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(Citation)

Fermentation, 11(2):45

(Issue Date)

2025-02

(Resource Type)

journal article

(Version)

Version of Record

(Rights)

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(URL)

<https://hdl.handle.net/20.500.14094/0100493558>



Article

Lipid Production in *Streptomyces jeddahensis* Is Enhanced by Glucose and Fatty Acid Derivatives, with Temperature Variations Influencing Gene Expression and Biosynthesis

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Abstract: The potential of *Streptomyces jeddahensis* for lipid production was investigated, focusing on its ability to utilize various carbon sources under different temperature conditions. Glucose and fatty acid derivatives (sodium hexanoate, sodium octanoate, and sodium decanoate) were tested at 30 °C, 37 °C, and 45 °C to optimize growth and lipid biosynthesis. The results revealed that 37 °C was the optimal temperature for lipid accumulation, with sodium octanoate leading to the highest lipid content. While growth declined at 45 °C, significant lipid production persisted, indicating an adaptive stress response. Gene expression analysis identified upregulation of key metabolic genes, including STSP_03080 (*DGAT*) and STSP_67970 (*fabD*), under thermal stress, highlighting enhanced fatty acid biosynthesis pathways. This study demonstrates the capacity of *S. jeddahensis* to efficiently convert medium-chain fatty acids into lipids, supporting its use as a sustainable and scalable platform for biodiesel production and other lipid-based industrial applications.

Keywords: *Streptomyces jeddahensis*; fatty acid derivatives; sodium hexanoate; sodium octanoate; sodium decanoate



Academic Editors: Kaloyan Petrov and Penka Petrova

Received: 25 December 2024

Revised: 18 January 2025

Accepted: 19 January 2025

Published: 21 January 2025

Citation: Apriliana, P.; Kahar, P.; Rachmadona, N.; Restu, W.K.; Kondo, A.; Ogino, C. Lipid Production in *Streptomyces jeddahensis* Is Enhanced by Glucose and Fatty Acid Derivatives, with Temperature Variations Influencing Gene Expression and Biosynthesis. *Fermentation* **2025**, *11*, 45. <https://doi.org/10.3390/fermentation11020045>

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1. Introduction

The increasing global demand for sustainable and renewable energy sources has driven significant interest in microbial lipid production as a promising avenue for developing eco-friendly alternatives to fossil-derived oils. Among the oleaginous microorganisms, certain species of *Streptomyces* have gained attention due to their ability to accumulate lipids, particularly triacylglycerols (TAGs), which serve as precursors for biodiesel and various industrial bioproducts. These microorganisms can produce lipids constituting 20% to 80% of their biomass under nitrogen-limiting conditions [1–4], making them a viable candidate for scalable lipid production. Oleaginous microbes, such as *Rhodococcus opacus* and *Cryptococcus curvatus*, have been extensively studied for their ability to accumulate TAGs [2,5]. For instance, *Rhodococcus* is known for its ability to degrade a wide range of hydrocarbons and accumulate lipids through its versatile central metabolism, which includes pathways like glycolysis, gluconeogenesis, and the tricarboxylic acid cycle [6,7]. These insights highlight the distinctive metabolic adaptations of *S. jeddahensis* that contribute to its potential for biodiesel

production. The process of TAG formation in *S. jeddahensis* is schematically illustrated in Figure 1.

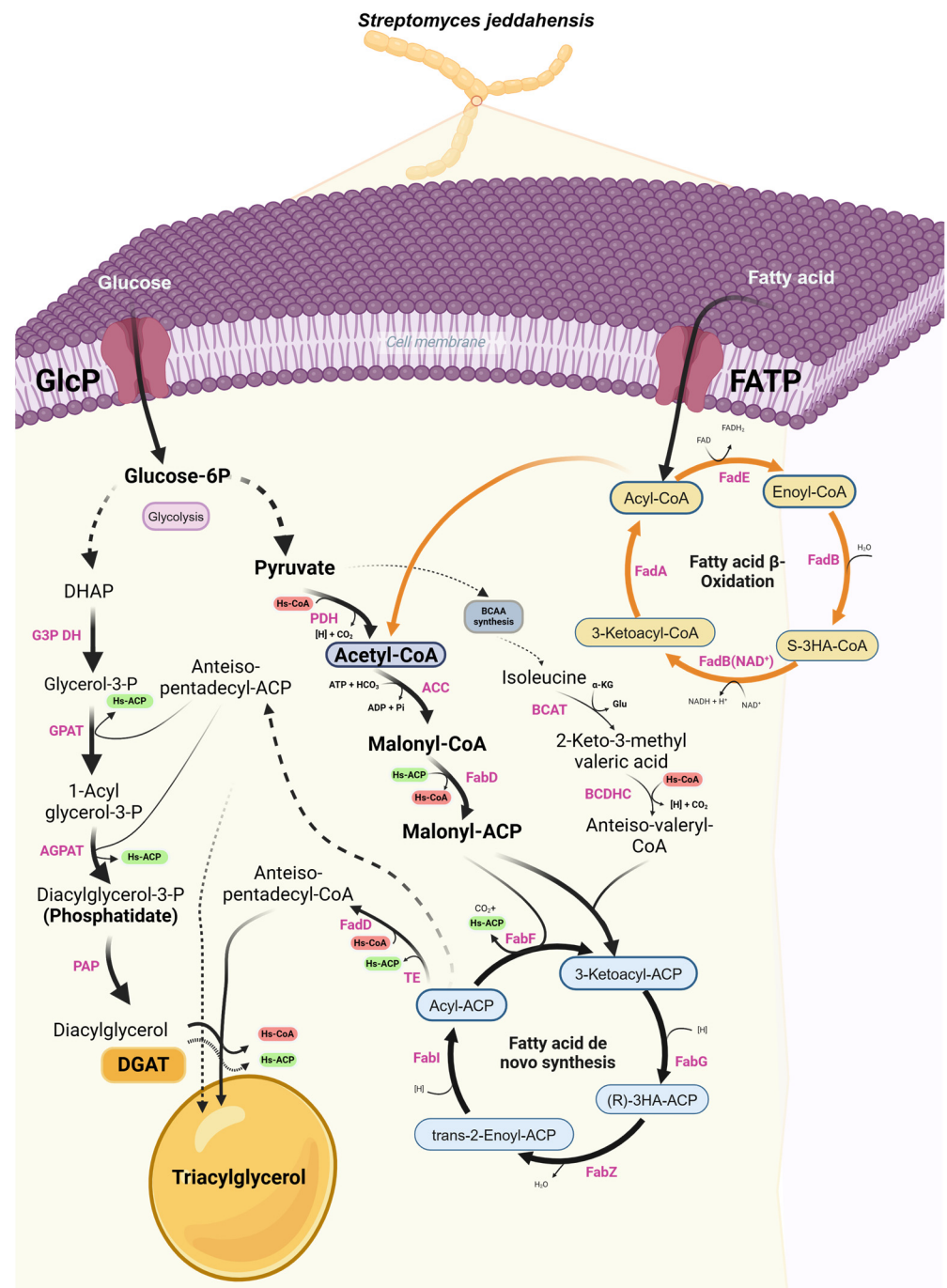


Figure 1. Schematic representation of the metabolic pathway rewiring for lipid production in *S. jeddahensis*.

This capability enables them to transform low-cost, renewable feedstocks into high-value lipids, highlighting their economic and environmental advantages [7,8]. Despite the substantial research focus on these organisms, *Streptomyces* species remain relatively underexplored. However, they demonstrate considerable potential for lipid production, particularly in synthesizing branched-chain fatty acids and high-valued lipids when cultivated with carbon sources such as glucose or cellobiose. One of the notable members of this genus, *S. jeddahensis*, stands out for its metabolic diversity and exceptional lipid accumulation capability, producing lipids up to 64% (*w/w*) of its dry cell weight. This

species can grow under a wide range of temperature conditions, up to 50 °C, which is advantageous for industrial-scale production. These features make it a novel and promising host for the biotechnological development of lipid-based products [9].

The process by which *Streptomyces* accumulates lipids is influenced by multiple factors, with two of the most crucial being the carbon source [10] and growth temperature [11,12]. Carbon sources are essential because they provide energy for microbial growth and serve as precursors for lipid biosynthesis. While glucose is commonly used as a carbon source in many microbial cultures [13,14], certain fatty acids have been shown to promote lipid accumulation in various microorganisms [15]. These fatty acids can directly impact metabolic pathways that lead to lipid formation, acting both as an energy source and as precursors for fatty acid biosynthesis. In particular, medium-chain fatty acids, like sodium hexanoate, are known to activate specific metabolic pathways, such as β -oxidation, which enhances lipid storage in many oleaginous microorganisms.

This study focuses on optimizing lipid production in *S. jeddahensis* using glucose and fatty acid derivatives, including sodium hexanoate, sodium octanoate, and sodium decanoate, as alternative carbon sources. Glucose is widely used due to its abundance, cost-effectiveness, and efficient metabolism through glycolysis to acetyl-CoA, a precursor for fatty acid synthesis [16]. Medium-chain fatty acids, such as hexanoate, octanoate, and decanoate, activate specific biosynthetic pathways, including β -oxidation, and enhance lipid accumulation [17–21]. By leveraging these substrates, this research aims to elucidate the metabolic potential of *S. jeddahensis* and its ability to serve as a sustainable platform for lipid production.

2. Materials and Methods

2.1. Medium, Bacterial Strains and Maintenance

The glucose and all other reagents were purchased from Sigma-Aldrich (Tokyo, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan). Sodium hexanoate, sodium octanoate, and sodium decanoate used in this study were purchased from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). The oleaginous microorganism used in the experiments was *Streptomyces jeddahensis* DSM 101878 sourced from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. The strains were maintained in TSB agar medium containing (g/L) peptone from casein, 17; peptone from soymeal, 3; D(+)-glucose, 2.5; NaCl, 5; K₂HPO₄, 2.5; and agar, 20.

2.2. Cultivation

In this study, the accumulation of lipids within *S. jeddahensis* cells was evaluated following a modified protocol adapted from Sass P et al. [22] conducted in 250 mL Erlenmeyer flasks. The experimental setup began by introducing approximately 1 cm² *S. jeddahensis* into 45 mL of tryptic soy broth (TSB) liquid medium. This initial culture was incubated at 30 °C for 2 days at 160 rpm, serving as the pre-seed culture. For the seed culture, 5 mL of pre-seed culture was transferred into 50 mL of TSB medium and incubated under the same conditions for 18 h. The fermentation phase seed culture with OD₆₀₀ = 1 was transferred into 60 mL of mineral salt medium (MSM) to facilitate the accumulation of triacylglycerols (TAGs). A range of carbon sources were examined, including glucose, sodium hexanoate, sodium octanoate, and sodium decanoate. MSM with reduced nitrogen content contains (g/L) urea, 0.25; yeast extract, 0.16; CaCl₂, 0.02; KH₂PO₄, 1.52; MgSO₄·7H₂O, 0.52; and Na₂HPO₄, 4, supplemented with different carbon sources: glucose, sodium hexanoate, sodium octanoate, and sodium decanoate at 2.5 g/L of each. Cultivation was performed on an orbital shaker at 160 rpm for 48 h. At different temperatures (30 °C, 37 °C, and 45 °C),

cells were harvested by centrifuge at $10,000\times g$ for 5 min at 4 °C and washed with MilliQ water. The cells were frozen at −20 °C overnight and freeze-dried for 48 h.

2.3. Dry Cell Weight Measurement

Cells from each culture were collected by centrifugation at $5000\times g$ for 5 min. The wet weight of the cell pellets was determined. Subsequently, the cell pellets were washed with sterile water. After washing, the cell pellets were reweighed. The collected cell pellets were then subjected to freeze-drying for 48 h. The cell dry weight was recorded after freeze-drying before the lipid contents were extracted.

2.4. Lipid Contents

To prepare bacterial cell samples for lipid extraction, 30 mg of freeze-dried cells were combined with 0.6 mm zirconia beads in a 2 mL Eppendorf tube. The cell mixture was treated with lysozyme (5 mg/mL) to lyse the bacterial cell walls and incubated for 30 min. After incubation, the samples were freeze-dried again. Chloroform (1 mL) and methanol (0.5 mL) were added to the mixture. The samples were disrupted using a Shake Master Neo, operating at a speed of 1500 rpm for 5 min thrice. This disruption process was performed in three consecutive cycles to ensure thorough mixing. Following the disruption, the samples were centrifuged at 14,000 rpm for 5 min to separate the supernatant from the pellet. The supernatant was carefully removed, and 1.5 mL MilliQ water was added to the pellet. The samples underwent another round of disruption under the same conditions as previously described. After the second disruption, the supernatant was discarded, and the remaining sample was dried in a block incubator set at 60 °C for 3 days. The final dry weight of the samples was recorded to calculate the lipid content by comparing it with the initial weight of the freeze-dried bacterial cells.

2.5. Fatty Acid Methyl Ester (FAME) Analysis

A gas chromatograph-mass spectrometer (GCMS-QP2020 NX, Shimadzu Corporation, Kyoto, Japan) was employed to analyze the cellular fatty acid composition. Chromatographic separation was achieved on a DB-23 column (60 m $\times$ 0.25 mm $\times$ 0.15 μ m; Agilent Technologies, Palo Alto, CA, USA). The temperature program for the analysis commenced at 50 °C, which was maintained for 2 min, followed by a ramp of 25 °C per minute to 175 °C, and subsequently, a slower ramp of 4 °C per minute to a final temperature of 230 °C. Approximately 30 mg of freeze-dried bacterial cells were accurately measured and combined with 0.6 mm zirconia in a 2 mL microcentrifuge tube. The mixture was then treated with 500 μ L of Reagent A and Reagent B, as specified by the fatty acid methylation kit's instructions (Fatty Acid Methylation Kit, Nacalai Tesque, Kyoto, Japan). Cell lysis was achieved by agitating the mixture with a Shake Master Neo at 1500 rpm for three cycles of 5 min each. Following lysis, the sample was incubated at 37 °C for 60 min on a heat block to allow for the methylation of glycerides and sterol esters.

In a subsequent step, 500 μ L of Reagent C (from the same kit) was added to the mixture, which was then subjected to the same cell disruption protocol followed by a 20 min incubation at 37 °C to promote the methylation of free fatty acids. After the methylation process, 1 mL of the kit's extraction solvent was added. The mixture was centrifuged at 14,000 rpm for 1 min, and the clear supernatant was transferred into a new microcentrifuge tube. For gas chromatography-mass spectrometry (GC-MS) analysis, 90 μ L of this supernatant was combined with 10 μ L of an internal standard (IS) solution in a glass vial. The IS was prepared by diluting 10 μ L of methyl benzoate in 990 μ L of hexane, yielding a final volume of 1 mL.

2.6. Bodipy Staining for Qualifying Lipid Inside the Cells

To visualize intracellular triacylglycerol lipids, BodipyTN 493/503 (4,4-difluoro-5,7-dimethyl-4-bora-3a, 4a-diaza-s-indacene) obtained from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA) was utilized for staining. The dye was initially dissolved in ethanol to prepare a 5 mg/mL stock solution, which was subsequently stored at -20°C in the absence of light. Cell cultures (approximately 100 μL each) were centrifuged at 13,000 rpm for 30 s, and the supernatant was discarded. The cell pellets were washed with 100 μL phosphate-buffered saline (PBS) at pH 7. Following supernatant removal, the pellets were resuspended in 100 μL of the same buffer. To this suspension, 1 μL of Bodipy staining solution was mixed thoroughly. The stained cells were incubated at room temperature for 10 min. A 10 μL sample of the stained cell suspension was placed on a microscope slide for observation with a Keyence BZ-X810 fluorescence microscope (Osaka, Japan).

2.7. qPCR Analysis of Gene Transcript Levels in *S. jeddahensis* Strain

qRT-PCR assays were used to investigate whether the gene was expressed. Firstly, *S. jeddahensis* strains were grown to $\text{OD}_{600} = 1$. cDNA from DNase-treated 0.5 μg of total RNA was synthesized in a 20 μL reverse transcription reaction containing random hexamers and SuperScript III reverse transcriptase (Invitrogen). All real-time PCR reactions were performed with a Bio-Rad iCycler (iQ5 system). All primers used are listed in Table 1. Changes in gene expression were calculated using the formula $2^{-\Delta\Delta\text{Cq}}$, as previously described [23]. Gene expression data for each run were organized into a matrix labeled as “countData,” where rows represented genes and columns corresponded to samples. Metadata containing information about the samples, referred to as “colData,” included details on sample conditions and biological replicates, which were numbered (1, 2, or 3). Differentially expressed genes (DEGs) were identified by analyzing expression level variations between two groups of samples using the DESeq2 package in R (<http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>) (accessed on 7 December 2024), which applies a negative binomial distribution. DEGs were defined based on a threshold of $|\log_2\text{-fold change (LFC)}| \geq 2$ and an adjusted $p\text{-value} \leq 0.05$ (equivalent to $-\log_{10}\text{ adjusted } p\text{-value} \geq 1.30$). A list of DEGs meeting these criteria was compiled. Furthermore, a protein–protein interaction (PPI) network analysis of the DEGs was performed using STRING version 11.5 (<https://string-db.org/>) (accessed on 7 December 2024).

Table 1. List of primers used for measuring gene expression via quantitative PCR.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
STSP_00760 (<i>plsC_1</i>)	CCGTGAACCACTCCCACAA	ACCTTCAGCTGGCCGATTC
STSP_00850	GCCAGTGCGTACCGAGATAG	CTCGCTATATGGCCTCGAC
STSP_01000 (<i>inhA_1</i>)	CAGGTTGCAGCGGATGTTCT	CGCAGTACGACTGGATGGG
STSP_01010 (<i>fabG1_1</i>)	CTCCAGTGATGTACGAGGCG	TCGACACCGACATGACCAAG
STSP_03080 (<i>DGAT</i>)	GATCGACGGGGTCCATCAC	TTCGCCGTGCTGTTCAAGTT
STSP_12530	TAGCCCCACTCGAAGTTGTC	CGGACCGTATCGCGTATCTG
STSP_17580 (<i>bkdA_1</i>)	GTTGACACCACGGAACATGC	GACGACTACGTCTTCCCGAC
STSP_19060 (<i>gpsA</i>)	GATGGTCTCCTCCAGGGTCA	CATGACCTTCTCCGACTCG
STSP_28680 (<i>accD5_1</i>)	CATCAAGCAGCTCCTGTCGT	CTCGATGACCTCGTGATGT
STSP_45110 (<i>tesB_2</i>)	GTACTTCGGCAGTGACTCGG	GCAGCCGATCTTCCATCTGT
STSP_45150 (<i>accA1_2</i>)	CGGAGAGCTATCTGTCCGTG	CTCGGCGAGGAATCCGTATC

Table 1. Cont.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
STSP_50580 (<i>FabZ</i>)	CCGACCTCTTTGGCGAACTT	GCCCAGACCTTCAACGTGAC
STSP_61390 (<i>ilvE</i>)	TGACGATCAAGTGGACGGAG	CGAAGATCTCCTGGGCGTAG
STSP_67970 (<i>fabD</i>)	GCGTACGTCTCGAACAAGGA	TCTGGAACGTCTCCATGCAC
STSP_67980 (<i>fabH_2</i>)	AGCTGATCAAGCAGACCGTG	GGAACCTGATGTCGCCGTTG
STSP_68010 (<i>fabF</i>)	CGATCGAGATGATCCGCACC	TGTTCTTGGACATCGCCATCA
STSP_72400 (<i>lcfB-5</i>)	CACGAGACGGTCTACATCGG	ATCGTCACAGCTCAACTCGG
STSP_74190	TACCTGGTCCGTCAGGTTGA	AAGTGGACGATCCAGTTCGG
<i>rpoB</i> ¹	GTAGTTGTGACCCTCCCACG	GACTACATCACCACCGCCAA

¹ Housekeeping gen.

3. Results and Discussion

3.1. Effect of Temperature and Carbon Source on *S. jeddahensis* Growth

Microorganisms require the uptake of carbon sources and other nutrients from their environment to sustain cell growth and metabolic balance [24]. Additionally, temperature plays a crucial role in regulating bacterial growth. This study evaluated the growth dynamics and metabolic efficiency of *S. jeddahensis* under varying carbon sources and incubation temperatures. These findings offer valuable insights into how temperature and carbon availability influence the bacterium’s growth and metabolism. Three different temperatures (30 °C, 37 °C, and 45 °C) were selected to represent a range conducive to optimal growth and metabolic activity, covering mild, moderate, and higher thermal conditions [25–27].

Glucose is commonly the preferred carbon source and is the primary energy source for many organisms [28]. Moreover, sodium hexanoate has been shown to support the growth of various bacterial strains, such as *Cupriavidus necator* [29]. Similarly, sodium octanoate is a potent carbon source for *C. necator* [30], supporting efficient biomass production without detrimental effects. Octanoate has been observed to promote effective biomass production, making it a favorable carbon source for bacterial growth. Several bacterial species can also utilize decanoate as a carbon and energy source [31]. The four carbon sources utilized in the study were glucose, sodium hexanoate, sodium octanoate, and sodium decanoate. The growth was monitored at three distinct temperatures: 30 °C, 37 °C, and 45 °C. Cell dry weight (CDW) measurements were recorded at 0, 12, 24, and 48 h, with triplicate samples taken at each time point to ensure reproducibility and reliability of the data.

The results demonstrated that *S. jeddahensis* exhibited the highest CDW values at 30 °C across all the tested carbon sources, indicating that this temperature is the most favorable for growth (Figure 2A,C,E,G). At this temperature, the bacterium showed robust growth, highlighting 30 °C as the optimal biomass production condition. On the other hand, at 45 °C, a significant decline in CDW was observed for all carbon sources, suggesting a critical threshold for thermal tolerance in this strain. This reduction in growth at higher temperatures may be attributed to cellular damage, protein denaturation, or impaired enzymatic activity, as previously noted by Schroda et al. [32]. Temperature sensitivity in *S. jeddahensis* could also be linked to the strain’s inability to maintain metabolic processes necessary for cell division and growth under thermal stress. Among the carbon sources tested, sodium octanoate emerged as the most efficient substrate for biomass production, with the highest variability in CDW observed at 30 °C (Figure 2E). Interestingly, sodium hexanoate and sodium octanoate were consumed within 24 h at 30 °C and 37 °C (Figure 2D,F). However, at 45 °C, the consumption rate slowed significantly, and these carbon sources were depleted much more slowly (Figure 2H). This further suggests elevated temperatures inhibit key metabolic processes such as carbon source uptake and utilization and enzyme activities crucial for substrate metabolism [33]. This research has consistently shown that

bacteria readily utilize medium-chain fatty acids like sodium hexanoate and sodium octanoate for growth, where these substrates were efficiently consumed by *S. jeddahensis*. This was evident in the present study [29,30]. In contrast, glucose displayed the most significant reduction in residual levels at 45 °C, with no measurable glucose remaining after 12 h (Figure 2B). The rapid glucose consumption at 45 °C suggests that glucose is rapidly metabolized under higher temperatures.

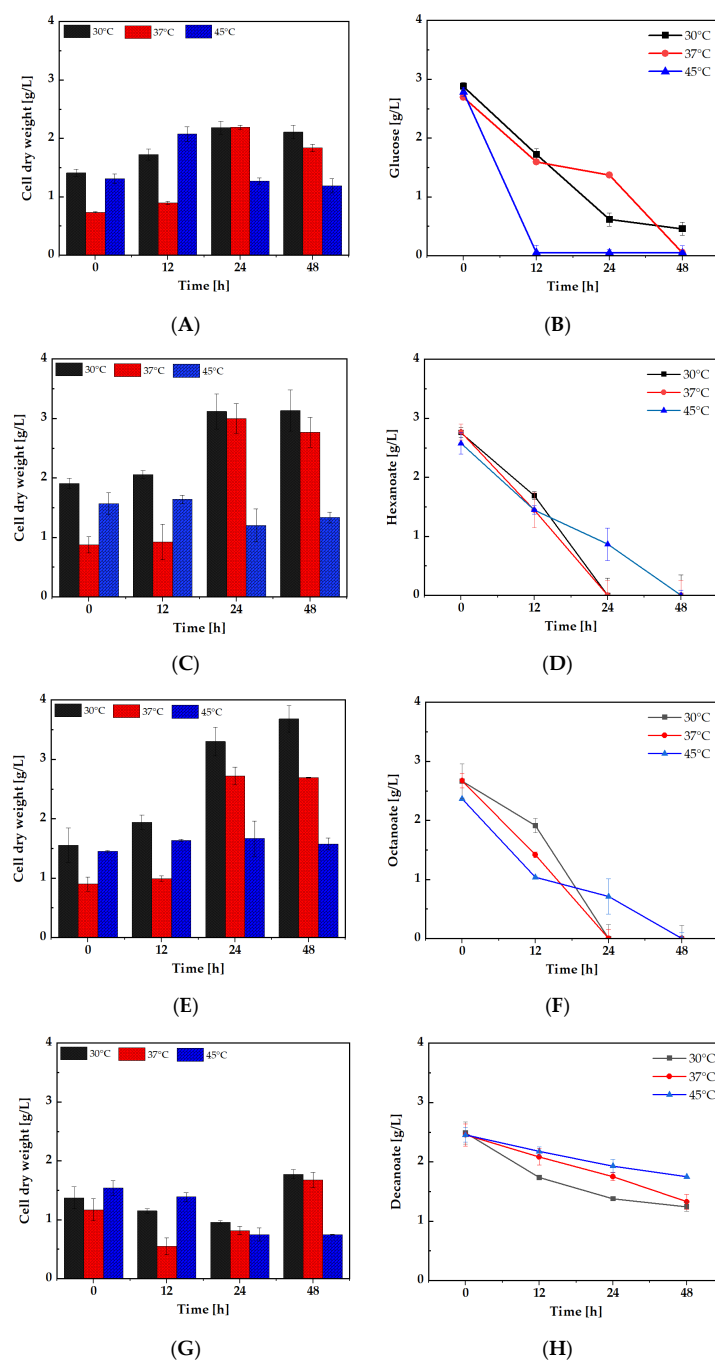


Figure 2. Fermentations of *S. jeddahensis* strains, cultivated in minimal salt medium (MSM) under different carbon sources. Fermentations were conducted for 48 h, with triplicate samples; results were measured from 1 mL samples, and standard deviations were reported. The strain was cultivated for 48 h, with triplicate samples taken. (A) Cell dry weight (CDW) in MSM containing 2.5 g/L glucose. (B) Glucose consumption. (C) CDW in MSM containing 2.5 g/L sodium hexanoate. (D) Sodium hexanoate consumption. (E) CDW in MSM containing 2.5 g/L sodium octanoate. (F) Sodium octanoate consumption. (G) CDW in MSM containing 2.5 g/L sodium decanoate. (H) Sodium decanoate consumption.

3.2. Lipid Accumulation in *S. jeddahensis*

Storage lipids play crucial roles in cells, including regulating membrane fatty acid composition, serving as a reservoir for reducing equivalents and metabolic intermediates, and acting as precursors for essential lipid biosynthesis, helping balance metabolism under fluctuating environmental conditions [34]. The experiment aimed to evaluate the lipid accumulation in *S. jeddahensis* cultured with different carbon sources (glucose, sodium hexanoate, sodium octanoate, and sodium decanoate) at three different temperatures (30 °C, 37 °C, and 45 °C). The lipid content was measured as a percentage of cell dry weight (% w/w), and Bodipy staining was used to visualize lipid accumulation within the cells. Figure 3 shows the observation of lipid accumulation in *S. jeddahensis* at 37 °C using Bodipy staining, glucose (Figure 3A), sodium hexanoate (Figure 3B), sodium octanoate (Figure 3C), and sodium decanoate (Figure 3D), indicating the presence of lipid accumulation within the cells. The other visualizations for 30 °C and 45 °C are shown in Figures S1 and S2, respectively.

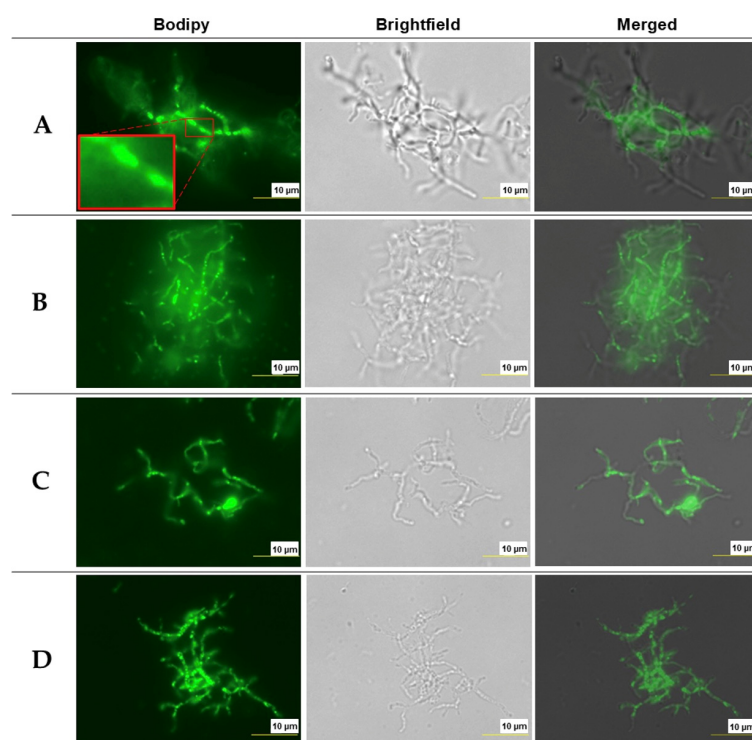


Figure 3. Observation of lipid accumulation in *S. jeddahensis* culture process at 37 °C. The panel displays images of both strains as observed through Bodipy staining, phase-contrast microscopy (Brightfield), and merged (bars, 10 µm). (A) Strain cultured in MSM containing 2.5 g/L glucose; the area within the red box is a cropped and magnified section, highlighting the lipids (green) inside the cell. (B) Strain cultured in MSM containing 2.5 g/L sodium hexanoate, (C) strain cultured in MSM containing 2.5 g/L sodium octanoate, and (D) strain cultured in MSM containing 2.5 g/L sodium decanoate.

The quantitative analysis of lipid content (% w/w) across different temperatures and carbon sources is shown in Figure 4. The results indicate that temperature and carbon source significantly affect lipid accumulation in *S. jeddahensis*. At 30 °C, lipid contents were generally lower across all carbon sources. Glucose resulted in a higher lipid accumulation. At 37 °C, accumulation appeared optimal, with sodium octanoate leading to the highest lipid content, followed by glucose and sodium hexanoate. At 45 °C lipid accumulation decreased at higher temperatures, with all carbon sources showing reduced lipid contents compared to 37 °C. The results demonstrate that the type of carbon source and the incubation temperature play crucial roles in lipid accumulation in *S. jeddahensis*. The highest lipid content was observed at 37 °C with sodium octanoate as the carbon source. This suggests

that medium-chain fatty acids (such as octanoate) are more efficiently converted into lipids by *S. jeddahensis* under optimal temperature conditions. Previous research has shown that oleaginous bacteria can accumulate significant amounts of lipids when provided with appropriate carbon sources and environmental conditions. For instance, a study by Yang et al. [35] highlighted the potential of oleaginous microbes for lipid accumulation and renewable energy generation. They found that the choice of carbon source and culture conditions, such as temperature and nutrient availability, significantly influenced lipid production. Similarly, Koreti et al. [36] reviewed the lipid accumulation capabilities of various oleaginous bacteria and emphasized the importance of optimizing culture conditions to enhance lipid yield. They noted that medium-chain fatty acids, like those used in this study, were particularly effective in promoting lipid accumulation. The enhanced lipid accumulation observed with sodium hexanoate and sodium octanoate can be attributed to the metabolic pathways involved in fatty acid synthesis. Oleaginous bacteria, including *S. jeddahensis*, can convert excess carbon into storage lipids, primarily triacylglycerols (TAGs). The efficiency of this conversion process is influenced by the chain length of the fatty acids provided as carbon sources. Bodipy staining, as used in this study, is a reliable method for visualizing lipid droplets within cells. Patel et al. [37] discussed the advantages of using Bodipy for lipid detection, noting its high specificity and photostability.

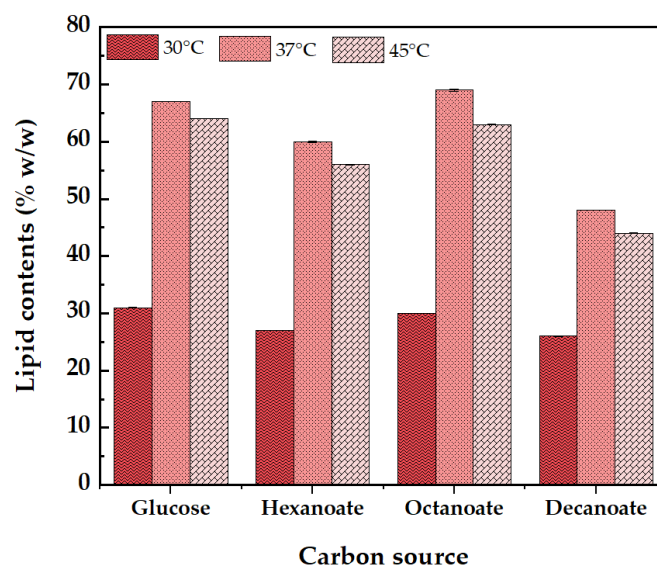


Figure 4. The bioconversion of various carbon sources into lipids was evaluated by cultivating *S. jeddahensis* for 48 h in MSM supplemented with different carbon sources at 30 °C, 37 °C, and 45 °C. The lipid content (% w/w) is represented as a bar, showing the average value and standard deviation (n = 3).

3.3. Total Fatty Acid in *S. jeddahensis*

The presented stacked bar chart (Figure 5) reveals the total fatty acid (total FA) composition of *S. jeddahensis* under varying carbon sources and incubation temperatures. The carbon sources used were glucose, sodium hexanoate, sodium octanoate, and sodium decanoate, and the cultures were incubated at three different temperatures: 30 °C, 37 °C, and 45 °C. This experiment highlights the interplay between carbon source utilization and environmental conditions in shaping fatty acid metabolism in *S. jeddahensis*. Each set of conditions induced specific shifts in the proportions of fatty acid types, underscoring the strain's metabolic flexibility. The stacked bar chart reveals the total (FA) composition of *S. jeddahensis* under varying carbon sources and incubation temperatures. The carbon sources used were glucose, sodium hexanoate, sodium octanoate, and sodium decanoate, and the cultures were incubated at three different temperatures: 30 °C, 37 °C, and 45 °C. This

experiment highlights the interplay between carbon source utilization and environmental conditions in shaping fatty acid metabolism in *S. jeddahensis*. Each set of conditions induced specific shifts in the proportions of fatty acid types, underscoring the strain's metabolic flexibility and potential applications in biotechnological industries. The type of carbon source strongly influenced the fatty acid composition of *S. jeddahensis*. Cultures grown on glucose, a simple carbon source, exhibited a balanced distribution of fatty acids, with C16:1 (cis-9) and 3-OH C14:0 dominating the profiles across all temperatures. This indicates that glucose promotes a generalist metabolic pathway, producing both medium- and long-chain fatty acids in relatively stable proportions.

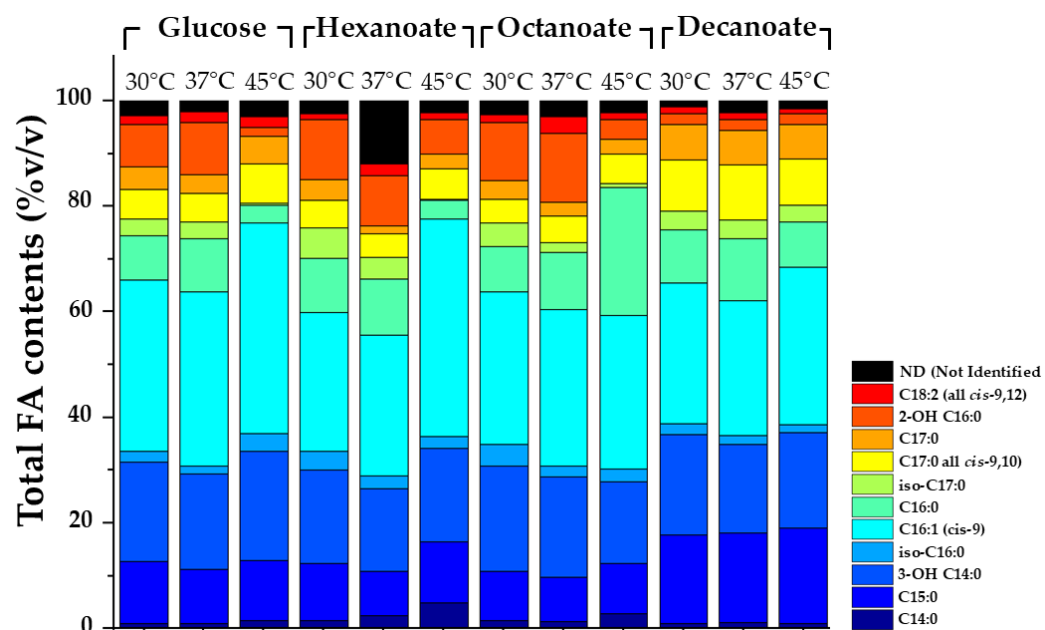


Figure 5. Total fatty acid (FA) contents of *S. jeddahensis* strains cultivated in minimal salt medium (MSM) supplemented with different carbon sources (2.5 g/L glucose, sodium hexanoate, sodium octanoate, or sodium decanoate) and incubated at varying temperatures (30 °C, 37 °C, and 45 °C) for 48 h. Experimental conditions are as follows: MSM + glucose at (30 °C, 37 °C, 45 °C), MSM + sodium hexanoate at (30 °C, 37 °C), MSM + sodium octanoate at (30 °C, 37 °C, 45 °C), MSM + sodium decanoate at (30 °C, 37 °C, 45 °C). Results highlight the impact of carbon source and temperature on Total FA production under defined growth conditions.

In contrast, cultures grown on sodium hexanoate displayed a marked increase in 2-OH C16:0 and C16:0, particularly at 30 °C. Hexanoate, a medium-chain fatty acid precursor, likely serves as a direct substrate for elongation and desaturation pathways, explaining the higher abundance of these medium-chain fatty acids. Similar trends were observed with sodium octanoate, where an elevated proportion of 2-OH C16:0 at 30 °C and 37 °C and C16:1 (cis-9) dominated across all temperatures, for C16:0 was noted at 45 °C as the highest compared to the other temperatures. Octanoate enhances the synthesis of longer-chain unsaturated fatty acids, reflecting its role in extending fatty acid chains through β -oxidation intermediates.

Sodium decanoate, a longer-chain fatty acid precursor, produced distinctive profiles characterized by increased C15:0 at all temperatures. This indicates that decanoate promotes the biosynthesis of polyunsaturated fatty acids under high-temperature conditions, suggesting a potential stress adaptation mechanism by *S. jeddahensis*. These results demonstrate the strain's ability to metabolize diverse carbon substrates into tailored fatty acid profiles, a feature that could be harnessed for industrial fatty acid production.

At 37 °C, a more balanced distribution of saturated and unsaturated fatty acids emerged. This intermediate temperature likely optimizes enzymatic activities, enabling efficient chain elongation and desaturation. Cultures grown on glucose and sodium octanoate showed increased proportions of long-chain unsaturated fatty acids, such as C18:2 (all *cis*-9,12), reflecting enhanced metabolic activity in fatty acid biosynthesis. At 45 °C, significant shifts in fatty acid composition were observed, particularly in cultures grown on sodium decanoate. Polyunsaturated fatty acids, such as C18:2 (all *cis*-9,12), became prominent, indicating an adaptive response to thermal stress. High temperatures likely induce the expression of desaturases, enzymes responsible for introducing double bonds into fatty acid chains. This adaptation enhances membrane fluidity, maintaining cellular integrity under heat stress. These findings align with previous studies that show elevated polyunsaturated fatty acid production in bacteria exposed to high temperatures.

The metabolic flexibility of *S. jeddahensis* revealed by this study highlights its potential for biotechnological applications. The ability to tailor fatty acid profiles by manipulating carbon sources and temperature conditions offers valuable insights into producing specific fatty acids for industrial purposes.

The GC-MS results also demonstrated elevated peaks at 37 °C and 45 °C compared to 30 °C. However, the specific compounds responsible for these peaks could not be identified due to their absence in the BAME standard utilized (Figures S3–S6). These observations suggest that high-temperature cultivation with sodium decanoate may have potential applications in the nutraceutical and pharmaceutical industries. The findings of this study are consistent with and extend those of previous research on bacterial fatty acid metabolism. For instance, Gorte et al. [38] demonstrated that the carbon source and temperature significantly influence microbial lipid profiles, mainly glucose, which promotes the production of saturated fatty acids.

3.4. Gene Expression Analysis

As previously mentioned, the lipid content was highest when sodium octanoate was used as the carbon source at 37 °C, even though the highest CDW was observed at 30 °C. Another interesting finding from the lipid content result is that the lipid content was still significant at 45 °C despite the cell dry weight not being very high (Figure 4). This phenomenon is likely due to multiple factors tied to metabolic and physiological adaptations of the strain. Because the strain has been screened from the desert soil environment, it may have evolved to store lipids as a survival mechanism under nutrient scarcity and elevated temperatures [9]. Its capacity to utilize different carbon sources can further enhance lipid production under varied growth conditions. Additionally, the presence of specific enzymes, such as wax ester synthase/acyl-CoA:diacylglycerol acyltransferase [39] involved in its lipid metabolism, indicates a complex metabolic network that supports lipid biosynthesis even under stressful environments. Bacterial responses to temperature changes highlight the induction of heat-shock proteins and other stress-related genes at higher temperatures [40]. We conducted a detailed gene expression analysis at 37 °C and 45 °C using sodium octanoate to understand the underlying mechanisms. We analyzed several genes that play critical roles in lipid metabolism, as detailed in Table 1, with *rpoB* as a housekeeping gene.

The STRING analysis emphasizes the functional importance of lipid biosynthetic genes in *S. jeddahensis* (Figure 6). The high connectivity of *accD5_1* (acetyl-CoA carboxylase) underscores its role as a key regulator of fatty acid biosynthesis, catalyzing the first committed step in the pathway. The strong interactions of *fabF* and *fabG*, which are involved in elongation and reduction steps in fatty acid synthesis, further highlight the metabolic prioritization of lipid production [41]. Proteins such as *plsC* (1-acyl-sn-glycerol-3-phosphate

acyltransferase) and *gpsA* (glycerol-3-phosphate dehydrogenase) were identified, underscoring their roles in glycerolipid biosynthesis. Environmental conditions, such as carbon sources and temperature, likely play a role in modulating the expression of these genes. Peripheral interactions involving *plsC_1* suggest its role in glycerolipid and polyketide biosynthesis. Specifically, *plsC_1*, a bacterial 1-acyl-sn-glycerol-3-phosphate acyltransferase, catalyzes the acylation of lysophosphatidic acid to produce phosphatidic acid, a precursor for membrane glycerophospholipids [42]. The bacterium's reliance on lipid biosynthesis under heat stress aligns with observations in other oleaginous microorganisms where lipid accumulation is a protective mechanism. Regulatory proteins like *pucR* were also identified, indicating a potential role in modulating stress responses, including regulating heat shock proteins under thermal stress [43].

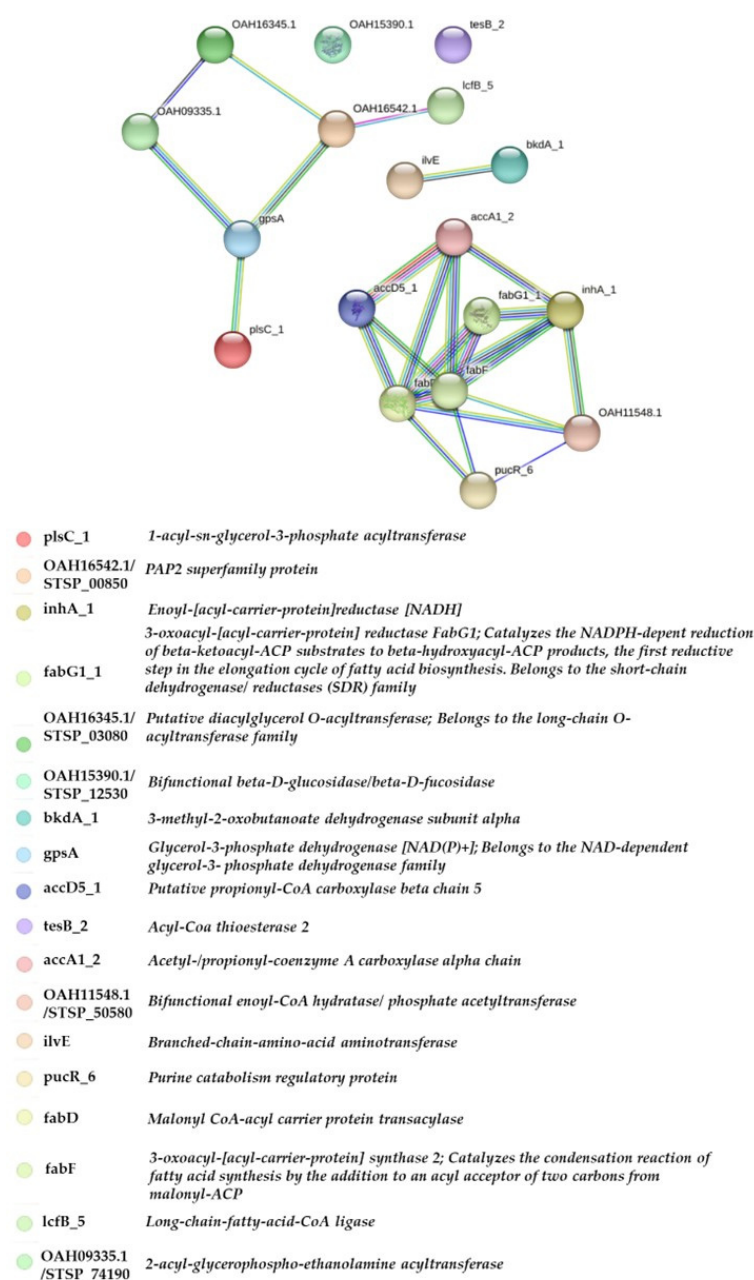


Figure 6. The STRING interaction network of the analyzed genes. Each filled node represents a gene, with edges between nodes indicating interactions between the protein products encoded by these genes. The edges are color-coded to represent different types of evidence supporting these interactions. Genes are color-coded based on their names and functional categories.

Regulatory proteins like *pucR* were also noted, indicating potential roles in modulating stress responses. Such regulatory elements mediate heat shock protein expression under thermal stress in other bacteria. These findings suggest that *S. jeddahensis* adapts to elevated temperatures by enhancing lipid biosynthesis pathways, with acyl carrier proteins playing a central role. Understanding these mechanisms lays a foundation for exploring thermally induced metabolic pathways and their industrial applications, such as biofuel production [35].

The RNA-Seq analysis compared the transcriptomes of *S. jeddahensis* at 37 °C and 45 °C using DESeq2. This analysis provided insights into temperature-dependent gene expression patterns, crucial for understanding the bacterium's metabolic adaptations under varying thermal conditions. The results were visualized using volcano plots, bar plots, and heatmaps, offering a comprehensive view of the differential gene expression data. The volcano plot (Figure 7A) highlights most genes with modest $\log_2$ fold changes (FC) and low statistical significance. However, a subset of genes displays substantial $\log_2$ FC values and high statistical significance (p -value < 0.05), suggesting their potential role as key metabolic regulators under specific conditions. Genes at the plot's extremes may encode enzymes critical for medium-chain fatty acid metabolism. Overall, only a few genes show significant differential expression. Features with high $|\log_2 \text{FC}|$ (far left or right) and high $-\log_{10}(p\text{-value})$ are significantly upregulated or downregulated. The bar chart (Figure 7B) illustrates differential gene expression from the experiment, highlighting upregulated genes (red), such as STSP_67970 (*fabD*) and STSP_03080 (*DGAT*), alongside downregulated genes (blue) like STSP_12530. This suggests *S. jeddahensis* undergoes significant metabolic and regulatory shifts to adapt to varying temperatures. The upregulated genes are likely involved in carbon metabolism, heat response, or biosynthetic pathways, consistent with responses observed under stress conditions, like the presence of different kinds of carbon source fatty acids and elevated temperatures. Conversely, downregulated genes may indicate suppression of specific pathways, such as glucose metabolism, in favor of fatty acid utilization [44].

For instance, research by Bucca et al. [45] on *S. coelicolor* under thermal stress identified similar upregulation in heat shock proteins' metabolism-related genes. This emphasizes conserved stress responses across *Streptomyces* species and provides insights into their adaptive mechanisms. The heatmap (Figure 7C) reveals distinct clusters of genes with higher expression (indicated in red) at 45 °C compared to 37 °C. For instance, genes such as STSP_12530, STSP_01010 (*fabG1_1*), and STSP_19060 (*gpsA*) exhibit elevated expression levels at 45 °C. These genes likely involve critical metabolic pathways such as fatty acid biosynthesis and stress response. Notably, STSP_28660 (*accD5_1*), a gene associated with acetyl-CoA carboxylase activity, also showed a significant increase in expression, suggesting that higher temperatures may enhance lipid metabolism, potentially to cope with membrane fluidity changes at elevated temperatures.

In contrast, specific genes demonstrated reduced expression at 45 °C. For example, STSP_45150 (*accA2_1*) and STSP_45110 (*tesB_2*) showed lower expression levels at higher temperatures. These genes are likely involved in alternative metabolic pathways, which might be suppressed in favor of other pathways better suited for adaptation to heat stress. The clustering of gene expression profiles further highlights the functional partitioning of genes based on temperature, suggesting a shift in the metabolic network of *S. jeddahensis* to adapt to environmental conditions. The results align with the hypothesis that temperature significantly influences secondary metabolism in *Streptomyces*. Higher temperatures may induce stress-response pathways and enhance the biosynthesis of secondary metabolites, which are critical for the organism's survival under suboptimal conditions. Sodium octanoate, as a carbon source, appears to amplify these effects by promoting the activation of

specific pathways related to lipid metabolism and energy production. This finding aligns with prior research that highlights the role of carbon source availability and temperature in regulating *Streptomyces* metabolic pathways.

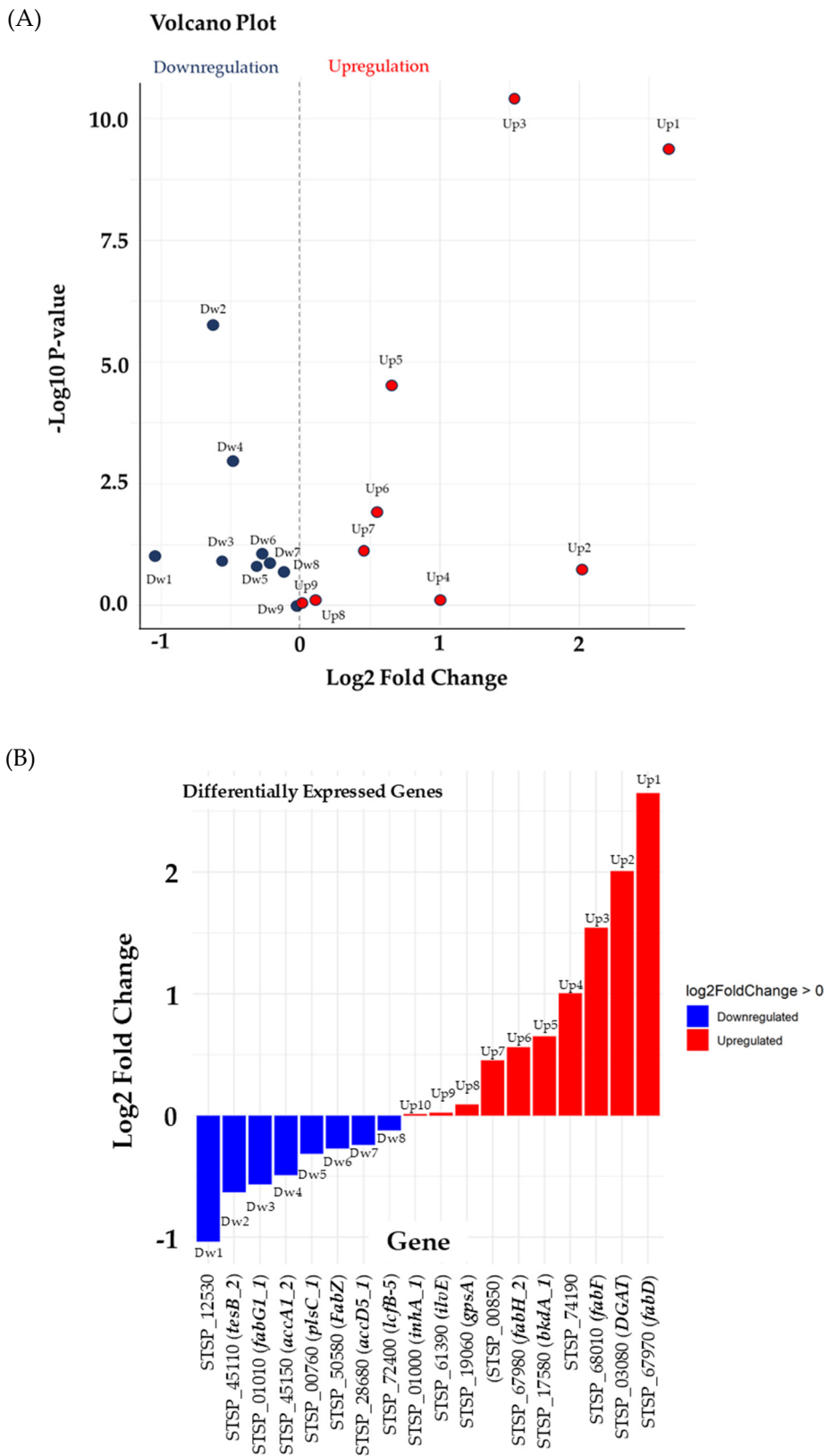


Figure 7. Cont.

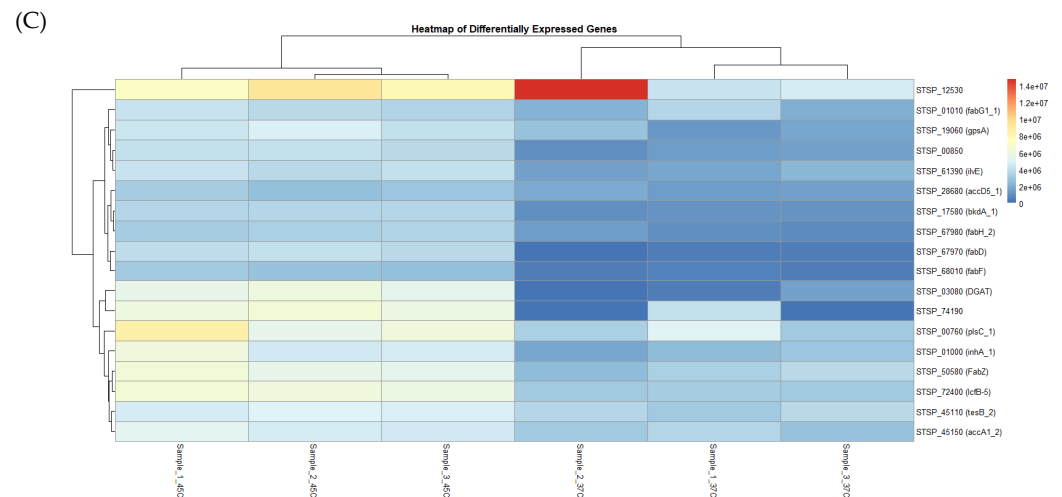


Figure 7. Visual gene expression using Deseq2. (A) Volcano plot of differential gene expression. The volcano plot shows gene expression changes between 37 °C and 45 °C. Dots represent significant genes ($\log_2FC > 1$ or < -1 , $p < 0.05$). (B) Bar chart of $\log_2 FC$ for selected genes. The bar plot highlights $\log_2$ fold changes between conditions, showing upregulation (red) and downregulation (blue). (C) The heatmap visualizes expression patterns across conditions, clustering genes like STSP_68000 and STSP_67970 based on significant temperature responsiveness. Red indicates high expression, and blue indicates low expression, respectively.

The expression of genes, such as STSP_03080 (*DGAT*), involved in triacylglycerol biosynthesis indicates a potential accumulation of storage lipids at 45 °C. This adaptive mechanism could serve as an energy reservoir to endure thermal stress. Similarly, the upregulation of STSP_61390 (*ilvE*), a gene associated with branched-chain amino acid metabolism, suggests an increased demand for amino acid precursors, which could play a role in maintaining protein stability and function under heat stress. Similar temperature-induced gene expression responses have been observed in other bacterial species. For instance, in *Escherichia coli*, the expression of heat shock proteins is regulated by RNA thermometers, which modulate gene expression in response to temperature changes [40]. These RNA thermometers are located in the 5'-untranslated region (5'-UTR) of genes encoding heat shock proteins and function as thermosensors to regulate gene expression at the post-transcriptional level [46]. The significantly upregulated genes at 45 °C likely contribute to maintaining membrane fluidity and protecting cellular integrity under thermal stress.

In addition to *acpP*, other genes were identified as being temperature-responsive. Among the upregulated genes were STSP_68010 (*fabF*) encoding 3-ketoacyl-ACP synthase II, STSP_67970 (*fabD*) encoding a transcriptional activator of the *fab* operon, STSP_74190 encoding 1-acylglycerol-3-phosphate acyltransferase (AGPAT), and STSP_03080 encoding diacylglycerol acyltransferase (DGAT). DGAT, a CoA-dependent acyltransferase enzyme, is key in synthesizing triacylglycerols (TAGs) in prokaryotes. Interestingly, DGAT exhibits dual activity, functioning both as an acyl-CoA:fatty alcohol acyltransferase (wax ester synthase, WS) and a diacylglycerol acyltransferase (DGAT), utilizing a wide range of acyl-CoA substrates for the synthesis of TAGs or wax esters (WEs) [47,48]. Conversely, some genes, such as STSP_12530, were downregulated in response to temperature changes. STSP_12530 encodes β -Glucosidase, an enzyme critical for bacterial metabolism, particularly in breaking down complex carbohydrates. β -Glucosidase hydrolyzes β -glycosidic bonds in compounds like cellobiose (a cellulose degradation product) and other oligosaccharides, converting them into glucose [49,50].

Lipid biosynthesis in bacteria depends on key metabolic intermediates, such as pyruvate, acetyl-CoA, and glycerol-3-phosphate. Pyruvate, the end product of glycolysis, serves as a critical switch point for carbon flux distribution within central metabolism. It can act as a precursor for sugar phosphate synthesis via gluconeogenesis or be utilized to replenish tricarboxylic acid (TCA) cycle intermediates through anaplerotic reactions, thereby supporting anabolic processes. Together, these pathways highlight bacteria's metabolic versatility and adaptability to optimize lipid production under varying environmental conditions.

This study found that *S. jeddahensis*'s ability to accumulate high levels of lipids, particularly when cultured with medium-chain fatty acids at optimal temperatures, suggests that this bacterium could be a valuable candidate for biodiesel production. Using renewable and cost-effective carbon sources, combined with optimized culture conditions, can enhance the feasibility and sustainability of microbial biodiesel production.

4. Conclusions

This study highlights *Streptomyces jeddahensis* as a robust and efficient microorganism for lipid production, particularly when cultured with medium-chain fatty acids under optimal conditions. Sodium octanoate emerged as the most effective substrate for lipid accumulation, with 37 °C as the optimal temperature for growth and biosynthesis. Despite reduced biomass at higher temperatures, lipid production remained significant due to stress-induced metabolic adaptations. The upregulation of lipid biosynthesis genes, such as STSP_03080 (*DGAT*) and STSP_67970 (*fabD*), emphasizes the bacterium's potential for industrial biofuel applications. These findings establish *S. jeddahensis* as a promising candidate for renewable energy production, with opportunities for further optimization through genetic engineering and process development to enhance its industrial scalability. Future studies should explore the use of alternative, cost-effective carbon sources, such as waste oils, molasses, and agricultural residues to assess their impact on both biomass growth and lipid accumulation. Additionally, investigating mixed-carbon media could provide a more sustainable approach for large-scale lipid production, offering further opportunities for optimization and industrial scalability through genetic engineering and process development.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fermentation11020045/s1>.

Author Contributions: Conceptualization, P.K., P.A. and C.O.; data curation, P.A. and P.K.; formal analysis, P.A., N.R. and W.K.R.; funding acquisition, C.O.; investigation, P.A. and P.K.; methodology, P.A. and P.K.; project administration, P.K. and C.O.; resources, P.A. and P.K.; supervision, P.K., C.O. and A.K.; validation, P.A. and P.K.; writing—original draft, P.A. and P.K.; writing—review and editing, P.A. and P.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the International Joint Program, Science and Technology Research Partnership for Sustainable Development (SATREPS) of the Japan Science and Technology Agency and the Japan International Cooperation Agency (JST and JICA), Japan.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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