Genome sequences of *Arthrobacter* spp. that use a modified sulfoglycolytic Embden-Meyerhof-Parnas pathway

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Abstract

Background

Sulfoglycolysis pathways enable the breakdown of the sulfosugar sulfoquinovose and environmental recycling of its carbon and sulfur content. Several pathways exist for the breakdown of sulfoquinovose that usually lead to production of C3-sulfonates (sulfolactate and 2,3-dihydroxypropanesulfonate) that are excreted and sustain secondary bacterial communities. The prototypical sulfoglycolytic pathway is a variant of the classical Embden-Meyerhof-Parnas pathway that has been described in gram-negative *Escherichia coli* and results in production of 2,3-dihydroxypropanesulfonate.

Results

We used enrichment cultures to discover new sulfoglycolytic bacteria from Australian soil samples. Two gram-positive *Arthrobacter* spp. were isolated that produced sulfolactate as the metabolic end-product. Genome sequences identified a modified sulfoglycolytic Embden-Meyerhof-Parnas (sulfo-EMP) gene cluster that retained the core sulfoglycolysis genes encoding metabolic enzymes, but featuring the replacement of the gene encoding sulfolactaldehyde (SLA) reductase with SLA dehydrogenase, and the absence of sulfoquinovosidase and sulfoquinovose mutarotase genes. The gene clusters were broadly conserved across a range of other sequenced Actinobacteria.

Conclusions

We report the first gram-positive soil bacteria that utilize sulfo-EMP pathways to metabolize SQ. Excretion of sulfolactate is consistent with an aerobic saprophytic lifestyle. This work broadens our knowledge of the sulfo-EMP pathway to include soil bacteria.

Background

Sulfoquinovose (SQ; 6-deoxy-6-sulfo-D-glucose) is a sulfosugar produced by photosynthetic organisms [1]. It is primarily found as the headgroup of the sulfoglycolipid sulfoquinovosyl diacylglycerol (SQDG) in photosynthetic tissues and membranes in plants, algae and cyanobacteria [1, 2]. The annual global production of SQ is estimated at 10^{10} tons per annum [3], and thus the degradation of this sulfosugar is an important arm of the global biogeochemical cycle. The degradation of sulfoquinovose occurs through pathways of sulfoglycolysis and provides access to its constituent carbon and generates ATP and reducing equivalents (NADH/NADPH) [4]. The sulfoglycolytic Embden-Meyerhof-Parnas (sulfo-EMP) [5, 6], Entner-Doudoroff (sulfo-ED) [7] and sulfofructose transaldolase (sulfo-SFT) [8, 9] pathways cleave the 6-carbon chain of SQ into two 3-carbon fragments, one of which is utilized in primary metabolism, while the other containing the sulfonate group is converted to either sulfolactate (SL) or 2,3-dihydroxypropanesulfonate (DHPS) and excreted. The sulfoglycolytic sulfoquinovose monooxygenase (sulfo-SMO) pathway results in cleavage of the C-S bond of sulfoquinovose and leads to production of glucose, and thus enables the complete breakdown of the SQ molecule [10]. The genes encoding these sulfoglycolytic pathways are found within clusters that typically contain genes encoding proteins for the import of SQ or its glycosides, the export of the end-products, SL or DHPS, and a specialized glycoside hydrolase termed a sulfoquinovosidase (SQase) [11, 12] that can cleave SQ glycosides to release SQ that can undergo sulfoglycolysis.

We report here the use of sequential enrichment culturing using minimal media containing SQ as sole carbon source to isolate new sulfoglycolytic bacteria from soil. We identify two *Arthrobacter* sp. strains, AK01 and AK04, and demonstrate that they grow on SQ and secrete SL into the growth media. We present the draft genome sequences of AK01 and AK04 that reveals that these *Arthrobacter* sp. contain a gene cluster encoding a sulfoglycolytic Embden-Meyerhof-Parnas pathway that differs from the prototypical pathway of *E. coli* through the lack of an identifiable candidate SQase, the replacement of SLA reductase with SLA dehydrogenase, and the presence of ABC transporters and TauE permeases, variations that are present within other sequenced Actinobacteria.

Results and Discussion

Isolation and characterization of sulfoglycolytic bacteria

Bacteria able to grow on SQ as sole carbon source were selected by using soil samples (collected from the University of Melbourne, Parkville campus) to inoculate a vitamin-enriched minimal media containing SQ as sole carbon source. Sequential subculturing into fresh SQ-minimal media, followed by plating onto LB agar and then regrowth in SQ-minimal media led to isolation of strains AK01 and AK04 that possessed a short rod-like appearance (**Fig. 1**). Strains AK01 and AK04 grew robustly on SQ with peak growth rates of 0.129 and 0.081 A₆₀₀/min and achieved stationary phase after approximately 25 and 40 h, respectively (**Fig. 2a,c**). The absorbance at stationary phase for cultures grown on SQ were approximately half of that for cultures grown on equimolar glucose. ¹³C-NMR analysis of culture medium of AK01 and AK04 grown on ¹³C₆-SQ (7.7 mM) media gave three signals corresponding to ¹³C₃-SL (**Fig. 2b,d, Table 1**). ¹³C NMR analysis of spent culture confirmed that substrate SQ is completely consumed by bacteria and metabolised to SL.

To investigate the genetic basis for SQ consumption by AK01 and AK04, DNA extracted from these bacteria were sequenced using the Illumina NextSeq platform. **Table 2** shows the key features of the two draft genomes. On the basis of 16S rRNA gene sequence analysis the organisms were assigned as *Arthrobacter* sp. The 16S rRNA genes of the two strains were 98.8% identical over 1520 bp, which suggests that these organisms are of the same species [13]. However, average nucleotide identity (ANI) between the two organisms is only 76.5%, conflicting with the 16S rDNA gene results and suggesting that these two organisms differ sufficiently to be considered separate species [14].

Genomic features related to SQ metabolism

Genome analysis of *Arthrobacter* spp. AK01 and AK04 revealed a cluster of genes that were assigned as encoding SQ degradation through the sulfo-EMP pathway (**Fig. 3a,b**). The prototypical sulfo-EMP pathway was identified in *E. coli* and involves a 10-gene cluster (*yihOPQRSTUV, csqR*) [5]. In *E. coli* these genes encode a transcription factor (CsqR) [15], putative transmembrane proteins for the import of SQ and export of the end-product of the pathway, DHPS (YihO, YihP). The enzymatic steps involve a sulfoquinovosidase (YihQ) for cleavage of SQ glycosides [12], sulfoquinovose mutarotase (YihR) for interconversion of SQ anomers [16], SQ-sulfofructose (SF) isomerase (YihS) that interconverts SQ, SF and sulforhamnose,[6] an ATP-dependent sulfofructose kinase (YihV) that converts SF to SF-1-phosphate (SFP) [6], sulfofructose-1-phosphate aldolase (YihT) which converts SFP to SLA and dihydroxyacetone phosphate [6], and a NADH-dependent sulfolactaldehyde reductase (YihU) to convert SLA to DHPS [17], which is excreted into the growth media (**Fig. 3d**).

Both *Arthrobacter* sp. contained genes encoding SQ-SF isomerase (YihS), SF kinase (YihV), and SFP aldolase (YihT). Consistent with the excretion of SL into the growth media, both strains lacked an SLA reductase homologue, but instead contained an SLA dehydrogenase homologue, annotated as GabD. GabD homologues within sulfoglycolytic gene clusters have been identified for bacteria that utilize the sulfo-ED [7, 18] and sulfo-SFT [8, 9] pathways, and in *Pseudomonas putida* SQ1 there is an NAD⁺/NADP⁺-dependent SLA dehydrogenase [7]. A proposed pathway for SQ metabolism in these *Arthrobacter* spp. is shown in **Figure 3c**. AK01 and AK04 represent the first characterized examples of sulfoglycolytic bacteria that use a sulfo-EMP pathway with a GabD SLA dehydrogenase. *E. coli* and related Enterobacteriaceae that contain SLA reductases are facultative anaerobes, and the presence of a reducing pathway for excretion of DHPS may support their anaerobic lifestyle. On the other hand, *Arthrobacter* are normally considered aerobes [19] (although anaerobic *Arthrobacter* have been described [20]), which is consistent with an oxidative pathway for excretion of SL.

SQ import and DHPS/SL export is poorly characterized, but several different strategies have been identified across sulfoglycolytic pathways. Both AK01 and AK04 contain genes annotated as ABC transporter cassettes and solute binding proteins. ABC transporter systems have been identified in *Agrobacterium tumefaciens* C58 (which uses the sulfo-SMO pathway) [Sharma, 2021 #8628] and *R. leguminosarum* SRDI858 (which utilizes a sulfo-ED pathway) [18]. The *A. tumefaciens* solute binding protein binds SQGro with high affinity [10]. We therefore propose that these *Arthrobacter* isolates utilize the solute binding protein and an ABC transporter system to import SQ or its glycosides. Strain AK04 also contains a TauE permease (a member of the 4-toluene sulfonate uptake permease (TSUP) system) [21]. TSUP proteins are poorly characterized permeases that are suggested to be involved with the transport of sulfur-containing organic compounds. TauE of *Cupriavidus necator* H16 is proposed to be involved in the export of sulfolactate [22], a function that is consistent with the excretion of SL by AK04. The absence of an obvious permease candidate in the AK01 gene cluster suggests that another protein may adopt this function in this strain.

Other key differences with *E. coli* includes the lack of identifiable SQ mutarotase and SQase encoding genes. Interestingly, the isolated strains could grow on the simple SQ glycoside, methyl α -sulfoquinovoside. This suggests that they may harbor an unidentified SQase without homology to known SQases.

A search for organisms with gene clusters related to strains AK01 and AK04 led to identification of other *Arthrobacter* strains with syntenic or closely related sulfo-EMP gene

clusters (**Fig. 4**). Other *Arthrobacter* spp. were identified that contained gene clusters with architectures identical to AK01; and both *Arthrobacter* spp. and *Pseudarthrobacter* spp. were identified with gene clusters identical to AK04. Non-identical but closely syntenic sulfo-EMP gene clusters were observed in select Actinobacteria including other *Arthrobacter* spp. and *Microbacterium* sp. No. 7 (both order Micrococcales), and a more distantly related cluster in the actinobacteria *Acrocarpospora corrugata* (order Streptosporangiales) and *Phytohabitans houttuyniae* (order Micromonosporales).

Conclusions

Two sulfoglycolytic soil bacteria belonging to the *Arthrobacter* genus (strains AK01 and AK04) were isolated by enrichment culture involving growth on SQ from soil. The stationary phase optical density of these bacteria when grown on SQ was approximately half that of growth on glucose, consistent with utilization of only three of the six carbons of SQ. Both possessed a variant of the sulfo-EMP pathway that uses an SLA dehydrogenase to produce SL that is secreted into the growth media, and which is proposed to arise from the occurrence of an SLA dehydrogenase (GabD). SL in turn becomes available for other members of the microbial community that specialize in its metabolism [23-27]. The observation that cells achieve stationary phase in SQ at approximately half the optical density as cells grown on glucose is consistent with utilization of only three of the six carbon in SQ. Prior to this work, the only sulfo-EMP pathway bacteria that have been characterized were a range of *E. coli* strains, which produce DHPS through the action of SLA reductase [5]. Notably, strains AK01 and AK04 lack genes assigned as encoding an SQase, but nonetheless could grow on MeSQ, indicating the presence of a non-specific SQase or a novel SQase that is not readily identified by sequence homology. This study highlights the core genes required for sulfoglycolysis (YihS, YihT, YihU, YihV) and constitutes the first examples of sulfo-EMP bacteria isolated from soil.

Methods

Bacterial growth media

Growth media was prepared using M9 minimal salts media (2 mL), trace metal solution (0.1 mL), and vitamin solution (0.01 mL) and contained 5 mM sulfoquinovose (SQ) as sole carbon source, made up to a final volume of 10 mL with water. M9 minimal salts media contains 0.45 M Na₂HPO₄, 0.11 M KH₂PO₄, 0.09 M NH₄Cl, 0.04 M NaCl, 0.1 M MgSO₄, 0.1 M CaCl₂; trace metal solution contains 0.4 mM FeCl₃, 0.08 mM CoCl₂, 0.08 mM CuCl₂, 0.08 mM MnCl₂, 0.55 mM ZnCl₂, 0.01 mM NiCl₂, 0.05 mM Na₂MoO₄ and 0.5 mM H₃BO₃; vitamin mixture contains 0.04 mM biotin, 0.05 mM calcium

pantothenate, 0.15 mM thiamine hydrochloride, 0.36 mM p-aminobenzoic acid, 0.81 mM nicotinic acid, 1.49 mM pyridoxamine dihydrochloride, 0.01 B12 (cyanocobalamin).

Isolation of *Arthrobacter* sp.: *Arthrobacter* sp. AK01 and AK04 was isolated from enriched culture, obtained from soil of the Botany Systems Garden (University of Melbourne).

Two soil samples were collected and approximately 1 g of soil was suspended in 5 mL of sterilized growth media containing 5 mM SQ as a sole carbon source. The culture was incubated at 30 °C for 4 d with agitation at 250 rpm. A subsample (100 μ L) was transferred into fresh vitamin supplemented M9 media and grown for a further 4 d. This step was repeated four times and after outgrowth of the final culture for 4 d, cells were plated onto LB agar plates (10 g/L tryptone, 5g/L NaCl, 5g/L yeast, 15g/L agar) and incubated overnight at 30 °C in dark. Single colonies were picked and inoculated into fresh vitamin supplemented M9 media containing 5 mM SQ and incubated at 28 °C while shaking at 250 rpm. Once the cultures were visibly turbid, cells were again plated onto LB agar, incubated overnight at 30 °C in dark and single colonies picked and inoculated again into fresh vitamin supplemented M9 media containing 5 mM SQ. Once cultures were visibly turbid, frozen stocks were prepared by diluting to 10% glycerol and freezing at -80 °C. Cell morphology was examined using scanning electron microscopy. Genomic DNA was isolated using the GenElute DNA extraction kit (Sigma) with inclusion of lysozyme and RNAase.

Phenotypic assays

Bacteria were grown in vitamin-supplemented M9 minimal media containing $^{13}\text{C}_6$ -SQ (7.7 mM) at 30 °C for 1 day with agitation at 250 rpm. After cultures became visibly turbid, the cells were sedimented by centrifugation (10,000 rpm, 10 min) and the supernatant was diluted with 50 % D₂O and transferred to a 5 mm NMR tube. ^{13}C NMR spectra were acquired using a 500 MHz instrument and are shown in Figure 1(a).

Genome sequence, assembly, and annotation

Genomic DNA was sequenced using an Illumina NextSeq at the Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, Australia. DNA was prepared for sequencing on the Illumina NextSeq platform using the Nextera XT DNA preparation kit (Illumina) with $\times 150$ bp paired end chemistry and with a targeted sequencing depth of $>50\times$. Draft genomes were assembled using Shovill v1.1.0 (<https://github.com/tseemann/shovill>) and annotated using Prokka v1.14.5 [28]. GC percentage and ANI calculations were performed using ANI calculator (<https://www.ezbiocloud.net/tools/ani>) [29]. Assembled genomes have been deposited at the NCBI (GenBank accession: AK01, SAMN23041292; AK04, SAMN23041293). General features for isolated bacteria are reported in Table 1. The protein sequences of putative sulfo-

proteins in AK01 and AK04 were used to search against the NCBI non-redundant database using BLASTp. Percentage identities for key sulfo proteins are given in **Table 4**.

Discovery of related sulfoglycolytic operons

Sequences for *E. coli* sulfoquinovosidase (NP_418314.1, locus tag b3878), SQ mutarotase (NP_418315.3, locus tag b3879), SQ isomerase (NP_418316.4, locus tag b3880), SF kinase (NP_418319.2, locus tag b3883), SFP aldolase (NP_418317.1, locus tag b3881), SLA reductase (NP_418318.1, locus tag b3882) and sulfo-EMP regulator (NP_418320.2, locus tag b3884) were submitted separately as queries to the NCBI BLASTp tool. The database searched was the non-redundant protein sequence (nr) database, with *E. coli* (taxid: 562) sequences excluded. Standard algorithm parameters were used, except the maximum target sequences was set to 10,000. The results were filtered, with only protein sequences with E-value $\leq 5.41e-44$ retained. The corresponding nucleotide accession numbers for each protein from all seven searches were extracted, and the seven lists combined and duplicates removed to give a list of candidate genome sequences. This list was converted into a MultiGeneBLAST reference library and searched using the *E. coli* sulfo-EMP gene cluster as a query. Scripts for this pipeline are available on GitHub (<https://github.com/jmui-unimelb/Gene-Cluster-Search-Pipeline>). Gene clusters found using this workflow were screened for clusters that contained a putative SQ isomerase, a putative SF kinase and a putative SFP aldolase, but lacked a homologous sulfoquinovosidase.

List of Abbreviations

ABC: ATP-binding cassette

DHPS: 2,3-dihydroxypropanesulfonate

NAD(P)H: reduced nicotinamide adenine dinucleotide (phosphate)

SF: sulfofructose

SL: sulfolactate

SLA: sulfolactaldehyde

SQ: 6-deoxy-6-sulfo-D-glucose

SQase: sulfoquinovosidase

SQDG: sulfoquinovosyl diacylglycerol

SQGro: sulfoquinovosyl glycerol

Sulfo-ED: sulfoglycolytic Entner-Doudoroff

Sulfo-EMP: sulfoglycolytic Embden-Meyerhof-Parnas

Sulfo-SFT: sulfoglycolytic sulfofructose transaldolase

Sulfo-SMO: sulfoglycolytic sulfoquinovose monooxygenase

TSUP: 4-toluene sulfonate uptake permease

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analysed during the current study are available in the GenBank database with accession codes SAMN23041292 (strain AK01) and SAMN23041293 (strain AK04). Scripts used for bioinformatic search pipeline are available on the GitHub repository, <https://github.com/jmui-unimelb/Gene-Cluster-Search-Pipeline>.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SJW conceived the study with input from SP. AK and PvdP performed enrichment culture and microbial characterization. JM synthesized 13C6-SQ. AK and JM performed bioinformatic analysis. MH and SP performed genome sequencing, assembly and analysis. AK and SJW wrote the paper with input and approval of the final draft.

References

- [1] Goddard-Borger ED, Williams SJ: Sulfoquinovose in the biosphere: occurrence, metabolism and functions. *Biochem J* 2017;474:827–49.
- [2] Benson AA, Daniel H, Wiser R: A sulfolipid in plants. *Proc Natl Acad Sci USA* 1959;45:1582–7.
- [3] Harwood JL, Nicholls RG: The plant sulfolipid - a major component of the sulphur cycle. *Biochem Soc Trans* 1979;7:440–7.
- [4] Benson AA, Shibuya I: Sulfocarbohydrate metabolism. *Fed Proc* 1961;20:79.

- [5] Denger K, Weiss M, Felux AK, Schneider A, Mayer C, Spiteller D, Huhn T, Cook AM, Schleheck D: Sulphoglycolysis in *Escherichia coli* K-12 closes a gap in the biogeochemical sulphur cycle. *Nature* 2014;507:114-7.
- [6] Sharma M, Abayakoon P, Epa R, Jin Y, Lingford JP, Shimada T, Nakano M, Mui JWY, Ishihama A, Goddard-Borger ED *et al*: Molecular Basis of Sulfosugar Selectivity in Sulfoglycolysis. *ACS Cent Sci* 2021;7:476-87.
- [7] Felux AK, Spiteller D, Klebensberger J, Schleheck D: Entner-Doudoroff pathway for sulfoquinovose degradation in *Pseudomonas putida* SQ1. *Proc Natl Acad Sci USA* 2015;112:E4298-305.
- [8] Frommeyer B, Fiedler AW, Oehler SR, Hanson BT, Loy A, Franchini P, Spiteller D, Schleheck D: Environmental and Intestinal Phylum Firmicutes Bacteria Metabolize the Plant Sugar Sulfoquinovose via a 6-Deoxy-6-sulfofructose Transaldolase Pathway. *iScience* 2020;23:101510.
- [9] Liu Y, Wei Y, Zhou Y, Ang EL, Zhao H, Zhang Y: A transaldolase-dependent sulfoglycolysis pathway in *Bacillus megaterium* DSM 1804. *Biochem Biophys Res Commun* 2020;533:1109-14.
- [10] Sharma M, Lingford, J.P., Petricevic, M., Snow, A.J.P., Zhang, Y., Jarva, M., Mui, J.W.-Y., Scott, N.E., Saunders, E.C., Mao, R., Epa, R., da Silva, B.M., Pires, D.E.V., Ascher, D.B., McConville, M.J., Davies, G.J., Williams, S.J., and Goddard-Borger, E.D.: Oxidative desulfurization pathway for complete catabolism of sulfoquinovose by bacteria. *ChemRxiv* 2021:10.33774/chemrxiv-2021-08j01.
- [11] Abayakoon P, Jin Y, Lingford JP, Petricevic M, John A, Ryan E, Wai-Ying Mui J, Pires DEV, Ascher DB, Davies GJ *et al*: Structural and Biochemical Insights into the Function and Evolution of Sulfoquinovosidases. *ACS Cent Sci* 2018;4:1266-73.
- [12] Speciale G, Jin Y, Davies GJ, Williams SJ, Goddard-Borger ED: YihQ is a sulfoquinovosidase that cleaves sulfoquinovosyl diacylglyceride sulfolipids. *Nat Chem Biol* 2016;12:215-7.
- [13] Kim M, Oh HS, Park SC, Chun J: Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346-51.
- [14] Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S: High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 2018;9:5114.
- [15] Shimada T, Yamamoto K, Nakano M, Watanabe H, Schleheck D, Ishihama A: Regulatory role of CsqR (YihW) in transcription of the genes for catabolism of the anionic sugar sulfoquinovose (SQ) in *Escherichia coli* K-12. *Microbiology (Reading, England)* 2019;165:78-89.
- [16] Abayakoon P, Lingford JP, Jin Y, Bengt C, Davies GJ, Yao S, Goddard-Borger ED, Williams SJ: Discovery and characterization of a sulfoquinovose mutarotase using kinetic analysis at equilibrium by exchange spectroscopy. *Biochem J* 2018;475:1371-83.
- [17] Sharma M, Abayakoon P, Lingford JP, Epa R, John A, Jin Y, Goddard-Borger ED, Davies GJ, Williams SJ: Dynamic Structural Changes Accompany the Production of Dihydroxypropanesulfonate by Sulfolactaldehyde Reductase. *ACS Catalysis* 2020;10:2826-36.
- [18] Li J, Epa R, Scott NE, Skoneczny D, Sharma M, Snow AJD, Lingford JP, Goddard-Borger ED, Davies GJ, McConville MJ *et al*: A Sulfoglycolytic Entner-Doudoroff Pathway in *Rhizobium leguminosarum* bv. *trifolii* SRI565. *Appl Environ Microbiol* 2020;86:e00750-20.
- [19] Jones D, Keddle RM: The Genus *Arthrobacter*. In: *The Prokaryotes: Volume 3: Archaea Bacteria: Firmicutes, Actinomycetes*. Edited by Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E. New York, NY: Springer New York; 2006: 945-60.
- [20] Eschbach M, Möbitz H, Rompf A, Jahn D: Members of the genus *Arthrobacter* grow anaerobically using nitrate ammonification and fermentative processes: anaerobic adaptation of aerobic bacteria abundant in soil. *FEMS Microbiol Lett* 2003;223:227-30.

- [21] Shlykov MA, Zheng WH, Chen JS, Saier MH, Jr.: Bioinformatic characterization of the 4-Toluene Sulfonate Uptake Permease (TSUP) family of transmembrane proteins. *Biochim Biophys Acta* 2012;1818:703-17.
- [22] Weinitschke S, Denger K, Cook AM, Smits THM: The DUF81 protein TauE in *Cupriavidus necator* H16, a sulfite exporter in the metabolism of C2 sulfonates. 2007;153:3055-60.
- [23] Rein U, Gueta R, Denger K, Ruff J, Hollemeyer K, Cook AM: Dissimilation of cysteate via 3-sulfolactate sulfo-lyase and a sulfate exporter in *Paracoccus pantotrophus* NKNCYSA. *Microbiology* (Reading, England) 2005;151:737-47.
- [24] Denger K, Huhn T, Hollemeyer K, Schleheck D, Cook AM: Sulfoquinovose degraded by pure cultures of bacteria with release of C₃-organosulfonates: complete degradation in two-member communities. *FEMS Microbiol Lett* 2012;328:39-45.
- [25] Cook AM, Denger K, Smits TH: Dissimilation of C3-sulfonates. *Arch Microbiol* 2006;185:83-90.
- [26] Denger K, Cook AM: Racemase activity effected by two dehydrogenases in sulfolactate degradation by *Chromohalobacter salexigens*: purification of (S)-sulfolactate dehydrogenase. *Microbiology* (Reading, England) 2010;156:967-74.
- [27] Denger K, Mayer J, Buhmann M, Weinitschke S, Smits TH, Cook AM: Bifurcated degradative pathway of 3-sulfolactate in *Roseovarius nubinhibens* ISM via sulfoacetaldehyde acetyltransferase and (S)-cysteate sulfolase. *J Bacteriol* 2009;191:5648-56.
- [28] Seemann T: Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068-9.
- [29] Yoon SH, Ha SM, Lim J, Kwon S, Chun J: A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281-6.

343 **Table 1.** ^{13}C NMR (125 MHz) data of $^{13}\text{C}_3$ -SL produced as metabolite from $^{13}\text{C}_6$ -SQ.

344 Samples contain 50 % D_2O to allow frequency lock.

chemical shift (d, ppm)	multiplicity	coupling constant (Hz)	assignment
55.19	d	$^1J_{\text{C1,C2}} = 36.9$	C3
62.42	s	NA	---
68.85	dd	$^1J_{\text{C2,C3}} = 52.7$ $^1J_{\text{C1,C2}} = 38.0$	C2
72.01	s	NA	---
178.69	d	$^1J_{\text{C2,C3}} = 54.6$	C1

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Table 2. Key features of the AK01 (GenBank accession: SAMN23041292) and AK04 (GenBank accession: AK01, SAMN23041293) draft genome assemblies.

	AK01	AK04
Genome size	5,105,913	4,700,363
Number of contigs	169	130
GC %	62.87	65.51
Number of ORFs	4734	4318
Number of putative genes	4669	4261
Number of putative tRNA	59	53
Number of putative rRNA	5	3
Number of putative tmRNA	1	1
Number of genes assigned a function (%)	2478 (53%)	2219 (52%)

a) AK01

b) AK04

Figure 1. Scanning electron microscopy image of *Arthrobacter* sp. a) strain AK01, b) strain AK04. Cell morphology was examined using a scanning electron microscope (Quanta 200 ESEM). Cells were grown in LB media for 3 days, fixed in 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), then in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and allowed to react for 20 min. Fixed cells were adhered onto poly-lysine coated slides and rinsed with water 3 times, then dehydrated by soaking in an ascending ethanol gradient (20-100%). The sample was critical point dried using a Leica CPD3000 and gold coated to thickness of 5 nm using Safematic CCU-010 compact coating unit. Images are at approximately 50,000x magnification with scale bar shown.

a) *Arthrobacter* sp. (AK04)

Locus Tag	03985	03986	03987	03988	03989	03990	03991	03992	03993	03994	03995
Gene Name	<i>glpR_2</i>	<i>iolG_20</i>	<i>yihV</i>	<i>yihT</i>	Protein	<i>ngcF</i>	<i>ngcG_6</i>	<i>gabD_2</i>	Transporter	Protein	<i>yihS</i>
Predicted Function	GlpR repressor	Myo-inositol 2-dehydrogenase	Sulfofructose Kinase	SFP Aldolase	Binding Protein	Uptake system Permease	Uptake system Permease	[NADP(+)] Reductase	TSUP family Transporter	Binding Protein	SQ-SF isomerase

b) *Arthrobacter* sp. (AK01)

Locus Tag	00271	00272	00273	00274	00275	00276	00277	00278	00279
Gene Name	<i>yihS</i>	<i>glpR_2</i>	<i>iolG_1</i>	<i>yihV</i>	<i>yihT</i>	Protein	<i>melD</i>	<i>melC</i>	<i>gabD_1</i>
Predicted Function	SQ-SF isomerase	GlpR repressor	Myo-inositol 2-dehydrogenase	Sulfofructose Kinase	SFP Aldolase	Binding Protein	Uptake system Permease	Uptake system Permease	[NADP(+)] Reductase

c) *Arthrobacter* spp.

d) *E. coli* K12

Figure 2. Proposed sulfoglycolytic Embden-Meyerhof-Parnas (sulfo-EMP) pathway for *Arthrobacter* spp. (a) Gene cluster encoding the sulfo-EMP pathway for *Arthrobacter* sp. AK04 (b) Gene cluster encoding the sulfo-EMP pathway for *Arthrobacter* sp. AK01 (c) Proposed sulfo-EMP pathway for *Arthrobacter* spp. (d) Comparison with EMP pathway for *E. coli* K12.

Figure 3. Growth curves of *Arthrobacter* strains a) AK01 and c) AK04 grown on minimal salts media containing 5 mM glucose or SQ. ^{13}C NMR (500 MHz) spectra of spent culture media of *Arthrobacter* strains b) AK01 and d) AK04 grown on $^{13}\text{C}_6$ -SQ (7.7 mM).

Figure 4. Distribution and architecture of sulfo-EMP gene clusters in *Arthrobacter* and related organisms. Syntenic relationship of sulfo-EMP gene clusters in *Arthrobacter* sp. AK01 and AK04 with homologous gene clusters. Colored links indicate $\geq 30\%$ protein sequence similarity. Genome accession codes: *Arthrobacter* sp. D2 (LUKB01000109.1), *Arthrobacter* sp. D4 (LUKC01000078.1), *Arthrobacter* sp. AAC2 (JAAGBD010000014.1), *Arthrobacter* sp. M5 (LVCB01000107.1), *Arthrobacter* sp. M6 (LVCC01000103.1), *Arthrobacter* sp. AK-YN10 (AVPD02000157.1), *Arthrobacter* sp. ATCC 21022 (CP014196.1), *Arthrobacter* sp. EpRS71 (LNUV01000003.1), *Arthrobacter* sp. ZXY-2 (CP017421.1), *Arthrobacter* sp. AG367 (VIVE01000010.1), *Arthrobacter* sp. AG258 (SOBI01000009.1), *Arthrobacter* sp. 4J27 (CAQI01000048.1), *Arthrobacter* sp. S39 (SIHX01000007.1), *Arthrobacter* sp. 1704 (SOBD01000016.1), *Arthrobacter* sp. BB-1 (VDEV01000010.1), *Arthrobacter* sp. FB24 (CP000454.1), *Arthrobacter* sp. KBS0703 (MVDG02000001.1), *Arthrobacter* sp. OV608 (FOEZ01000003.1), *Arthrobacter* sp. PGP41 (CP026514.1), *Arthrobacter* sp. SLBN-53 (VFMZ01000001.1), *Arthrobacter* sp. SLBN-83 (VFMX01000001.1), *Arthrobacter* sp. SLBN-112 (VFMU01000001.1), *Arthrobacter* sp. SLBN-122 (VFMS01000001.1), *Arthrobacter* sp. SLBN-179 (VFNR01000001.1), *Arthrobacter* sp. Soil761 (LMSF01000007.1), *Arthrobacter* sp. Soil764 (LMSI01000008.1), *Pseudarthrobacter phenanthrenivorans* (CP002379.1), *Pseudarthrobacter phenanthrenivorans* (RBNH01000003.1), *Pseudarthrobacter phenanthrenivorans* (VHJD01000009.1), *Pseudarthrobacter* sp. AG30 (QEHL01000024.1), *Arthrobacter* sp. MYb23 (PCPR01000010.1), *Arthrobacter* sp. KBS0703 (MVDG02000001.1), *Microbacterium* sp. No.7 (CP012697.1), *Acrocarpospora corrugata* (BLAD01000050.1), *Phytohabitans houttuyniae* (BLPF01000004.1), *Pseudoruegeria haliotis* (PVTDO1000003.1).