Tandem sulfofucolytic-sulfolactate sulfolyase pathway for catabolism of the rare sulfosugar sulfofucose

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Abstract

The rare sulfosugar sulfofucose (SFuc, 6-deoxy-6-sulfo-D-galactose) is catabolized by *Paracoccus onubensis* strain Merri via a novel tandem pathway that couples sulfofucolytic and sulfolactate sulfolyase reactions. We isolated *P. onubensis* from soil and demonstrated its ability to utilize SFuc as the sole carbon source, leading to complete degradation of SFuc and release of sulfite. Proteomics and metabolomics analysis revealed a sulfofucolytic variant of the Entner–Doudoroff (ED) pathway, wherein SFuc is converted to sulfolactaldehyde (SLA) through oxidation, lactone hydrolysis, and dehydration steps. The SLA is subsequently degraded to pyruvate and sulfite via a downstream biomineralization pathway involving sulfolactate (SL) as an intermediate. *In vitro* reconstitution of the key enzymatic steps validated the pathway, identifying four enzymes: SfcH (dehydrogenase), SfcD (lactonase), SfcF (dehydratase), and SfcE (aldolase), that collectively convert SFuc to SLA and pyruvate. Our findings provide insight into microbial utilization of rare sulfosugars and expand the known metabolic versatility of the genus *Paracoccus*.

Significance

Sulfosugars, such as sulfoquinovose, are integral to the global sulfur cycle, but the metabolism of rarer sulfosugars, like sulfofucose, remains poorly understood. This study identifies a unique microbial catabolic pathway for sulfofucose degradation, comprising a sulfofucolytic ED pathway followed by biomineralization to sulfite. The identification of this tandem pathway in *P. onubensis* highlights the metabolic flexibility of bacteria to adapt carbohydrate catabolic pathways for processing structurally similar sulfosugars. This work sheds light on the ecological role of sulfofucose metabolism and broadens our understanding of bacterial sulfur metabolism, offering potential implications for biogeochemical sulfur cycling and biotechnological applications.

Introduction

Sulfur is an essential macronutrient and is incorporated into a wide range of organosulfur compounds that serve as metabolic currencies and facilitate sulfur exchange between producer and consumer organisms.[1, 2] The biosynthesis and catabolism of these organosulfur metabolites form intricate biochemical networks within the global sulfur cycle.[3, 4] Organosulfur compounds of global significance include the sulfur-containing amino acids cysteine and methionine, the osmoprotectants dimethylsulfoniopropionate[5] and dimethyloxosulfonium propionate,[6] the short-chain organosulfonates 2,3-dihydroxypropanesulfonate (DHPS) and sulfolactate (SL),[7 8] and plant and cyanobacterial sulfolipid (sulfoquinovosyl diglycerol; SQDG), which contains the sulfosugar sulfoquinovose (SQ, 6-deoxy-6-sulfo-D-glucose).[9]

Over the past three decades, extensive work has elucidated the biosynthetic route to SQ in plants and cyanobacteria, as well as the diverse bacterial pathways used for its degradation (**Figure 1a**).[9-12] SQ breakdown occurs via multiple sulfoglycolytic and sulfolytic pathways. Glycoside hydrolases known as sulfoquinovosidases initiate catabolism by cleaving SQ from glycoconjugates such as SQDG and sulfoquinovosyl glycerol.[13-15] In sulfoglycolytic bacteria, SQ is partially degraded into short-chain C2 and C3-organosulfonates, which are excreted and subsequently utilized by secondary consumers. These secondary organisms—facultative anaerobes or aerobes—cleave the C–S bond using sulfolyases and release sulfite to enable carbon assimilation.[16-19] In anaerobic environments, short-chain organosulfonates serve as terminal electron acceptors or sulfur sources, a process facilitated by glycyl radical enzymes.[20] Some organisms combine both sulfoglycolytic and short-chain catabolism pathways, allowing complete SQ degradation to sulfite.[21-23] In contrast, sulfolytic bacteria can directly cleave the C–S bond in SQ, releasing sulfite and converting the carbon backbone to glucose for entry into central carbon metabolism.[19, 24]

While SQ metabolism is well-characterized, much less is known about other sulfosugars. One such compound is sulfofucose (SFuc, 6-deoxy-6-sulfo-D-galactose), a rare sulfosugar reported in the glycolipids of algae from the order *Fucacaea*,[25] and in cell surface N-linked glycans from the archaeon *Thermoplasma acidophylum* (**Figure 1b**).[26] Sulfofucose differs from sulfoquinovose only at the configuration of carbon-4. Despite this minimal structural difference, no biosynthetic or catabolic pathways for sulfofucose have been identified.

Here, we report the isolation of *Paracoccus onubensis* strain Merri, a bacterium capable of using SFuc as its sole carbon source. Using uniformly ^{13}C-labeled SFuc, we show that the entire carbon skeleton is metabolized and that sulfite is released as the end-product. Comparative proteomics revealed increased abundance of proteins associated with

organosulfur metabolism during growth on SFuc. Metabolomics analysis of cell lysates identified a pathway intermediate consistent with an Entner–Doudoroff (ED) like pathway for SFuc metabolism to sulfolactaldehyde (SLA), and a sulfolyase pathway for biomineralization via SL. Finally, recombinant expression and *in vitro* reconstitution of four enzymes allowed demonstration of the individual steps of a sulfofucolytic ED pathway that converts SFuc to SLA and pyruvate. These findings support a tandem pathway comprising oxidative cleavage of SFuc to SLA, followed by canonical biomineralization to sulfite via SL sulfolyase.

Results

Isolation and characterization of a *Paracoccus onubensis* bacterium able to grow on sulfofucose.

A soil sample from Joe's Garden, an inner-Melbourne market garden that has been continuously farmed by Chinese and Italian gardeners for 150 years, was used to inoculate a vitamin-enriched defined media containing 10 mM SFuc as sole carbon source. Sequential subculturing, repeated plating onto SFuc-containing media agar plates, followed by regrowth in SFuc liquid media led to isolation of strain Merri. This strain grew on glucose and SFuc to similar peak absorbance, but did not grow on SQ (**Figure 2a**). Genomic DNA was extracted from strain Merri and was sequenced using the Illumina NextSeq platform. Analysis of the 16S rRNA gene revealed close similarity to *Methylarcula* and *Paracoccus* species, both members of the family Rhodobacteraceae. In silico DNA:DNA hybridization against other sequenced *Methylarcula* and *Paracoccus* genomes revealed *Paracoccus onubensis* to be the most closely related genome (94% average nucleotide identity). As such, the strain was designated as *P. onubensis* strain Merri and the general features of the draft genome are shown in **Table 1**.

To trace SFuc catabolism, we examined the fate of its carbon and sulfur components upon metabolism by *P. onubensis* strain Merri. To examine the fate of sulfur, spent culture media from strain Merri grown on SFuc were analyzed. SFuc consumption was tracked using a reducing sugar assay, while sulfate and sulfite production were quantified with colorimetric and turbidometric assays, respectively. Most SFuc was consumed over 200 h, accompanied by the production of sulfate and sulfite (**Figure 2b**). Sulfite levels peaked at 160 hours before declining, a pattern consistent with the known oxygen sensitivity of sulfite.[24] This suggests that sulfite is initially produced and subsequently undergoes autooxidation to sulfate. ^{13}C NMR analysis of the culture medium of *P. onubensis* strain Merri grown to mid log-phase (5 d) on $^{13}C_6$-SFuc revealed complete consumption of the substrate, and production of ^{13}C-bicarbonate (**Figure 2c**); extended growth to stationary phase (14 d) revealed no distinct carbon-containing compounds, indicating complete utilization of the complete carbon chain (data not shown).

Identification of genetic loci expressed upon growth on sulfofucose

To identify proteins potentially involved in SFuc metabolism, we cultured *P. onubensis* strain Merri using either SFuc or glucose as the sole carbon source and analyzed the resulting changes in the proteome (**Figures 3a, b**). Growth on SFuc led to significant increases in abundance of proteins primarily encoded within three genomic regions, designated clusters 1–3.

Cluster 1 (Locus IDs MDP0925965–0925971) comprises seven genes, all of which showed increased protein abundance (**Figure S1**). These genes encode enzymes annotated for roles in gluconeogenesis and the pentose phosphate pathway. Notably, previous studies in *E. coli* have shown that sulfoglycolysis via the sulfo-EMP pathway elevates gluconeogenic flux due to the generation of the C3-phosphate DHAP;[27] similarly, a sulfofucolytic pathway in *P. onubensis* would require gluconeogenesis to supply hexose-phosphate intermediates. Cluster 3 (MDP0926751–0926761) includes nine proteins with significantly increased abundance, associated with fumarate and tartrate metabolism—likely linked to the Krebs cycle (**Figure S1**). This cluster also harbors gene MDP0926757, annotated as a hypothetical protein but homologous to the TauE transmembrane sulfite exporter.[28]

Cluster 2 (MDP0926256–0926279) contains 24 genes, many of which were increased in abundance in SFuc-grown cells (**Figure 3c, Table S1**). Some of these genes are annotated with functions related to organosulfonate metabolism, and belong to protein families (PFAM) associated with SL biomineralization in *C. salexigens*[29] (**Table S2**). Among the proteins of increased abundance were MDP0926266, homologous to SLA dehydrogenase (SlaB, oxidizes SLA to SL);[18, 30] MDP0926258, annotated as SL sulfolyase B (SuyB); and MDP0926257, encoding a short protein homologous to SL sulfolyase A (SuyA).[31] Additionally, homologues of SL dehydrogenases SlcC (MDP0926259) and ComC (MDP0926260) were increased in abundance, which could act as a two-component SL racemase.[29] Together, these proteins likely form a C3-organosulfonate biomineralization pathway that involves SlaB-catalyzed oxidation of SLA to *S*-SL, racemization to *R*-SL catalyzed by SlcC/ComC, and SuyAB catalyzed cleavage of *R*-SL to yield pyruvate and sulfite, with the latter exported via TauE.

Turning to the initial steps of SFuc metabolism, several genes within cluster 2 are homologous to those involved in known sulfosugar breakdown pathways. Two key groups of interest were identified. The first includes homologues of sulfo-EMP pathway proteins: MDP0926268 and MDP0926269, belonging to PF00294 and PF01791, respectively—protein families encompassing sulfofructose kinase and sulfofructose-1-phosphate aldolase (**Table S3**).[17] Possibly, these could act on SFuc-derived intermediates, such as sulfotagatose and sulfotagatose-1-phosphate, with the latter producing SLA and DHAP. While no aldose–ketose isomerase is encoded within this cluster, a homologue is encoded elsewhere in the genome (protein MDP0925592) belonging to protein family PF07221, the same as SQ-sulfofructose isomerase within the sulfo-EMP pathway.[17] However, none of these three proteins showed significantly increased abundance in SFuc-grown cells.

The second group includes MDP0926275, MDP0926278, and MDP0926279—which were all increased in SFuc-grown cells and are associated with PF13561, PF03328, and PF09450, respectively. These protein families include enzymes from the sulfoglycolytic ED pathway: SQ dehydrogenase, 2-keto-3-deoxysulfogluconate (KDSG) aldolase and sulfogluconolactone (SGL) lactonase, respectively (**Table S4**).[18] A fourth enzyme required for a complete sulfoglycolytic ED pathway—SG dehydratase (belonging to PF00920)—was not encoded within cluster 2 or elsewhere in the genome. However, the protein encoded by MDP0926279 in cluster 2 was increased in abundance and belongs to protein families PF13378 and PF02746, families to which D-galactonate dehydratase belongs, which bears close structural resemblance to sulfogalactonate. Thus, we propose that protein MDP0926279 may be capable of dehydrating a sulfofucolytic ED intermediate such as sulfogalactonate (SGal). Associated with these genes was a likely ABC transporter cassette and substrate binding protein, which we assign as a putative SFuc importation system.

Collectively, these data support the existence of either an EMP-like or ED-like sulfofucolysis pathway. In the proposed sulfofucolytic EMP pathway, SFuc would be isomerized to sulfotagatose (by protein MDP0925592), phosphorylated to sulfotagatose-1-phosphate (by protein MDP0926268), and then cleaved via a retroaldol reaction (by protein MDP0926269) to yield SLA and DHAP. Alternatively, a sulfofucolytic ED pathway could involve the oxidation of SFuc to sulfogalactonolactone (SGalL) (by protein MDP0926275), followed by lactone hydrolysis to form SGal (by protein MDP0926279). This intermediate would undergo dehydration to 3-deoxy-2-ketosulfogalactonate (KDSGal) (by protein MDP0926277), and retroaldol cleavage (by protein MDP0926278) to generate SLA and pyruvate. In either pathway, SLA would be further metabolized by the SlaB–SlcC/ComC–SuyAB biomineralization system encoded in cluster 2, while DHAP or pyruvate would enter central carbon metabolism. Sulfite released by biomineralization would be exported by TauE homologue MDP0926575 in cluster 3.

Cell-free lysate analysis reveals sulfofucose-stimulated dehydrogenase activity

To explore enzymatic activities in SFuc catabolism, cell-free lysates were prepared from *P. onubensis* strain Merri grown in minimal medium with 5 mM SFuc. Cells were harvested in mid-log phase, lysed by sonication, and clarified by centrifugation.

$NAD(P)^+$-dependent dehydrogenase activity was assessed by monitoring NAD(P)H formation at 340 nm. An increase in absorbance was observed only when lysate and SFuc were present, indicating SFuc-stimulated dehydrogenase activity (**Figure S2**). To test for isomerase activity, ^{1}H NMR spectroscopy was used to monitor reactions in lysate supplemented with recombinant shrimp alkaline phosphatase, which prevented

phosphorylation and potential downstream turnover of sulfotagatose. After 24 hours of incubation with SFuc, no new resonances consistent with sulfotagatose were detected (data not shown), suggesting the absence of a SFuc–sulfotagatose isomerase. These findings support an ED-like sulfofucolytic pathway in which SFuc undergoes initial $NAD(P)^+$-dependent oxidation.

Metabolomics analysis: detection of sulfogalactonate and sulfolactate

To investigate the metabolic fate of SFuc in *P. onubensis* strain Merri, we performed metabolomic analysis on cell lysates from cultures grown with SFuc as the sole carbon source. Using ion chromatography-mass spectrometry (IC-MS), we screened for intermediates indicative of sulfofucolytic EMP- or ED-like pathways. Metabolites were extracted from mid-log phase cell lysates and were analyzed using ion chromatography coupled to high-resolution mass spectrometry.

In SFuc-grown samples, we observed peaks with retention times, *m/z* values and fragmentation patterns matching those of authentic standards for sulfogalactonate (SGal, *m/z* 259) and SL (*m/z* 169) (**Figures 4, S3**). In contrast, no signals corresponding to expected intermediates of an EMP-like pathway—such as sulfotagatose or sulfotagatose-1-phosphate—were detected extending the lack of isomerase activity noted in lysate assays. The detection of SGal supports an ED-like pathway, where SFuc is oxidized to sulfofuconolactone, followed by hydrolysis to yield SGal. The presence of SL, the product of SLA oxidation, supports downstream metabolism involving the SlaB–SlcC/ComC–SuyAB biomineralization pathway. Combined with SFuc-stimulated $NAD(P)^+$-dependent dehydrogenase activity, this supports the operation of an ED-like sulfofucolytic pathway in *P. onubensis*.

***In vitro* demonstration of an Enter-Doudoroff pathway for sulfofucose**

To demonstrate the biochemical steps of sulfofucolysis, genes encoding the candidate enzymes MDP0926275, MDP0926279, MDP0926278, and MDP0926280 were heterologously expressed in *E. coli*, and the corresponding proteins were purified. Based on the roles subsequently demonstrated in SFuc catabolism, these genes were subsequently renamed *sfcH* (dehydrogenase), *sfcD* (lactonase), *sfcF* (dehydratase), and *sfcE* (aldolase), reflecting their membership in a sulfofucose degradation pathway. Incubation of SfcH with SFuc and either $NADP^+$ or NAD^+ produced a compound with *m/z* 241, two mass units lower than SFuc, consistent with oxidation to SGalL (**Figure 5**). MS^2 fragmentation of this species matched that of the stereoisomer SGL.[18] Notably, this enzyme displayed greater activity on

NAD^+, a preference for this cofactor as seen with SQ dehydrogenase from *P. putida*.[32] Subsequent addition of SfcD along with Ca^{2+} and Zn^{2+} converted the oxidized intermediate into a compound with identical retention time and MS^2 fragmentation pattern as chemically synthesized SGal. When chemically synthesized SGal was incubated with SfcF, a new species with *m/z* 241 (18 mass units lower than sulfogalactonate) was detected, consistent with conversion to KDSGal, and which displayed a fragmentation pattern consistent with the stereoisomer KDSG.[18] Finally, addition of SfcE and Mg^{2+} to this reaction mixture yielded a product with retention time and MS^2 fragmentation matching that of chemically synthesized SLA. Separately, analysis of this reaction mixture by IC-MS demonstrated formation of pyruvate (**Figure S4**).

Identification of possible sulfofucolytic pathways in proteobacteria

To identify candidate sulfofucolytic ED pathways, we used tools from the Enzyme Function Initiative (EFI) and performed a BLAST search using the sequence of sulfogalactonate (SGal) dehydrogenase as a query.[33, 34] Homologous sequences were retrieved and used to construct a sequence similarity network (**Figure 6**). We then examined the genome neighborhoods of these homologues for the presence of genes encoding additional enzymes associated with the sulfofucolytic ED pathway—specifically, those from Pfam families PF08450 (SGalL lactonase), PF13378 and PF02746 (SGal dehydratase), and PF03328 (KDSGal aldolase). This analysis identified two clusters of proteins. Gene neighborhood diagrams from the parent bacteria showed that some clusters encode all four core enzymes of the proposed pathway, along with an ABC transporter system. Other clusters lacked a lactonase candidate but often included a gene encoding an aldose-1-epimerase, which may function as a SFuc mutarotase, potentially facilitating the first step in SFuc catabolism.

Discussion

In this study, we identify a novel sulfofucolytic Entner–Doudoroff (ED) pathway in *Paracoccus onubensis*, enabling the degradation of the rare sulfosugar sulfofucose (SFuc) (**Figure 7**). This pathway is characterized by the cleavage of the C6 backbone of SFuc into two C3 fragments: SLA and pyruvate. SLA is then biomineralized through a pathway involving oxidation to sulfolactate then sulfolyase catalysed elimination of sulfite to give pyruvate. The pyruvate outputs of this pathway are used in central carbon metabolism, while the sulfite is excreted into the culture media. This finding adds to the known metabolic versatility of the genus Paraccoccus, as *Paracoccus pantotrophus* strain NKNCYSA has previously been reported to degrade a range of organosulfonates, including cysteate,

taurine, homotaurine, isethionate, and SL,[35] suggesting a broad organosulfonate catabolic potential within this taxonomic grouping.

Metabolomic analysis of SFuc-grown *P. onubensis* cells revealed the presence of sulfogalactonate (SGal), a diagnostic intermediate indicative of an oxidative sulfofucolytic pathway. We identified four enzymes (SfcH, SfcD, SfcF and SfcE) that collectively enable the stepwise conversion of SFuc through an Entner–Doudoroff–like pathway: oxidation to sulfogalactonolactone (SGalL), hydrolysis to SGal, dehydration to 2-keto-3-deoxy-sulfogalactonate (KDSGal), and aldol cleavage to yield SLA and pyruvate. These steps mirror the enzymatic sequence of the canonical sulfoglycolytic ED pathway used for sulfoquinovose (SQ) catabolism, with the key distinction being the stereochemistry at C4 of the sugar.

All but one of the enzymes in the sulfofucolytic ED pathway belong to the same protein families as those in the canonical sulfoglycolytic ED pathway for sulfoquinovose catabolism.[18] The key difference lies in the dehydratase step: while sulfoquinovose degradation employs SG dehydratase (PF00920), the sulfofucolytic pathway utilizes SGal dehydratase, which belongs to a distinct family (PF13378, PF02746). This divergence provided a basis for a targeted bioinformatic search, enabling identification of related gene clusters in other bacteria. Using this signature, we identified a small subset of Proteobacteria harboring syntenic gene clusters that encode homologues of the sulfofucolytic ED pathway. Notably, like that of strain Merri, all of these clusters were associated with ABC transporter systems, which may mediate SFuc uptake. ABC transporters have previously been involved in SQ uptake in sulfo-ED pathways,[18, 36] and have been characterized biochemically and structurally.[24, 37, 38]

Comparative proteomics of strain Merri identified a cluster of genes that encoded homologues of proteins capable of the biomineralization of the C3-organosulfonate SLA. Specifically, these genes encode homologues of SLA dehydrogenase (SlaB), which oxidizes SLA to SL using NAD^+/$NADP^+$ as cofactor,[18] the two component $NAD(P)^+$-dependent SL racemases SlcC/ComC,[29] and SL lyase (SuyAB), which cleaves SL to yield pyruvate and sulfite.[31] Metabolomic analysis of lysates from SFuc-grown cells revealed the presence of SL, while culture supernatants contained sulfite, consistent with the activity of this pathway. Together, these data provide strong support for a SlaB–SlcC/ComC–SuyAB biomineralization pathway, proceeding via the sequence *S*-SLA → *S*-SL → sulfopyruvate → *R*-SL → pyruvate + sulfite.

SFuc and SQ differ only in the configuration at the C4 position, rendering their corresponding intermediates epimeric: SGalL vs. SGL, SGal vs. SG, and KDSGal vs. KDSG.

Despite this difference, the aldolase-catalyzed cleavage of both KDSG and KDSGal generates the same *R*-stereoisomer of SLA. This is because the stereocenter in SLA originates from carbon 5 of the sugar, while the configuration at carbon 4 is lost during cleavage, and so does not affect the stereochemistry of the product.

The protein family for SGal dehydratase, PF13378, PF02746, is distinct from that used in the sulfo-ED pathway, enabling a bioinformatic search to identify other candidate sulfofucolytic ED pathway organisms. Interestingly, several of the identified organisms lacked an obvious lactonase homologue, raising the possibility that they utilize a variant of the pathway that bypasses the lactone hydrolysis step. This difference is reminiscent of the De Ley–Doudoroff pathway—a variant of the Entner–Doudoroff pathway—in which D-galactose is oxidized by a NAD^+-dependent dehydrogenase to D-galactonate, followed by dehydration to 2-keto-3-deoxygalactonate and subsequent aldol cleavage to yield pyruvate and glyceraldehyde.[39, 40] By analogy, these bacteria may encode a lactonase-independent sulfofucolytic ED pathway in which SFuc is directly oxidized to sulfogalactonate, thereby bypassing the need for SGalL hydrolysis.

The occurrence of SFuc has only been described in two reports, including within glycoconjugates produced by an alga and an archaeon.[25, 26] This limited number of reports suggests it is a rare sulfosugar, although it is possible that it may have been misidentified for SQ in mass spectrometric based studies and could have a broader distribution than the limited literature reports imply. The origin of strain Merri is Joe's Garden, a riparian market garden situated on the banks of Merri Creek in Melbourne, Australia, with a history of flooding, suggesting a possible aquatic origin of either the bacterium or its substrate. Possibly, within either the soil or aquatic niche, this bacterium may encounter SFuc that is liberated from these known or other, as yet unknown SFuc-containing glycoconjugates. No candidate sulfofucosidases were identified in this work, and we note that previous work has shown that sulfoquinovosidases from *E. coli* and *Agrobacterium tumefaciens* are inactive on α-PNP-sulfofucoside.[15]

In conclusion, this work identifies a bacterium, *Paracoccus onubensis*, capable of complete catabolism of the rare sulfosugar SFuc. We provide evidence for a two-part metabolic route: an oxidative sulfofucolytic Entner–Doudoroff (ED) pathway that cleaves SFuc into C3-organosulfonate intermediates, and a downstream biomineralization pathway involving SL lyase–mediated conversion of SL to sulfite. While most enzymes in the sulfofucolytic ED pathway share homology with those in the known sulfoglycolytic ED pathway, the dehydratase step is catalyzed by a protein from a distinct family (PF13378, PF02746). This divergence enabled the identification of a small set of bacteria predicted to

possess similar SFuc catabolic pathways. Notably, *P. onubensis* did not grow on SFuc-containing glycosides, indicating the absence of a sulfofucosidase capable of liberating free SFuc from glycoconjugates. Future studies could focus on identifying and characterizing such sulfofucosidases, which would expand our understanding of how SFuc is accessed and degraded in diverse environmental contexts.

Materials and Methods

Specialist chemicals

Sulfolactate and sulfolactaldehyde were synthesized as reported.[41, 42] The synthesis of sulfofucose, $^{13}C_6$-sulfofucose, sulfotagatose and sulfogalactonate will be reported elsewhere.

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 10 mM SFuc as sole carbon source, made up to a final volume of 10 ml with deionized water. M9 minimal salts media contains 0.45 M Na_2HPO_4, 0.11 M KH_2PO_4, 0.09 M NH_4Cl, 0.04 M NaCl, 0.1 M $MgSO_4$, 0.1 M $CaCl_2$. Trace metal solution contains 0.4 mM $FeCl_3$, 0.08 mM $CoCl_2$, 0.08 mM $CuCl_2$, 0.08 mM $MnCl_2$, 0.55 mM $ZnCl_2$, 0.01 mM $NiCl_2$, 0.05 mM Na_2MoO_4 and 0.5 mM H_3BO_3. 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). Growth curves were measured using a custom built MicrobeMeter portable spectrophotometer.[43]

Isolation of *Paracoccus onubensis* strain Merri

Paracoccus onubensis strain Merri was isolated by substrate-guided enrichment culturing, from soil sampled at Joe's Market Garden (34 Edna Grove, Coburg, Victoria 3058; 37.7451° S, 144.9813° E). Approximately 1 g of soil was suspended in 5 ml of sterilized M9 media (supplemented with trace metals and vitamins) containing 10 mM SFuc as sole carbon source. The culture was incubated at 30 °C for 4 d with agitation at 250 rpm. A subsample (100 µL) was transferred into fresh M9 media (supplemented with trace metals and vitamins) and grown for a further 4 d. This step was repeated five times and after outgrowth of the final culture for 4 d, cells were plated onto SFuc-agar plates (20 mM SFuc, 15 g/l agar) and incubated at 30 °C in the dark. After 3 d, a single colony was picked and inoculated into fresh M9 media (supplemented with trace metals and vitamins) containing 10 mM SFuc and incubated at 30 °C while shaking at 250 rpm. Once the culture was visibly turbid, cells were again plated onto SFuc-agar plates, incubated at 30 °C in dark and single colony picked and inoculated again into fresh vitamin supplemented M9 media containing 10 mM SFuc. Once the culture was visibly turbid, frozen stocks were prepared by diluting to 5% DMSO and freezing at -80 °C. This strain could grow on racemic DHPS (5 mM), but did not grow on SQ (5 mM), or *S/R*-SL (5 mM).

Genome sequence, assembly and annotation

P. onubensis strain Merri was grown in 10 ml M9 media supplemented with 10 mM SFuc for 5 days and then the cell pellet was harvested by centrifugation. Genomic DNA was isolated using the GenElute DNA extraction kit (Sigma) with inclusion of lysozyme and RNAase, then it was sequenced using Illumina NextSeq platform (Peter Doherty Institute, Australia). DNA was prepared for sequencing using the Nextera XT DNA preparation kit (Illumina) with ×150 bp paired end chemistry and with a targeted sequencing depth of >50×. A draft genome was assembled using Shovill v1.0.9 (https://github.com/tseemann/shovill) and annotated using PGAP as part of the NCBI Genome submission pipeline. GC percentage and ANI calculations were performed using ANI calculator (https://www.ezbiocloud.net/tools/ani).[44]

^{13}C-NMR analysis of spent culture media from growth of *P. onubensis* strain Merri on ($^{13}C_6$)-sulfofucose

M9 minimal media (supplemented with trace metals and vitamins) (4 ml) containing 10 mM ($^{13}C_6$)-SFuc was inoculated with *P. onubensis* strain Merri and grown to mid-log phase (5 days) and stationary phase (14 days) at 30 °C (250 rpm). The final A_{600} was 0.3 (5 days) and 0.52 (14 days). Cells were sedimented by centrifugation at 5,000 × *g* for 3 min, and the supernatant was collected. 540 µL of culture supernatant was diluted with 60 µL D_2O and ^{13}C-NMR spectra acquired using a 500 MHz spectrometer. A control sample contained 10 mM ($^{13}C_6$)-SFuc in M9 minimal media prepared in the same way for NMR analysis.

Analysis of sulfofucose, sulfite and sulfate in *P. onubensis* culture

M9 minimal media (supplemented with trace metals and vitamins) 50 mL containing 10 mM SFuc was inoculated with *P. onubensis* strain Merri and grown at 30 °C at 250 rpm in a 500 mL baffled conical flask. Samples were withdrawn at various time points, cells were pelleted by centrifugation for 5 min at 5,000 *g*, and supernatant samples were frozen and stored at -80 °C prior to analysis for reducing sugar, sulfate or sulfite content.

Reducing sugar assay: The reducing sugar assay was performed according to the procedure of Blakeney and Mutton.[45] Alkaline diluent was prepared by the addition of sodium hydroxide (20 g, 0.50 mol) to a solution of 0.10 M trisodium citrate (50 mmol, 500 ml) and 0.02 M calcium chloride (13 mmol, 500 ml). PAHBAH working solution was prepared by dissolving 4-hydroxybenzhydrazide (PAHBAH) (0.25 g, 1.6 mmol) in alkaline diluent (50 ml).

The PAHBAH working solution should be made fresh shortly before use. To determine reducing sugar concentration, 90 µL of PAHBAH working solution and 10 µL of culture supernatant were combined in a 96-well half-area microplate. The mixture was heated at 98 °C for 5 min, and the absorbance read at 415 nm using a UV/visible spectrophotometer. Concentrations of SFuc were determined by reference to a standard curve constructed using SFuc. Analyses were performed in duplicate and errors are reported as standard deviation.

Turbidometric sulfate assay: The sulfate assay was performed according to the procedure of Sörbo.[46] This assay uses a barium sulfate–polyethylene glycol (Ba-PEG) reagent, which contains polyethylene glycol (PEG) to stabilize $BaSO_4$ crystals and a small amount of preformed $BaSO_4$ seed crystals to improve the reproducibility and linearity of the assay. Ba-PEG reagent was prepared by dissolving $BaCl_2$ (42 mg, 0.20 mmol) and polyethylene glycol 6000 (0.75 g) in deionized water (5.0 ml). A small amount of Na_2SO_4 (10 µl, 50 mM) was added to this solution, with efficient magnetic stirring to generate preformed $BaSO_4$ seed crystals. The Ba-PEG reagent must be prepared fresh on the day of use. Individual sulfate assays were conducted as follows: In an Eppendorf tube, 50 µl of 0.5 M HCl was added to 50 µl culture supernatant, followed by Ba-PEG reagent (50 µl). The mixture was mixed vigorously and then 90 µl was transferred to a 96-well half-area microplate. The absorbance of the sample at 400 nm was measured using a UV/visible spectrophotometer. Concentrations of sulfate were determined by reference to a standard curve constructed using 0-10 mM Na_2SO_4. Analyses were performed in duplicate and errors are reported as standard deviation.

Colorimetric fuchsin sulfite assay: The fuchsin sulfite assay was performed according to the procedures of Brychkova et al.[47] and Kurmanbayeva et al.[48] This method requires three freshly pre-prepared solutions: Reagents A, B, and C. Reagent A was prepared by adding 1 ml Schiff's reagent to 8 ml deionised water, followed by addition of 5 M HCl until the solution turned colorless. Reagent B was prepared by diluting formaldehyde (36% in H_2O, 0.32 ml) in deionised water (9.68 ml) at 0 °C. Reagent C was prepared by dilution of Reagent A (1 ml) in deionised water (7 ml), prior to the addition of solution reagent B (1 ml). Individual sulfite assays were performed by adding Reagent C (100 µl) to the cell supernatant (10 µl). The sample was incubated at 25 °C for 10 min and then transferred to a 96 well half area microplate. The absorbance of the sample at 570 nm was determined by using a UV/visible spectrophotometer. Concentrations of sulfite were determined by reference to a standard curve constructed using Na_2SO_3. Analyses were performed in duplicate and errors are reported as standard deviation.

Comparative proteomics

Six independent cultures of *P. onubensis* strain Merri were grown in 10 mM SFuc and another six independent cultures were grown in 10 mM glucose (both in M9 media supplemented with trace metals and vitamins). The cultures were harvested at mid-log phase (A_{600} approx 0.25), and bacteria were collected by centrifugation at 4000 × *g* for 10 min. The pelleted cells were washed by resuspension with fresh 10 ml ice-cold phosphate-buffered saline (PBS) and re-pelleting three times, with the supernatant discarded each time. Cells were resuspended and transferred to a 1.5 ml tube using ice-cold PBS (1 ml), and collected by centrifugation for 2 min at 10,000 × *g*, and then snap-frozen in liquid nitrogen.

Frozen whole bacterial pellets were prepared using the in-StageTip preparation approach as previously described.[49] Cells were resuspended in 4% sodium deoxycholate (SDC), 100 mM Tris pH 8.0 and boiled at 95 °C with shaking for 10 min to aid solubilisation. Samples were allowed to cool for 10 min and then boiled for a further 10 min before the protein concentration was determined by bicinchoninic acid assays (Thermo Fisher Scientific). 100 µg of protein equivalent for each sample was reduced/alkylated with the addition of tris-2-carboxyethylphosphine hydrochloride and iodoacetamide (final concentrations 20 mM and 60 mM, respectively), by incubating in the dark for 1 h at 45 °C. Following reduction/alkylation, samples were digested overnight with trypsin (1/33 w/w Solu-trypsin, Sigma) at 37 °C with shaking at 1000 rpm. Digests were then halted the addition of isopropanol and trifluoroacetic acid (TFA) (50% and 1%, respectively) and samples were cleaned up using home-made SDB-RPS StageTips prepared according to previously described protocols.[49-51] SDB-RPS StageTips were placed in a Spin96 tip holder 2 to enable batch-based spinning of samples and tips conditioned with 100% acetonitrile; followed by 30% methanol, 1% TFA followed by 90% isopropanol, 1% TFA with each wash spun through the column at 1000 *g* for 3 min. Acidified isopropanol / peptide mixtures were loaded onto the SDB-RPS columns and spun through, and tips were washed with 90% isopropanol, 1% TFA followed by 1% TFA in deionised water. Peptide samples were eluted with 80% acetonitrile, 5% ammonium hydroxide and dried by vacuum centrifugation at room temperature, then were stored at -20 °C.

Prepared digested proteome samples were re-suspended in Buffer A* (2% acetonitrile, 0.01% TFA) and separated using a two-column chromatography setup composed of a PepMap100 C18 20-mm by 75-µm trap and a PepMap C18 500-mm by 75-µm analytical column (Thermo Fisher Scientific). Samples were concentrated onto the trap column at 5 µl/min for 5 min with Buffer A (0.1% formic acid, 2% DMSO) and then infused into an Orbitrap Fusion Eclipse at 300 nl/minute via the analytical columns using a Dionex Ultimate 3000 UPLCs (Thermo Fisher Scientific). 125-minute analytical runs were undertaken by altering the buffer composition from 2% Buffer B (0.1% formic acid, 77.9%

acetonitrile, 2% DMSO) to 22% B over 95 min, then from 22% B to 40% B over 10 min, then from 40% B to 80% B over 5 min. The composition was held at 80% B for 5 min, and then dropped to 2% B over 2 min then was held at 2% B for another 8 min. The Fusion Eclipse Mass Spectrometer was operated in a data-dependent mode with a single Orbitrap MS scan (375-1500 *m/z* and a resolution of 120k) was acquired every 3 seconds followed by Orbitrap MS/MS higher energy collisional dissociation (HCD) scans of precursors (stepped NCE 25,30,40%, with a maximal injection time of 80 ms with the Automatic Gain Control set to 400% and the resolution to 30k).

Identification and LFQ analysis were accomplished using MaxQuant (v1.6.17.0)[52] using the in-house generated proteome of *P. onubensis* strain Merri (RES21-00833.faa) allowing for oxidation on methionine. The LFQ and "Match Between Run" options were enabled to allow comparison between samples. The resulting data files were processed using Perseus (v1.4.0.6)[53] to compare the growth conditions using Student's t-tests as well as Pearson correlation analyses. For LFQ comparisons independent replicates were grouped and missing values imputed based on the observed total peptide intensities with a range of 0.3σ and a downshift of 1.8σ.

Cell free lysate analysis

P. onubensis strain Merri cells were grown in vitamin-supplemented M9 minimal media (250 mL) containing 5 mM SFuc. Cell pellets were collected at mid log phase by centrifugation at 4000 × *g* for 10 min at 5 °C washed thrice with cold PBS buffer (150 mM), thrice with cold water, and frozen on liquid nitrogen. Cells were defrosted and resuspended in 100 mM Tris-HCl buffer (pH 7.0, 100 mM) and lysed by sonication with 30% powder in 3 x 30 sec intervals. Samples were cooled on ice for 1 minute in-between cycles. Samples were clarified by centrifuge at 25,000 × *g* for 10 min at 5 °C and the cell lysate was used without further treatment.

Sulfofucose isomerase assay: Potential SFuc isomerase reactions were monitored by ^{1}H NMR spectroscopy using a 500 MHz Bruker NEO system. A 700 μL sample containing 50 μL of strain Merri cell lysate, 50 units of recombinant shrimp alkaline phosphatase (Sigma), in 50 mM sodium phosphate, 150 mM NaCl (pH 7.01) was incubated for 20 min. SFuc was added to a final concentration of 10 mM and samples were incubated for 24 h at 25 °C. Reactions were quenched by heating at 80 °C for 3 min, concentrated *in vacuo* and the residue was dissolved in D_2O for ^{1}H NMR analysis.

Sulfofucose dehydrogenase assay: In a 96-well plate, 200 μL solutions contained 1.1 mM SFuc, 6.5 mM $MgCl_2$, and 0.5 mM NAD^+ or $NADP^+$ in Tris-HCl (100 mM, pH 7.5). Reactions

were initiated by the addition of 20 μL of strain Merri cell lysate. Absorbance was measured at 340 nm for 15 mins.

Metabolomic analysis of *P. onubensis* strain Merri

Three independent replicates of *P. onubensis* strain Merri were grown to mid log phase in vitamin-supplemented M9 minimal media with either 5 mM SFuc or glucose as the sole carbon source. Samples were grown to the following A_{600} values: SFuc: 1.126, 1.130, 1.123 Glucose: 1.06, 1.01, 1.10. Cultures were centrifuged (4000 rpm, 30 cm rotor, 10 min, 5 °C) and cell pellets were washed three times with PBS buffer (10 mM, NaCl 150 mM, pH 7.0) and then three times with deionised water. Cells were snap frozen with liquid nitrogen prior to extraction.

____ and _____ were added to the frozen cell pellets and cells were lysed by sonication followed by mixing at XXX. Samples were centrifuged at 4,000 × *g* for 45 min and the aqueous phase was separated and evaporated to dryness. The residues were reconstituted in XXX and the metabolome of *P. onubensis* strain Merri was monitored by ion chromatography-mass spectrometry (IC-MS). IC-MS analysis was conducted on a ThermoFisher Scientific LC ID-X Orbitrap equipped with a Dionex IonPac AS11-HC column (ThermoFisher Scientific).

The IC used solvent A, ; solvent B: . The elution gradients were from X% B to X% B over 45 min with flow rate of 0.50 ml min^{-1} and injection volume of 1 µL. The mass spectrometer was operated with selected ion monitoring in negative ionization mode to monitor for pyruvate. Ion chromatograms where then extracted and data analysed using Thermo Scientific FreeStyle using 1.8 SP.

Cloning, protein expression and purification of SfcD, SfcE, SfcF, and SfcH.

The codon-harmonized protein-coding regions of SfcD, SfcE, SfcF, and SfcH genes from *P. onubensis* strain Merri (MDP0926279, MDP0926278, MDP092627 and MDP0926275 respectively) were synthesized and cloned between *Nco*I and *Xho*I in pET-28a with an N-terminal hexahistidine tag, followed by the TEV protease recognition site. For protein production, each plasmid encoding SfcE, SfcF, and SfcG was transformed in *E. coli* BL21(DE3) and expressed. The cells were grown in lysogeny broth with 50 mg/ml kanamycin at 37 °C and when they reached an A_{600} of 0.6–0.8 were induced with 0.4 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG). After incubation at 18°C for 16 h, cells were

harvested by centrifugation at 6000 × *g*. Due to low solubility of the SfcD protein, the plasmid encoding SfcD was expressed in ArcticExpress (DE3) (Agilent). The transformed cells were grown in LB media at 30 °C and when they reached an A_{600} of 0.6–0.8 were induced with 0.1 mM IPTG and incubated at 11 °C for 24 h then harvested as above. The cell pellets were resuspended in buffer A (20 mM Tris·HCl, 500 mM NaCl, 25 mM imidazole, pH 7.5), disrupted using a sonicator (Omni Sonic Ruptor 400), and clarified by centrifugation at 20000 × *g*. The soluble fraction was applied to Ni Sepharose 6 Fast Flow (Cytiva) resin and the bound protein was eluted with 300 mM imidazole in buffer A. Pooled fractions were concentrated and buffer exchanged into 20 mM Tris·HCl, 200 mM NaCl, pH 7.5.

Analysis of the products of reactions catalysed by SfcD, SfcE, SfcF and SfcH

SFuc dehydrogenase (SfcH): A 0.5 mL solution contained SFuc (2 mM), and either NAD^+ (3 mM) or $NADP^+$ (3 mM) in Tris-HCl buffer (50 mM, pH 8.0). SfcG (1.67 µM) was added to initiate the reaction and the solution was incubated at 25 °C for 24 h, then halted by heating at 80 °C for 3 min. Samples were frozen until LC-MS analysis.

SGalL lactonase (SfcD): A 0.3 mL solution contained 100 µL of the final SfcG solution from above, $CaCl_2$ (0.5 mM), and $ZnCl_2$ (0.5 mM) in Tris-HCl buffer (50 mM, pH 8.0). The reaction was initiated by adding SfcG (2.67 µM) and the solution was incubated at 25 °C for 24 h, then halted by heating at 80 °C for 3 min. Samples were frozen until LC-MS analysis.

SGal dehydratase (SfcF): A 0.5 mL solution contained chemically-synthesized SGal (2 mM) and $MgCl_2$ (1 mM) in Tris-HCl buffer (50 mM, pH 8.0). The reaction was initiated by adding SfcF (1.11 µM) and incubated at 25 °C for 24 h, then halted by heating at 80 °C for 3 min. Samples were frozen until LC-MS analysis.

KDSGal aldolase (SfcE): A 0.5 mL solution contained 100 µL of the final SfcF solution from above, and either $MgCl_2$ (1 mM), $MnCl_2$ (1 mM), or $ZnCl_2$ (1 mM) in Tris-HCl buffer (50 mM, pH 8.0). The reaction was initiated by adding SfcE (1.68 µM) and incubated at 25 °C for 24 h, then halted by heating at 80 °C for 3 min. Samples were frozen until LC-MS analysis.

LC-MS analysis: 20 µL of each sample was diluted with 60 µL of 1:1 ACN/H_2O and used for LC-MS analysis. An Aglient 6490 QQQ mass spectrometer equipped with an Aglient LC module was used for HPLC–MS analysis. A SeQuant ZIC-HILIC 3.5 µm 100 Å 150 x 2.1 mm column was used at room temperature. HPLC used solvent A, 10 mM NH_4OAc in 10% acetonitrile; solvent B: acetonitrile. The elution gradients were from 90% B to 40% B over 50 min; then 40% B for 2 min; back to 90% B over 1 min with flow rate of 0.50 ml min^{-1} and injection volume of 1 µL. The mass spectrometer was operated in negative ionization mode.

Prior to analysis, the sensitivity for each multiple reaction monitoring (MRM)-MS^2 transition was optimized for each analyte.

Retention times and MS^2 fragmentation patterns of the authentic reference standards were as follows:

For SFuc: Retention time: 25.3 and 25.8 min. ESI–MS/MS *m/z* of $[M-H]^-$ 243, product ions 283, 153, 123, 81.

For SGalL: Retention time: 13.1 min. ESI–MS/MS *m/z* of $[M-H]^-$ 241, product ions 197, 179, 161, 81.

For SGal: Retention time: 27.0 min. ESI–MS/MS *m/z* of $[M-H]^-$ 259, product ions 241, 213, 197, 183, 179, 161, 81.

For KDSGal: Retention time: 22.6 min. ESI–MS/MS *m/z* of $[M-H]^-$ 241, product ions 223, 205, 179, 161, 81.

For SLA: Retention time: 2.1 min. ESI–MS/MS *m/z* of $[M-H]^-$ 153, product ions 81, 71.

IC-MS analysis: Pyruvate was detected by IC-MS using a ThermoFisher Scientific LC ID-X Orbitrap equipped with a Dionex IonPac AS11-HC column (ThermoFisher Scientific). The IC used solvent A, ; solvent B: . The elution gradients were from X% B to X% B over 45 min with flow rate of 0.50 ml min^{-1} and injection volume of 1 µL. The mass spectrometer was operated with selected ion monitoring in negative ionization mode to monitor for pyruvate $[M-H]^-$ *m/z* 87. Ion chromatograms where then extracted and data analysed using Thermo Scientific FreeStyle using 1.8 SP.

Bioinformatic analysis

The protein sequence of SfcF (UniProt accession code: Q5LUS2) from *P. onubensis* strain Merri was used as a query for BLAST search in the Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST webtool).[14] This search retrieved 1001 sequences with ≥ 62.2% identity. Sequence similarity networks (SSNs) were generated at different alignment scores (AS = 120, 140, 160, 180, 200) and visualised in Cytoscape[15] 3.10.3 using the yFiles Organic Layout, with nodes representing individual sequences.

Genome neighborhoods were analyzed using the Enzyme Function Initiative-Genome Neighborhood Tool (EFI-GNT), with nodes color-coded according to colocation with SfcD, SfcE, SfcF, SfcH homologues. Analysis at AS = 160 identified two clusters containing 7

sequences with SfcF and at least one homologue of SfcD, SfcE or SfcH.

Further analysis of the SSN at AS = 160 using EFI-GNT retrieved neighboring genes within a ±10 open reading frame window.

Data Availability

The assembled genome has been deposited at the NCBI (GenBank accession: Bioproject PRJNA993841), release date Aug 7, 2023.

The mass spectrometry proteomics data has been deposited in the Proteome Xchange Consortium[54] via the PRIDE partner repository (https://proteomecentral.proteomexchange.org/cgi/GetDataset) with the data set identifier: PXD043488 Username: reviewer_pxd043488@ebi.ac.uk Password: DXxRoG1Y.

Supplementary material

Supplementary Figures S1-S6

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

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Table 1. Key features of the *Paracoccus onubensis* strain Merri (GenBank accession: Bioproject PRJNA993841) draft genome assembly.

	Strain Merri
Genome size	5,082,950
Number of contigs	74
GC %	60.1
Number of ORFs	4913
Number of putative genes	4966
Number of putative tRNA	49
Number of putative rRNA	3
Number of putative tmRNA	1
Number of genes assigned a function (%)	2847 (57%)

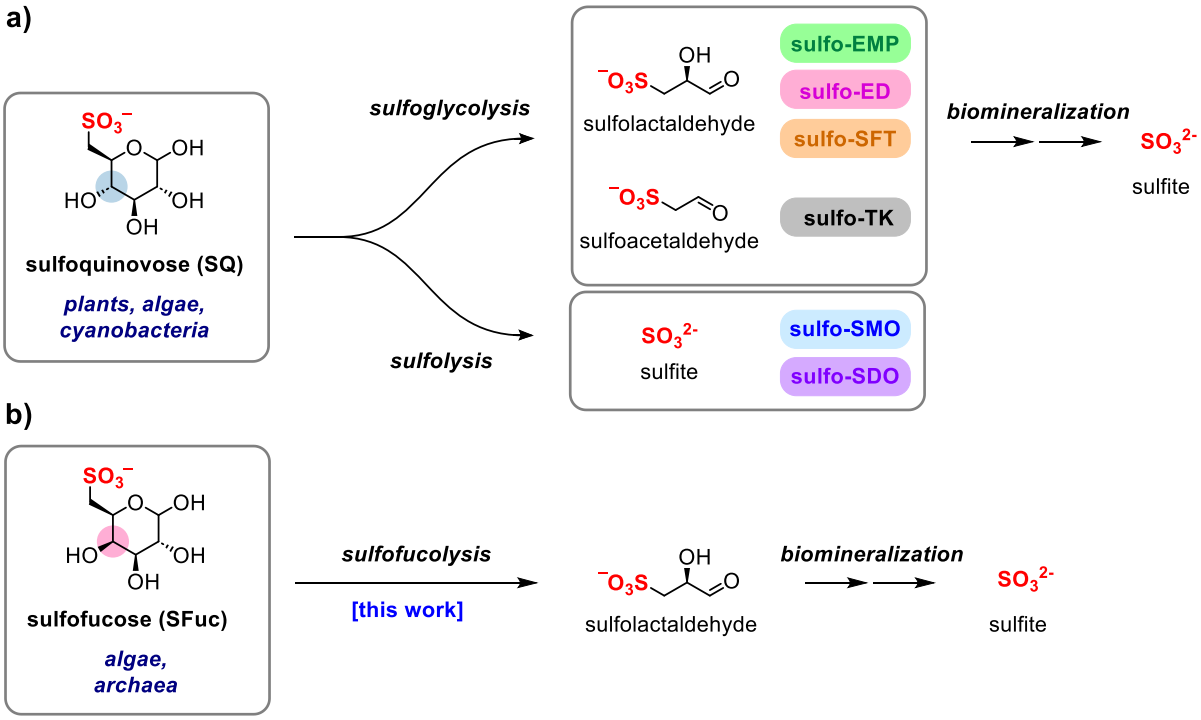

Figure 1. Breakdown of the sulfosugars sulfoquinovose and sulfofucose. (a) Degradation of sulfoquinovose to sulfoacetaldehyde (SA) or sulfolactaldehyde (SLA) by sulfoglycolysis (sulfoglycolytic Embden−Meyerhof−Parnas (sulfoEMP); sulfoglycolytic Entner−Doudoroff (sulfo-ED); sulfoglycolytic sulfofructose transaldolase (sulfo-SFT); sulfoglycolytic transketolase (sulfo-TK) pathways) or sulfolysis to sulfite (sulfolytic SQ monooxygenase (sulfo-SMO); and sulfolytic SQ dioxygenase (sulfo-SDO) pathways). (b) Sulfofucose has been identified in glycoconjugates from algae and archaea, this work discloses a two-phase sulfofucolysis−biomineralization breakdown pathway.

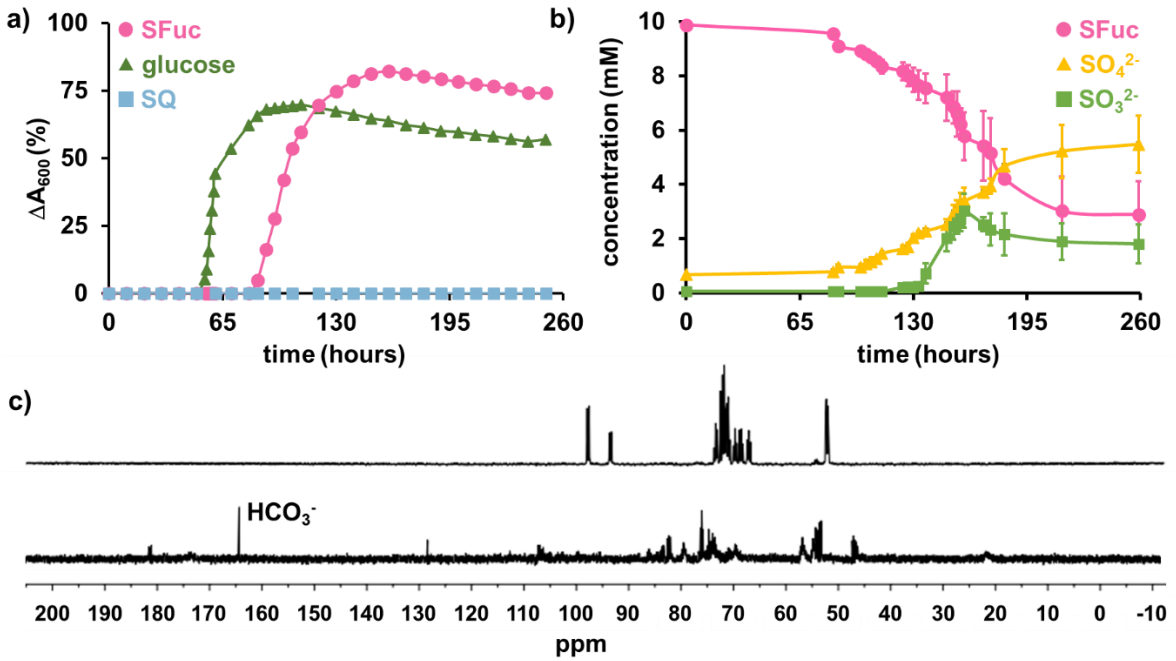

Figure 2. *P. onubensis* strain Merri grows on SFuc as a sole carbon source. (a) Growth of strain Merri on glucose, SFuc, and SQ. Turbidity was measured at A_{600} nm, and cells were cultured in M9 minimal media containing 5 mM of each carbon source, supplemented with a vitamin mix and trace metals. (b) Strain Merri uses SFuc as a carbon source and produces sulfite. Independent replicates show the change in concentration of SFuc (open circles), sulfite (open squares) and of sulfate (closed triangles) throughout growth of strain Merri on SFuc. Analyses were performed in duplicate, and errors are reported as standard deviations. (c) ^{13}C NMR spectra of M9 minimal media containing 10 mM $^{13}C_6$-SFuc (top) and after 5 days of growth of strain Merri at the mid-log phase (bottom).

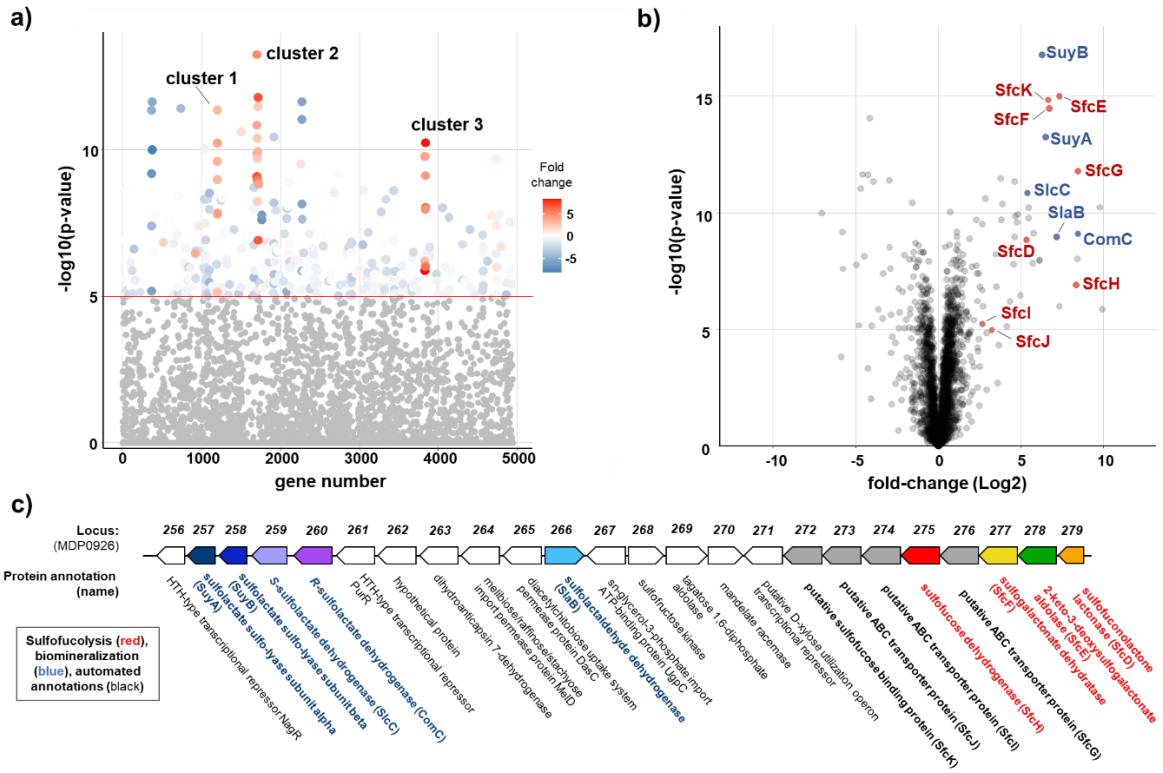

Figure 3. Genome and proteome analysis reveals a gene cluster for organosulfonate metabolism in *P. onubensis* strain Merri. Cells were cultured in vitamin-supplemented minimal media with either SFuc or glucose, and analyzed via comparative proteomics. (a) Manhattan plot highlighting three differentially expressed gene clusters. (b) Volcano plot showing proteins identified in the SFuc degradation pathway (red, SfcD, SfcE, SfcF, SfcG); blue, SlaB, SuyAB). (c) Gene neighborhood diagram of "cluster 2", associated with organosulfonate metabolism. Genes annotated in red are putative sulfofucolytic genes; those in blue are putative biomineralization genes. Genes in plain black text represent automated genome annotations; bold black text are a putative SFuc binding protein and ABC transporter cassette.

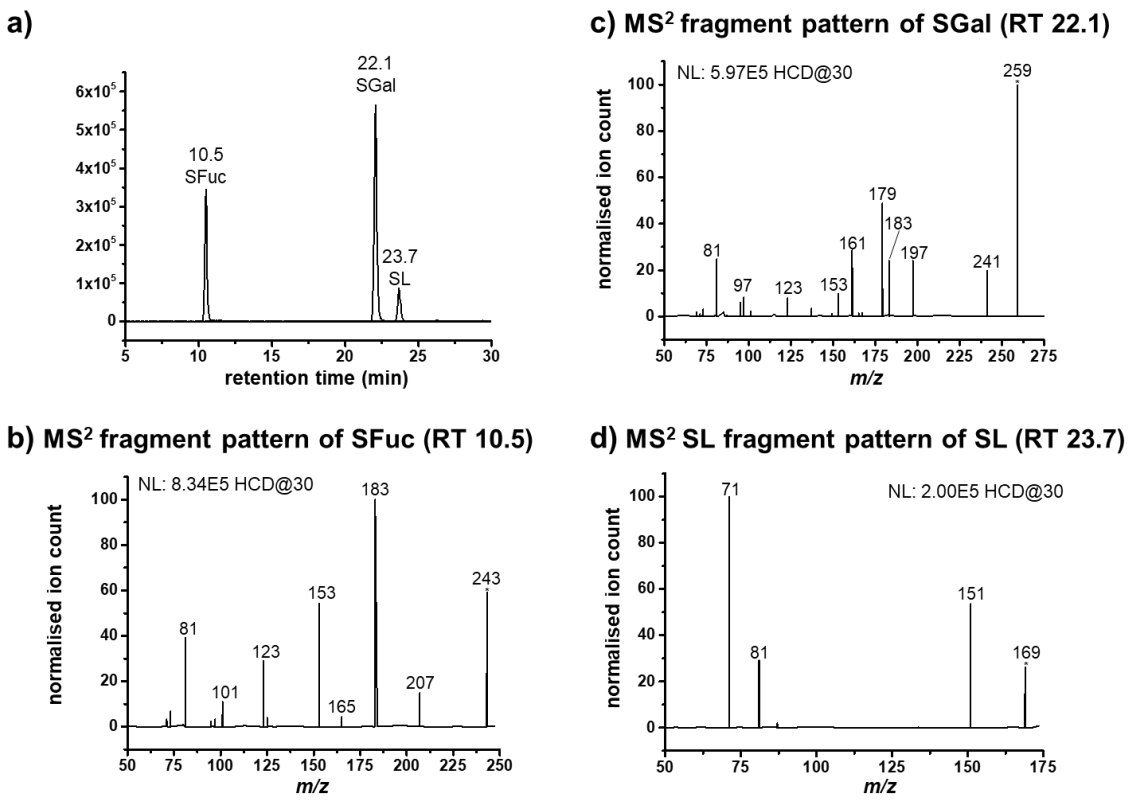

Figure 4. Metabolomic analysis of *P. onubensis* strain Merri grown on sulfofucose. Strain Merri cells were grown on SFuc until mid-log phase, harvested, lysed and metabolites were extracted from cells. Targeted LC-IC-MS was employed, and potential sulfofucolytic metabolites were screened for using SIM scans. (a) Extracted ion chromatograph showing key organosulfur metabolites within *P. onubensis* cells. (b) MS^2 fragment pattern of SFuc. (c) MS^2 fragment pattern of SGal. (d) MS^2 fragment pattern of SL

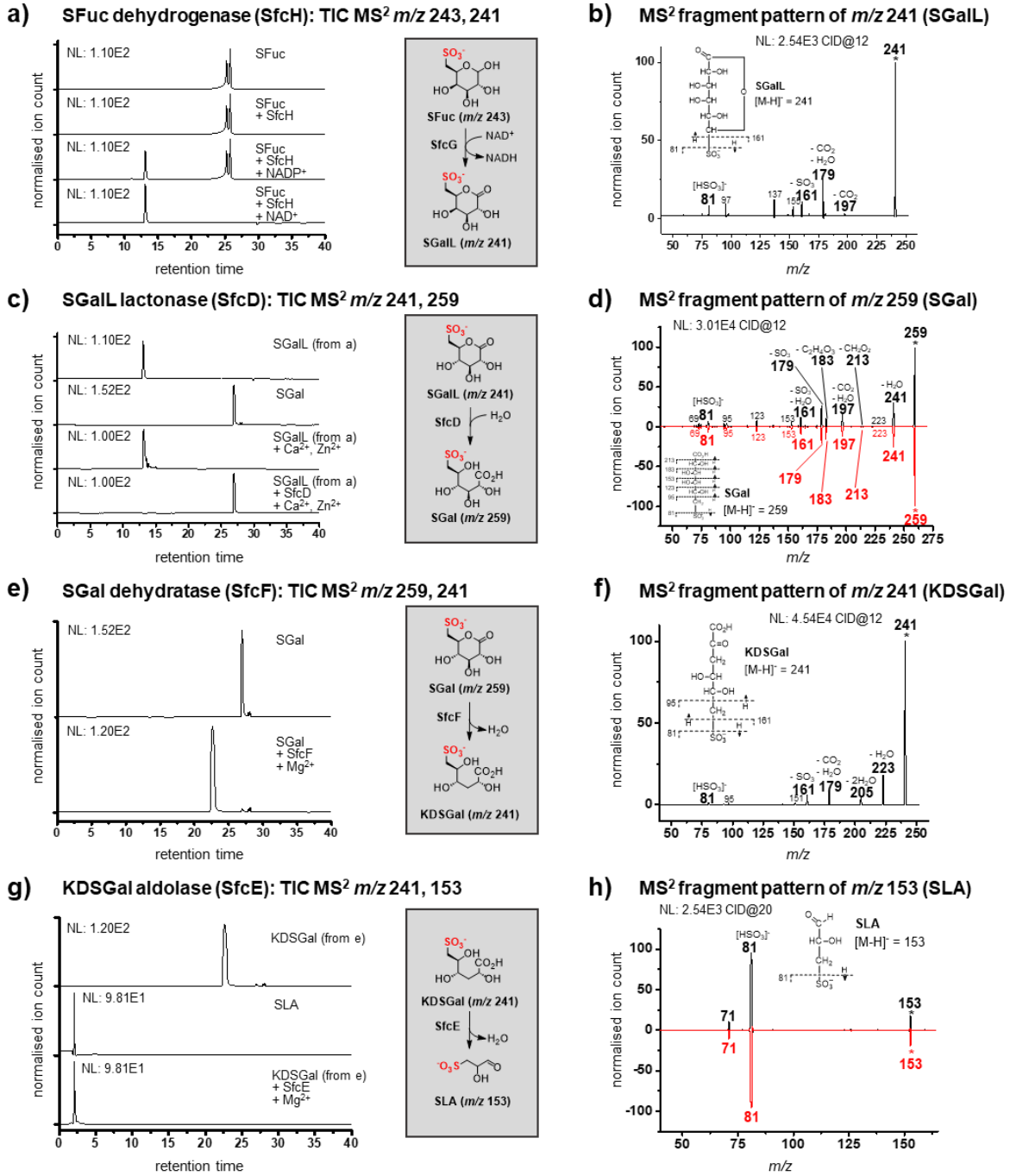

Figure 5. In-vitro reconstitution of individual steps of the sulfofucolytic ED pathway. The enzymatic conversions of SFuc to SGalL, SGalL to SGal, SGal to KDSGal, and KDSGal to SLA, catalyzed by SfcH, SfcD, SfcF and SfcE, respectively. Targeted MRM-MS analysis was used to monitor analytes. (a) Ion chromatograms of SFuc (*m/z* 243) and assays containing SFuc with SfcH and NAD(P)$^+$. (b) MS^2 fragment pattern of SGalL *m/z* 241. (c) Ion chromatograms of assays containing SGalL treated with SfcD, $CaCl_2$, and $ZnCl_2$. (d) MS^2 fragment pattern of experimentally obtained SGal (black) and chemically synthesized (red) *m/z* 259. (e) Ion chromatograms of SGal and assays containing SGal with SfcF and $MgCl_2$. (f) MS^2 fragment pattern of KDSGal *m/z* 241. (g) Ion chromatograms of assays containing

KDSGal treated with SfcE and $MgCl_2$. (h) MS^2 fragment pattern of experimentally obtained SLA (black) and chemically synthesized (red) *m/z* 153. Pyruvate, the second product of the SfcE reaction, was confirmed by ion chromatography-mass spectrometry.

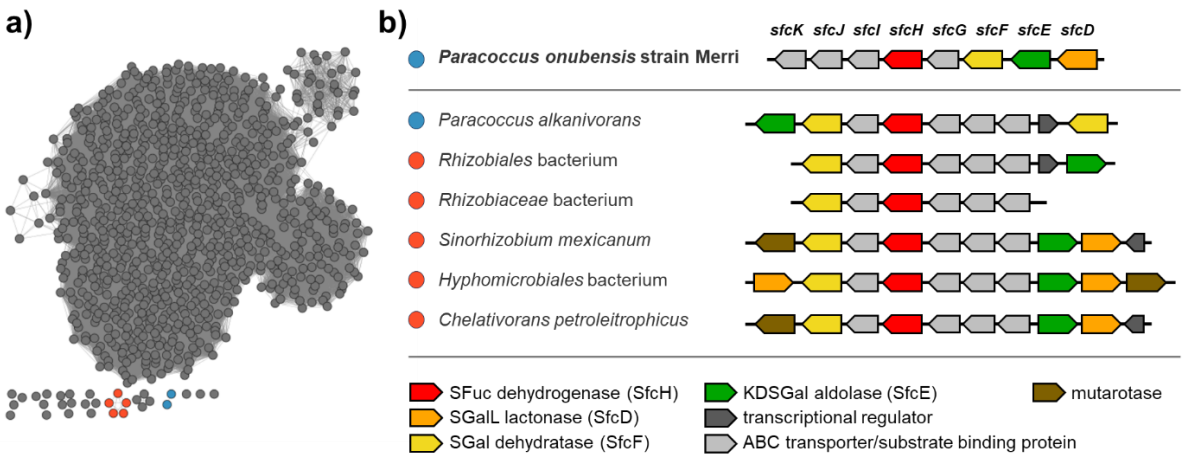

Figure 6. Proposed sulfofucolytic ED pathway gene clusters distributed across assorted proteobacteria. (a) Sequence similarity network (SSN) of SGal dehydrogenase at an alignment score of 160. Colored clusters represent organisms harboring gene clusters containing homologues of sulfofucolytic ED pathway genes *sfcD*, *sfcE*, *sfcF* and *sfcH*. (b) Gene clusters encoding sulfofucolytic ED pathways. Colored circles at left correspond to colored clusters in part (a). UniProt protein accession codes for SfcF homologues are as follows: *Paracoccus onubensis* strain Merri (A0A418T7C2), *Paracoccus alkanivorans* (A0A3M0MGN3), *Rhizobiales* bacterium (A0A1M2Z1S0), *Rhizobiales* bacterium (A0A522R0Q2), *Sinorhizobium mexicanum* (A0A859QZ22), *Hyphomicrobiales* bacterium (A0A8I1P1S0), *Chelativorans petroleitrophicus* (A0A9X2XAH4).

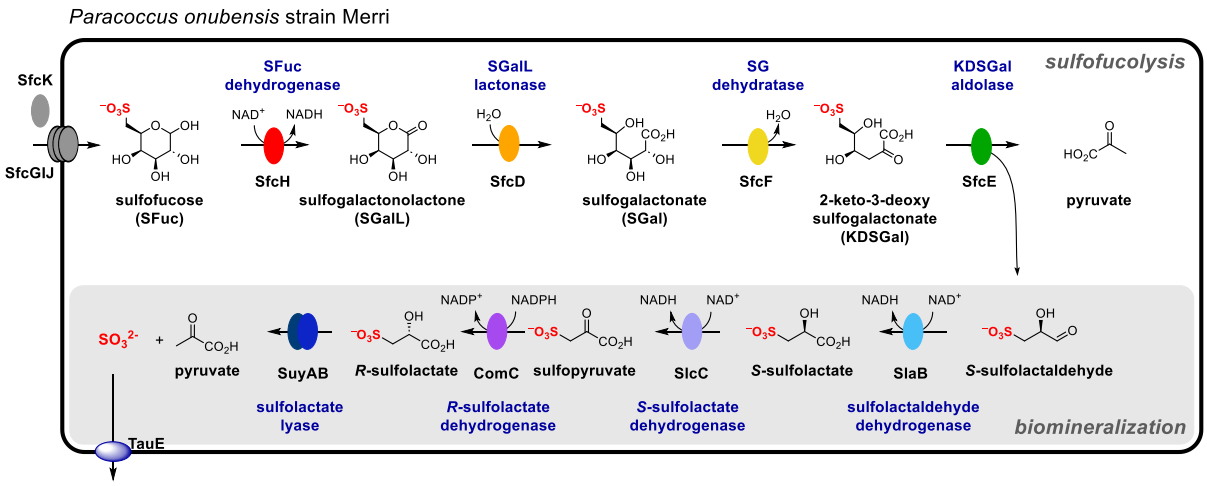

Figure 7. Proposed sulfofucolytic and biomineralization pathway within *P. onubensis* strain Merri. The identified sulfofucolytic steps involve SFuc dehydrogenase (SfcH) which facilitates the conversion of SFuc to SGalL. SGalL lactonase (SfcD) which facilitates the hydration of SGalL to SGal. SGal is selectively dehydrated to KDSGal by SGal dehydratase (SfcF) and KDSGal is lysed to yield SLA and pyruvate by KDSGal aldolase (SfcE). The resultant SLA then undergoes a biomineralization process in which *S*-SLA is oxidised to *S*-SL by SLA dehydrogenase (SlaB), racemized by SlcC/ComC to *R*-SL, and sulfite is liberated by SL lyase (SuyAB).