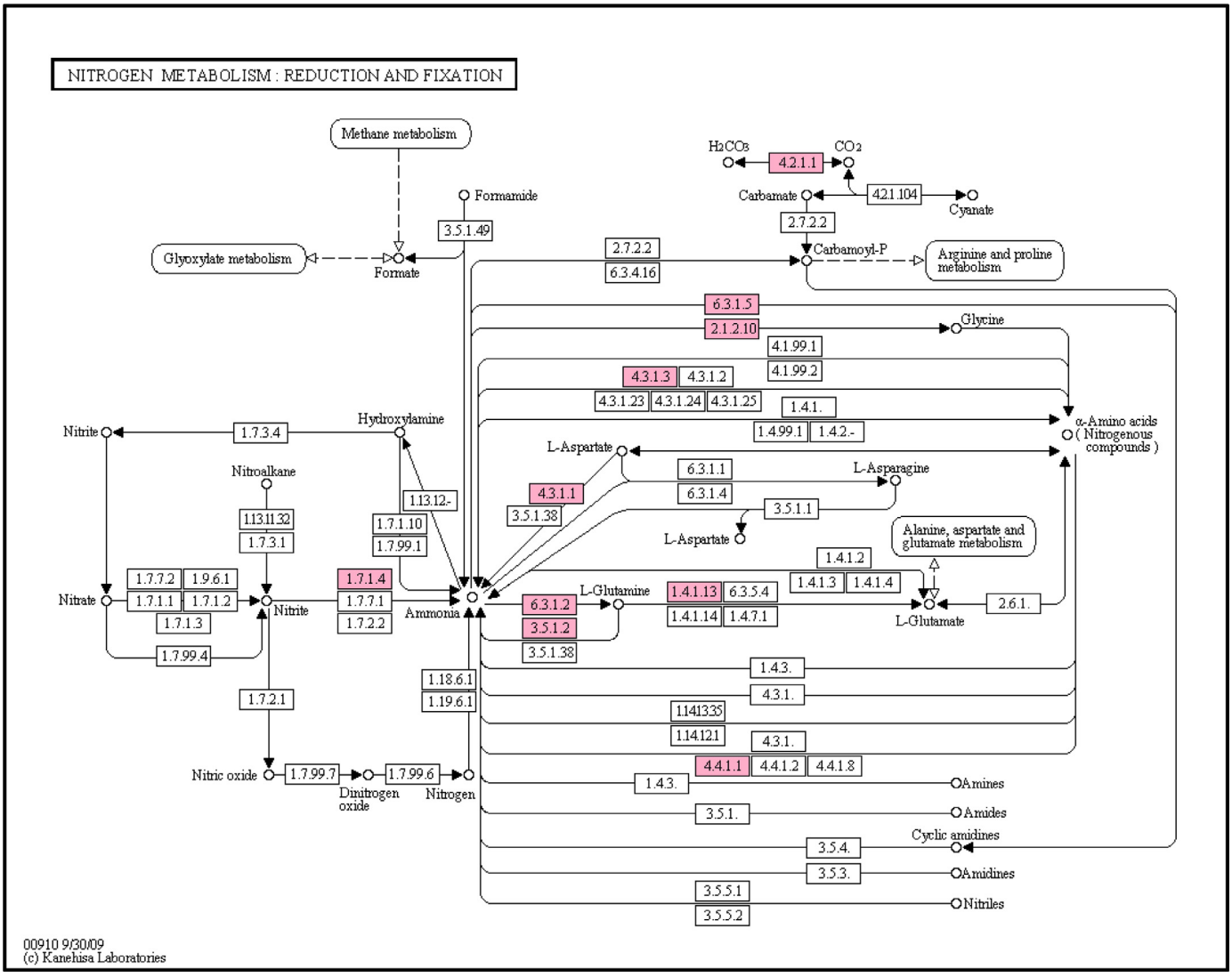

Fig. 5. Pathway for nitrogen metabolism. The presence of enzymes in the genome *Stenotrophomonas maltophilia* strain P13 are pink yellow.

3.2. 16S rRNA gene sequencing and phylogenetic analysis

Based on their morphology, biochemical characteristics and microscopy, the genetic identification was done by 16S rRNA gene sequencing. Based on identity standard of as a minimum 97% for the 16S rRNA gene sequencing result, it suggested that the strain P13 isolate showed close similarity to *Stenotrophomonas maltophilia* strain XS 8–4 with 99.7% ANI (average nucleotide identity). A phylogenetic tree of strain P13 and close relatives was prepared (Fig. 2).

3.3. Fatty acid methyl ester (FAME) analysis

The fatty acid analysis of the strain P13 was performed at 20 °C. Fatty acid was extracted and analysis were carried out following the instructions of the Microbial Identification System (MIDI). The results have shown the predominance fatty acid were C16:0 (7.72%), isoC11:0 (0.72%), isoC15:0 (2.40%), *ante*isoC15:0 (30.26%), and C12:03-OH (2.32%). By analyzing the results using RTSBA6 Sherlock libraries, it was found that the Similarity Index (SI-Index) of strain P13 was SI-Index 0.00. If the similarity index is <0.03 then the species might be novel with corresponding RTSBA6 libraries (Sherlock, MIDI). The comparison of various fatty acids of strain P13 and its closest relatives are shown in Table 2 and Supplementary Fig 5.

On comparing the overall data obtained by various techniques the results have shown that the strain P13 grows well between 4 °C to 40 °C on the other hand its closest neighbor *Stenotrophomonas maltophilia* KCTC 1773^T were not able to grow at this temperature ranges. Similarly, P13 strain assimilates salicin and weakly assimilates other sugar such as cellobiose, gentibiose, lactose, maltose, and turanose whereas its neighbor strain KCTC 1773^T were not able to assimilate above mentioned sugars. Thus, these results indicate that our isolate might be unique as compared to other strains. The major fatty acid present in P13 were C16:0 (7.72), anteisoC15:0 (30.26), and C12:03-OH (2.32), however these fatty acids were absent in the closest strain KCTC 1773^T. Thus on the basis of fatty acids comparison between P13 and its closest neighbor KCTC 1773^T, the strain P13 also showed varied fatty acid profile (Table 2).

3.4. Whole genome sequencing analysis

The genome sequencing yielded a sum of 2689,565 reads, with a total genome size of 73,319 bp and 69.9% ($G+C$) content. Over 90.66% of the score passed >=30 phred score. The average raw read was 150 bp. The RAST revealed N50 values as 6727 bp. The assembly of genomes comprise 548 contigs PEGs (protein encoding genes). The genome contains 2985 coding sequences with 55 rRNA predicted genes and strain P13 was devoid of plasmid (Supplementary Table 3).

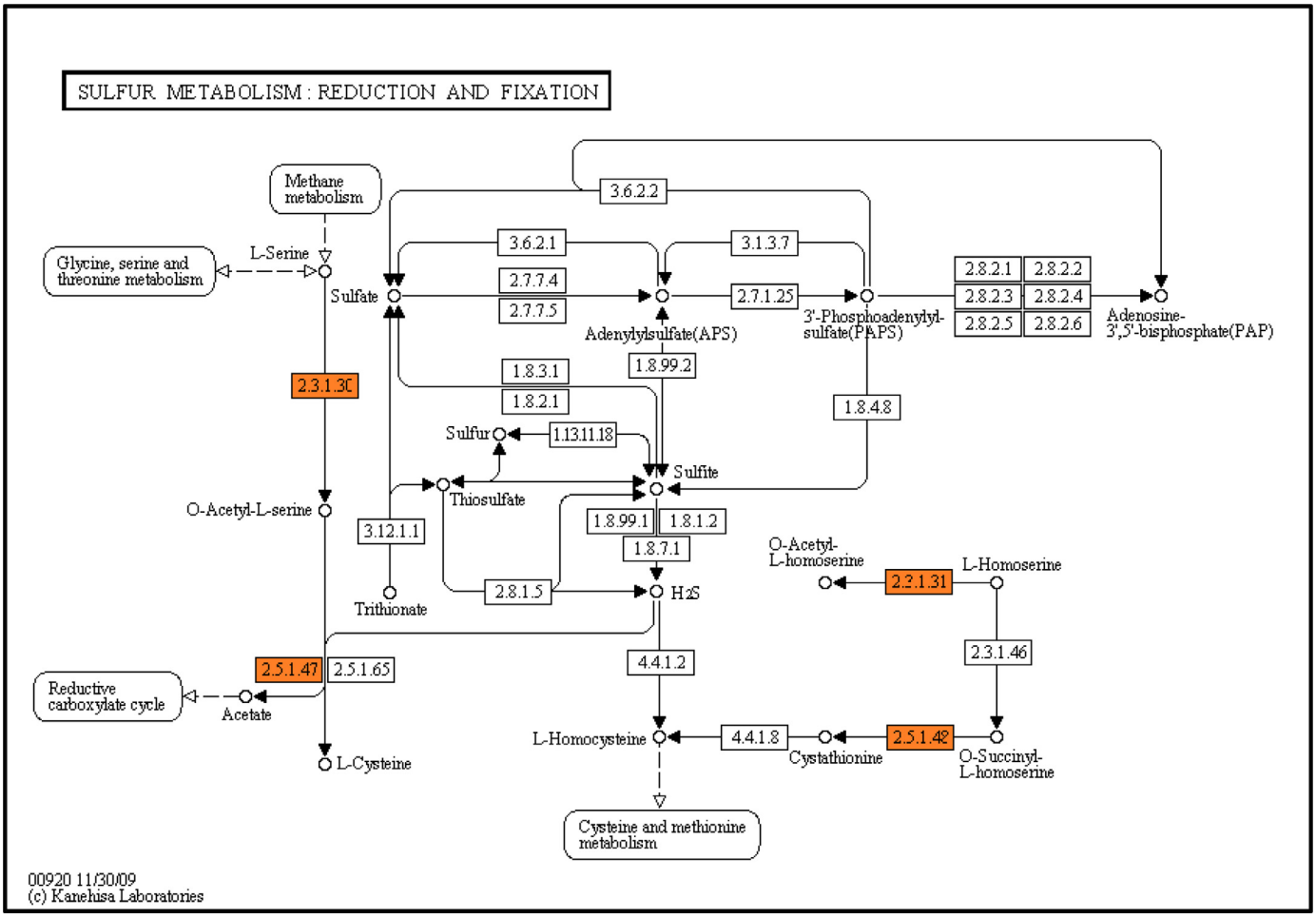

Fig. 6. Pathway for sulfur metabolism. The presence of enzymes in the genome *Stenotrophomonas maltophilia* strain P13 are orange yellow.

The circular representation of *Stenotrophomonas maltophilia*strain P13 complete assembled genome is given in Fig. 4. The coding protein identified were classified into 27 functional categories, based on the classification of COG.

3.5. *Functional annotation of* Stenotrophomonas maltophilia *strain P13*

To investigate the function of the 2985 coding sequences, the KEGG database and RAST web server were used. The 1489 genes annotated by KEGG database were classified into biological processes, cellular components, and molecular function. The top five categories were amino acids and derivatives (278 genes), carbohydrates (227 genes), protein metabolism (156 genes), cofactor, vitamins, prosthetic groups, pigments (112 genes), and nucleosides and nucleotides (93 genes) (Fig. 3).

Alignment of the genes with the KEEG pathways showed the presence of various nitrogen and sulfur metabolic enzymes in the bacterium strain. In the metabolism of nitrogen, the ammonia assimilation in bacteria is mediated by two phenomena: 1) the reductive amination of 2-oxoglutarate yields glutamate, catalysed by glutamate dehydrogenase (GDH; EC 1.4.1.2), and 2) glutamine formation from glutamate and ammonium, catalysed by glutamine synthetase (GS; EC 6.3.1.2), following by the transfer of the amid group to 2-oxoglutarate, catalysed by glutamate synthase (GOGAT; EC 1.4.1.13), which results in the net synthesis of one glutamate molecules (Brown et al., 1974; Dalton, 1979; Brana et al., 1986). Some bacteria seems to have only one mechanism either first or second one. In others, like *Escherichia coli*, where the two system are present, the high K_m of glutamate dehydrogenase for ammonium makes the first pathways useful only in the presence of relatively high ammonium concentrations. At lower concentrations, assimilation occur via the ATP-driven Glutamine synthetase-glutamate synthase (GS-GOGAT) pathways, taking advantage of the lower K_m of Glutamine synthetase for ammonia (Tyler, 1978). Since ammonia can be directly assimilated in to amino acids, few pathways such as glutamate, alanine/aspartate and other cellular components are known for its assimilation. The enzymes for instance glutamate dehydrogenase (GDH), glutamine synthetase (GS), and glutamate synthase are the major catalyzing agents for these reaction (Prabha et al., 2019). Reads associated with the enzyme Glutamine synthetase type 1 (EC 6.3.1.2), Glutamate synthase [NADPH] small chain (EC 1.4.1.13), Glutamate-ammonia-ligase adenylyltransferase (EC 2.7.7.42), Glutamate synthase [NADPH] large chain (EC 1.4.1.13), Ferredoxin-dependent glutamate synthase (EC 1.4.7.1), Glutamate-ammonia-ligase adenylyltransferase (EC 2.7.7.42), Nitrogen regulation protein NR(II) (EC 2.7.3) and ammonium transporter indicate prominent assimilation of ammonia by *Stenotrophomonas maltophilia* P13 in glacier ecosystem (Supplementary Table 4; Fig. 5).

Abundant sulfur metabolism-related genes, e.g., Thioredoxin-disulfide reductase (TR, TrxR; EC 1.8.1.9), Galactosylceramide and sulfatide metabolism, were detected in this metagenomic using the seed database (Fig. 6). The role of enzymes related to sulfur metabolism have been described from microbial communities inhibiting various habitats (Prabha et al., 2019). Their existence in metagenomic data reveals a balanced sulfur metabolic capability by *Stenotrophomonas maltophilia* P13 in glacier ecosystem.

Nitrogen fixation is an important aspect of plant growth promoter (Li et al., 2017). Presence of Nitrogen regulation protein NR(II) (EC 2.7.3) and ammonium transporter suggests that this strain has the

ability to fix nitrogen and assimilate atmospheric ammonia. Some conserved metabolism genes related to aromatic compounds such as tryptophan, tyrosine, and phenylalanine were found which indicates the PGP activity of the strain (Nogales et al., 2017). 3-oxoadipate pathway is one of the signature molecular marker for bacteria or microbes promoting plant growth (Harwood and Parales, 1996). Genes such as *cat*A, *cat*C, *CoT*a and *CoT*b responsible for catechol metabolism were found in strain P13. Many *Pseudomonas* sp. are known to be useful as PGP (plant growth promoters) as they contains the assimilatory genes required for NPK (Nitrogen-Phosphorous-Potassium) elements (Bargaz et al., 2018; Selvakumar et al., 2015). In strain P13, genes were also found pertaining to the cause. Genes for nitrogen and ammonium assimilation (GS, *Gln*E, NADPH GOGAT, *am*T and *Nsr*R), genes for phosphorous metabolism (*Pho*H, *Pho*P, *Pho*U, PPK, EPP, *Pho*R, *Pho*B, *pts*A, *pts*B and *pts*C), and genes for potassium synthesis (*Kdp*A, *Kdp*B, *Kdp*C, *Kdp*D, *Kup* and MSC) were found respectively in the reported strain P13.

In summary, *Stenotrophomonas maltophilia* strain P13 provides a basis for comprehending the properties which makes it with potential application as a potential component of agriculture as plant growth promoter.

Declaration of Competing Interest

None to be declared.

CRediT authorship contribution statement

Mingma Thundu Sherpa: Conceptualization, Investigation, Methodology, Writing - original draft, Data curation, Formal analysis. **Sayak Das:** Investigation. **Ishfaq Nabi Najar:** Investigation. **Nagendra Thakur:** Writing - review & editing, Visualization, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2020.100012.

References

Adegoke, A.A., Stenstrom, T.A., Okoh, A.I., 2017. Stenotrophomonas maltophilia as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. Front. Microbiol. 8, 2276. https://doi.org/10.3389/fmicb.2017.02276.

Assih, E.A., Ouattara, A.S., Thierry, S., et al., 2002. Stenotrophomonas acidaminiphila sp. nov., a strictly aerobic bacterium isolated from an up flow anaerobic sludge blanket (UASB) reactor. Int. J. Syst. Evol. Microbiol. 52, 559–568. https://doi.org/10.1099/ijs.o.o1869-0.

Aziz, R.K., Bartels, D., Best, A.A., et al., 2008. . The RAST server: rapid annotation using subsystems technology. BMC Genom. 9, 75. https://doi.org/10.1186/1471-2164-9-75.

Bargaz, A., Lyamlouli, K., Chtouki, M., et al., 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front Microbiol. 9, 1606. https://doi.org/10.3389/fmicb.2018.01606.

Boetzer, M., Henkel, C.V., Jensen, H.J., et al., 2011. Scaffolding preassembled contigs using SSPACE. Bioinformatics 27, 578–579. https://doi.org/10.1093/bioinformatis/btq683.

Brana, A.L., Paiva, N., Demain, A.L., 1986. Pathways and regulation of ammonium assimilation in streptomyces clavuligerus. J. Gen. Microbiol. 132, 1305–1317. https://doi.org/10.1099/00221287-132-5-1305.

Brown, C.M., Macdonald-Brown, D.S., Meers, J.L., 1974. Physiological aspects of microbial nitrogen metabolism. Adv. Microb. Physiol. 11, 1–52. https://doi.org/10.1016/S0065-2911(08)60268-4.

Carattoli, A., Zankari, E., Garcia-Fernandez, A., et al., 2014. In-silico detection and typing of plasmids using plasmid finder and plasmid multi-locus sequence typing. Antimicrob. Agents Chemother. 58, 3895–3903. https://doi.org/10.1128/ACC.02412-14.

Crossman, L.C., Gould, V.C., Dow, J.M., et al., 2008. . The complete genome, comparative and functional analysis of Stenotrophomonas maltophilia reveals an organism heavily shielded by drug resistance determinants. Genome Biol 9 (40), R74. https://doi.org/10.1186/gb-2008-9-4-r74.

Dalton, H., 1979. Utilization of inorganic nitrogen by microbial cells. In: Quayle, JR (Ed.), International Review of Biochemistry, 21. University Park Press, Baltimore, pp. 258–292.

Dunne, C., Crowley, J.J., Moenne-Loccoz, Y., et al., 1997. . Biological control of pythium ultimum by Stenotrophomonas maltophilia W81 is mediated by an extracellular proteolytic activity. Microbiology 143, 3921–3931. https://doi.org/10.1099/00221287-143-12-3921.

Erickson, K., 2010. The jukes-cantor model of molecular evolution. Ecol. Primus. 20, 438–445. https://doi.org/10.1080/10511970903487705.

Finkmann, W., Altendorf, K., Stackebrandt, E., Lipski, A., 2000. Characterisation of N2O-producing Xanthomonas - like isolates from bio-filters as Stenotrophomonas nitritireducens sp. nov., Luteimonas mephitis gen. nov., sp. nov. and Pseudoxanthomonas broegbernensis gen. nov., sp. nov. Int. J. Syst. Evol. Microbiol. 50, 273–282. https://doi.org/10.1099/00207713-50-1-273.

Golding, C.G., Lamboo, L.L., Beniac, D.R., et al., 2016. The scanning electron microscope in microbiology and diagnosis of infectious disease. Sci. Rep. 6, 26516. https://doi.org/10.1038/srep26516.

Harwood, C.S., Parales, R.E., 1996. The beta-ketoadipate pathway and the biology of self-identity. Annu Rev Microbiol. 50, 553–590. https://doi.org/10.1146/annurev.micro.50.1.553.

Heylen, K., Vanparys, B., Peirsegaele, F., et al., 2007. Stenotrophomonas terrae sp. nov. and Stenotrophomonas humi sp. nov., two nitrate-reducing bacteria isolated from soil. Int. J. Syst. Evol. Microbiol. 57, 2056–2061. https://doi.org/10.1099/ijs.0.65044-0.

Jakobi, M., Winkelmann, G., Kaiser, D., et al., 1996. Maltophilin: a new antifungal compound produced by Stenotrophomonas maltophilia R3089. J. Antibiot. (Tokyo). 49 (11), 1101–1104. https://doi.org/10.7164/antibiotics.49.1101.

Kunitsky, C., Osterhout, G., Sasser, M., 2006. Identification of microorganisms using fatty acid methyl ester (FAME) analysis and the MIDI Sherlock microbial identification system identification system. Encyclo. Rapid Microbiol. Meth. 3, 1–18.

Lagesen, K., Hallin, P., Rodland, E.A., et al., 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35, 3100–3108. https://doi.org/10.1093/nar/g-km160.

Laslett, D., Canback, B., 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic. Acids. Res. 32 (1), 11–16. https://doi.org/10.1093/nar/g-kh152.

Li, H.B., Singh, R.K., Singh, P., et al., 2017. Genetic diversity of nitrogen-fixing and plant growth promoting Pseudomonas species isolated from sugarcane rhizosphere. Front Microbiol. 8, 1268. https://doi.org/10.3389/fmicb.2017.01268.

Liu, Q., Tian, J.H., Liu, H.C., et al., 2020. Cryobacterium ruanii sp. nov. and Cryobacterium breve sp. nov., isolated from glaciers. Int. J. Syst. Evol. Microbiol. 70 (3), 1918–1923. https://doi.org/10.1099/ijsem.0.003994.

Nogales, J., García, J.L., Díaz, E., 2017. Degradation of aromatic compounds in Pseudomonas: a systems biology view.. In: Rojo, F. (Ed.), Aerobic Utilization of hydrocarbons, Oils and lipids. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-39782-5_32-1.

Najar, I.N., Sherpa, M.T., et al., 2018. Geobacillus yumthangensis sp. nov., a thermophilic bacterium isolated from a north-east Indian hot spring. Int. J. Syst. Evol. Microbiol. 68, 3430–3434. https://doi.org/10.1099/ijsem.0.003002.

Palleroni, N.J., Bradbury, J.F., 1993. Stenotrophomonas, a new bacterial genus for Xanthomonas maltophilia (Hugh 1980) Int. J. Syst. Bacteriol. 43, 606–609. https://doi.org/10.1099/002-07713-433606.

Patel, R.K., Jain, M., 2012. NGS QC Toolkit. A toolkit for quality control for next generation sequencing data. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0030619.

Prabha, R., Singh, D.H., Gupta, S., et al., 2019. Rhizosphere metagenomics of Paspalum scrobiculatum L. (Kodo millet) reveals rhizobiome multi-functionalities. Microorganisms 7, 608. https://doi.org/10.3390/microorganisms712-06.

Reddy, G.S.N., Pradhan, S., Manorama, R., et al., 2010. Cryobacterium pindariense sp. nov., a psychrophilic bacterium from a Himalayan glacier. Int. J. Syst. Evol. Microbiol. 60, 866–870. https://doi.org/10.1099/ijs.0.011775-0.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425. https://doi.org/10.1093/oxfordjournals.m-olev.a040454.

Selvakumar, G., Panneerselvam, P., Bindu, G.H., Ganeshamurthy, A.N., 2015. Pseudomonads: plant growth promotion and beyond. In: Arora, N. (Ed.), Plant Microbes symbiosis: Applied facets. Springer, New Delhi https://doi.org/10.1007/978-81-322-2068-8_10.

Sherpa, M.T., Najar, I.N., Das, S., et al., 2018. Bacterial diversity in an alpine debris-free and debris-cover accumulation zone glacier ice, North Sikkim. Indian J. Microbiol. 58, 470–478. https://doi.org/10.1007/12088-018-0747-8.

Sherpa, M.T., Najar, I.N., Das, S., et al., 2018. The diversity of Pseudomonas species from the accumulation zone of Kanchengayao Glacier, North Sikkim, India. J. Pure Appl. Microbiol. 13, 339–348. https://doi.org/10.22207/JPAM.13.1.37.

Stothard, P., Wishart, D.S., 2005. Circular genome visualization and exploration using CGView,". Bioinformatics 21 (4), 537–539.

Tamura, K., Dudley, J., Nei, M., et al., 2007. Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1580–1596. https://doi.org/10.1093/molbe-v/msm092.

Tyler, B., 1978. Regulation of the assimilation of nitrogen compounds. Ann. Rev. Biochem. 47, 1127–1162.

Wattam, A.R., Davis, J.J., Assaf, R., et al., 2017. . Improvement to PATRIC, the all-bacterial bioinformatics database and analysis resources center. Nucleic Acids Res. 45, 535–542. https://doi.org/10.1093/nar/gkw1017.

Wolf, A., Fritze, A., Hagemann, M., Berg, G., 2002. Stenotrophomonas rhizophila sp. nov., a novel plant-associated bacterium with antifungal properties. Int. J. Syst. Evol. Microbiol 52, 1937–1944. https://doi.org/10.1099/00207713-52-6-1937.

Xiang, S.R., Yao, T.D., An, L.Z., et al., 2004. . Bacterial diversity in Malan ice core from the Tibetan Plateau. Folia Microbiol. 49, 269–276. https://www.biomed.cas.cz/mbu/folia.

Zerbino, D.R., Birney, E., 2008. Velvet algorithms for de novo short read assembly using de Brujin graphs. Genome Res. 18, 821–829. https://doi.org/10.1101/gr.074492.107.

Zhang, Z., Yuen, G.Y., 1999. Biological control of Bipolaris sorakiniana on tall fescue by Stenotrophomonas maltophilia strain C3. Phytopathol 89, 817–822. https://doi.org/10.1094/PHYTO.1999.89.9.817.

Zhang, Z., Yuen, G.Y., Sarath, G., et al., 2001. . Chitinases from the plant disease biocontrol agent, Stenotrophomonas maltophilia C3. Phytopathol 91, 204–211. https://doi.org/10.1094/PHYTO.2001.91.2.204.

Zhang, X., 2003. Diversity of 16S rDNA and environmental factor influencing microorganisms in Malan ice core. Chin. Sci. Bull. 48, 1146–1152. https://doi.org/10.1360/02wd0409.

Zhu, B., Liu, H., Tian, W.X., et al., 2011. . Genome sequence of Stenotrophomonas maltophilia RR-10, isolated as an endophyte from rice root. J. Bacteriol. 194, 1280–1281. https://doi.org/10.1128/JB.06702-11.