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Activity of lactic acid-producing *Streptomyces* strain CSK1against *Staphylococcus aureus*

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Abstract

This study aimed to evaluate an active *Streptomyces* strain CSK1 previously isolated from UAE soils to produce inhibitory bioactive compounds under different nutritional and growth conditions against Gram positive *Staphylococcus aureus*. Using a One Strain Many Compounds (OSMAC) strategy, specifically media optimization on *Streptomyces* strain CSK1 to enhance antibacterial activity against *S. aureus*, results indicated an average inhibition zone diameter ranging between 13 and 20 mm after 7 days of growth in ISP4 broth. Moreover, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the water soluble of evaporated ethyl acetate crude extract revealed values of 0.79 and 6.29 mg mL^{-1}, respectively. GC-MS analysis of the crude ethyl acetate extract confirmed three compounds namely lactic acid, butyl lactate and lactide. The production of these compounds by *Streptomyces* strain CSK1 may stress their valuable importance in food and pharmaceutical industries.

Keywords:GC-MS; Lactic acid; MIC; *Staphylococcus aureus*; *Streptomyces*.

1. Introduction

Streptomyces are Gram positive filamentous bacteria that belong to Actinobacteria that are abundant in soil. They are known to produce many natural products that are important for the biotechnology industry. They produce various compounds that have been commercialized such as antibiotics, antifungal and anti-parasite, and antitumor agents (Moore *et al*., 2022). *Streptomyces* have a complex life cycle that includes vegetative and reproductive growths. The cycle starts with spore germination, then substrate mycelium develops followed by the aerial mycelium, the apical aerial hypha grows and sporulates, upon maturation, the spores are dispersed. The synthesis of secondary metabolites is tightly linked to the *Streptomyces* complex life cycle (Khushboo *et al*., 2021; Manteca and Yagüe, 2018).

Secondary metabolites are chemicals that exhibit biological activity and produced in small concentrations. They are not essential for bacterial growth, which distinguishes them from primary metabolites (Keswani *et al*., 2019). Their diversity contributes to the microorganisms' ecological role. Secondary metabolites were found to have a significant influence on human health and are used in the pharmaceutical and food industries. *Streptomyces* produce secondary metabolites like cyclic and linear peptides and linear polyketides that are known for their antibacterial activity (Lacey and Rutledge, 2022). Genetic analysis has identified that up to 30 secondary metabolite pathways can be found in *Streptomyces* strains that are mostly not expressed when grown in culture (Choudoir *et al*., 2018; de Rop *et al*., 2022; Otani *et al*., 2022).

One of the methods used to activate the silent secondary metabolites pathways in *Streptomyces* is One Strain Many Compounds (OSMAC) approach. This strategy uses different culture conditions to induce and maximize the production of secondary metabolites. Cultural conditions include media chemical compositions, fermentation, and environmental factors. Secondary metabolites can be expressed or repressed through media chemical composition such as carbon and nitrogen sources. Fermentation and incubation conditions such as temperature and incubation period affect the quality of the natural products. pH is an environmental factor that is important for secondary metabolites synthesis and cellular metabolism. Developing high-quality natural products at an industrial scale with the use of affordable substrates is facilitated by optimizing the production conditions (Al Farraj *et al*., 2020; Chen *et al*., 2022; Hug *et al*., 2018; Khattab *et al*., 2016; Yun *et al*., 2018).

The aim of this study is to optimize the cultural conditions of an active *Streptomyces* strain CSK1previously isolated from UAE soil to produce inhibitory bioactive compounds against *Staphylococcus aureus*. The crude extract of *Streptomyces* strain CSK1 was analyzed using GC-MS to identify the active substrates.

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2. Materials and methods

2.1. Microbial test organisms

Test organisms that were used for the antimicrobial activity test included six bacterial strains and *Candida albicans* (ATCC® 66027). The bacterial Gram-positive strains are *Staphylococcus aureus* (ATCC® 29213), *Staphylococcus epidermidis* (ATCC® 14990), *Streptococcus pneumoniae* (ATCC® 6301) and *Bacillus subtilis* (ATCC® 6051). The bacterial Gram-negative strains are *Escherichia coli* (ATCC ® 25922) and *Pseudomonas aeruginosa* (ATCC® 55638).

2.2. Isolation and Characterization of the Streptomyces CSK1 strain

The *Streptomyces* strain CSK1 was isolated previously from a cultivated soil sample from Dubai, United Arab Emirates (Al-Joubori, 2020). It was characterized according to the International *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966). The isolate was grown on oatmeal agar (Himedia, India) and incubated at 28 °C for fourteen days. The colour of the aerial mycelium, substrate mycelium and diffusible soluble pigments was determined.

2.3. Antimicrobial activity of the Streptomyces CSK1 strain

The antimicrobial activity of the *Streptomyces* strain CSK1 was tested using the well diffusion method (Bauer *et al*., 1966; Hudzicki, 2009). The microbial test organisms illustrated above were inoculated on nutrient agar (Himedia, India) plate at 37 °C for 24 hours. Bacterial suspension was prepared using direct colony transfer and the turbidity was adjusted to 0.5 McFarland standard. The adjusted test bacterial suspension was cultured on fresh Mueller Hinton agar (Himedia, India) plate using sterile cotton swab. Six mm diameter wells were made using a sterile Pasteur pipette in the Mueller Hinton agar plate. The wells were filled with 55 µL of the supernatant culture broth. The plates were incubated overnight at 37 °C then the inhibition zones were recorded after 24 hours.

2.4. Optimization of cultural conditions

Streptomyces strain CSK1 culture conditions were optimized for the highest antibiotic production. The conditions that were tested were the following: Culture media broth (Nutrient media (Himedia, India), Tryptone - yeast extract media or ISP1 (Himedia, India), Inorganic salts-starch media or ISP4 [Ingredients per Liter; Starch 10g, K_2HPO_4 1g, $MgSO_4.7H_2O$ 1g NaCl 1g, $CaCO_3$ 2g, $(NH_4)_2SO_4$ 2g, Trace salts solution 1mL]), Carbon sources [concentration of 1%] (Glucose, Arabinose, xylose, sucrose, fructose, mannitol, sorbitol, starch, inositol, glycerol, carboxymethylcellulose, and Glucosamine), Nitrogen sources [concentration of 0.2%] (Ammonium sulphate, Casein, yeast extract, sodium nitrate, potassium nitrate, and Glycine), pH (5.0, 6.0, 7.2, 8.0 and 9.0) and temperature (25, 28, 31, 34, and 37 °C). *Streptomyces* strain CSK1 was grown in submerged cultures in 250 mL containing 50 mL culture broth. A seed culture of the *Streptomyces* isolate was prepared by growing the isolate on oatmeal agar plate for 4 days. The whole aerial mycelium was scrapped and suspended in sterile water. Flasks were inoculated with 1.0 mL of the spore suspension and incubated in an orbital shaker incubator at 100 rpm for 7 days. During the growth period, 1.0 mL of the culture broth was taken on days 0, 1, 2, 3, 4, 6, and 7 for antimicrobial activity testing.

2.5. Fermentation, extraction of metabolites

A seed culture of the *Streptomyces* strain CSK1 was prepared as described above where 5.0 mL of the spore suspension of the seed culture are inoculated in four 1.0 L flasks containing 250 mL of the optimal culture media. The flasks were incubated in an orbital shaker at 100 rpm for 4 days .

After incubation, cell free broth was obtained through filtration using 0.22 µm filter unites (Millipore, USA). The metabolites were extracted through liquid-liquid extraction using ethyl acetate as the organic solvent. Ethyl acetate was added to the cell free broth (v/v) and mixed vigorously. The mixture was shaken for 30 minutes and then was allowed to stand in a separatory funnel to settle into two layers. The organic layer was evaporated using a rotary evaporator (Rotavapor R-300, Buchi) at 45 °C with 200 rpm at 200 mbar. The crude ethyl acetate extract was collected and used for further analysis.

2.6. Antimicrobial activity of the ethyl acetate crude extract

The ethyl acetate crude extract was dissolved in 1.0 mL distilled water and filter-sterilized using 0.22 µm syringe filter. The antimicrobial activity of the ethyl acetate crude extract was performed using well diffusion method as described above. The crude extract was tested against *S. aureus*. Lactic acid was used as a positive control, and pure ethyl acetate and ISP4 broth were used as negative controls. The antibacterial activity of the crude extract against *S. aureus* was tested using the microbroth dilution according to the clinical and laboratory standards institute (CLSI) (CLSI, 2006; Elshikh *et al*., 2016). In a 96 well plate, the crude extract was serially diluted with Mueller Hinton broth starting from well one at 37.73 mg/mL followed by two-fold dilutions from 25.15 to 0.025 mg/mL in wells 2 through 12 performed in triplicates (columns A, B, and C). Positive control of tested *S. aureus* growth and negative control presenting only Mueller Hinton medium were assessed in columns G and H. The crude extract was incubated with 100 µL Mueller Hinton broth containing (5.0×10^5 CFU/mL) of *S. aureus* overnight at 37 °C. The minimum inhibitory concentration (MIC) was detected using resazurin (0.015%) as an indicator of bacterial growth. The minimum bactericidal concentration (MBC) was assessed using 10 µL of crude extract with concentrations ranging from 0.196 to 6.29 mg/mL that showed inhibition of growth of *S. aureus* in the MIC method.

2.7. GC-MS analysis of the ethyl acetate crude extract

The GC-MS analysis of the ethyl acetate crude extract was performed using a GC-MS QP2010 ultra, Shimadzu-Japan. The carrier gas used was helium at a flow rate of 0.9 ml/min. The column used is Rxi-5ms, Restek with 30 m length, 0.25 mm thickness and 0.25 mm diameter. The process was as following: hold 2 minutes at 70 °C, followed by 300 °C with heating rate 5°C/min, holding for 8.4 min at 300 °C. The temperature of the injector was 280 °C. Mass spectrum was determined at 70 eV with a scan interim of 1666 U/sec and the fractions between 35 and 500 amu. The component spectrums were compared to

a database of known component spectrums stored in the GC-MS. The mass results were analyzed according to three libraries namely Wiley9, NIST14, and NIST14s.

Streptomyces Strain CSK1	
Aerial Mycelium	White
Substrate Mycelium	Grey
Soluble pigment	No pigments
Antimicrobial Activity	
Escherichia coli (ATCC® 25922)	-
Pseudomonas aeruginosa (ATCC® 55638)	-
Staphylococcus aureus (ATCC® 29213)	19.67 mm ± 1.15
Staphylococcus epidermidis (ATCC® 14990)	25.67 mm ± 0.58
Streptococcus pneumoniae (ATCC® 6301)	-
Bacillus subtilis (ATCC® 6051)	-
Candida albicans (ATCC® 66027)	-

2.8. Statistical Analysis

Analyses of variance (ANOVA) for all data were performed. Two-way ANOVA was used to compare between the means ($\alpha = 0.05$).

3. Results

3.1. Characterization and antimicrobial activity of the Streptomyces CSK1 strain

The *Streptomyces* strain CSK1 was grown on oatmeal agar for 14 days. The mature *Streptomyces* colonies have white aerial mycelium and grey substrate mycelium, with no soluble pigments produced (Figure 1). The isolate was screened for antimicrobial activity using well diffusion method against various organisms (Table 1). The isolate showed an inhibitory activity against Gram positive organisms mainly *Staphylococcus aureus* and *Staphylococcus epidermidis* (Figure 2).

Table 1. *Streptomyces* strain CSK1 characteristics and antimicrobial activity.

(-) indicates that there is no antimicrobial activity against the organism. The data in the positive antimicrobial activity are represented by the average zone of inhibition of triplicate samples (mm) ± the standard deviation.

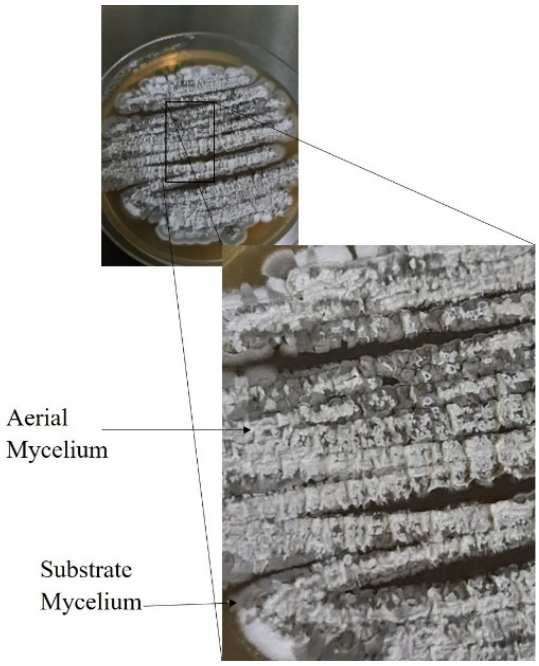

Figure 1. *Streptomyces* strain CSK1 grown on oatmeal agar for 14 days.

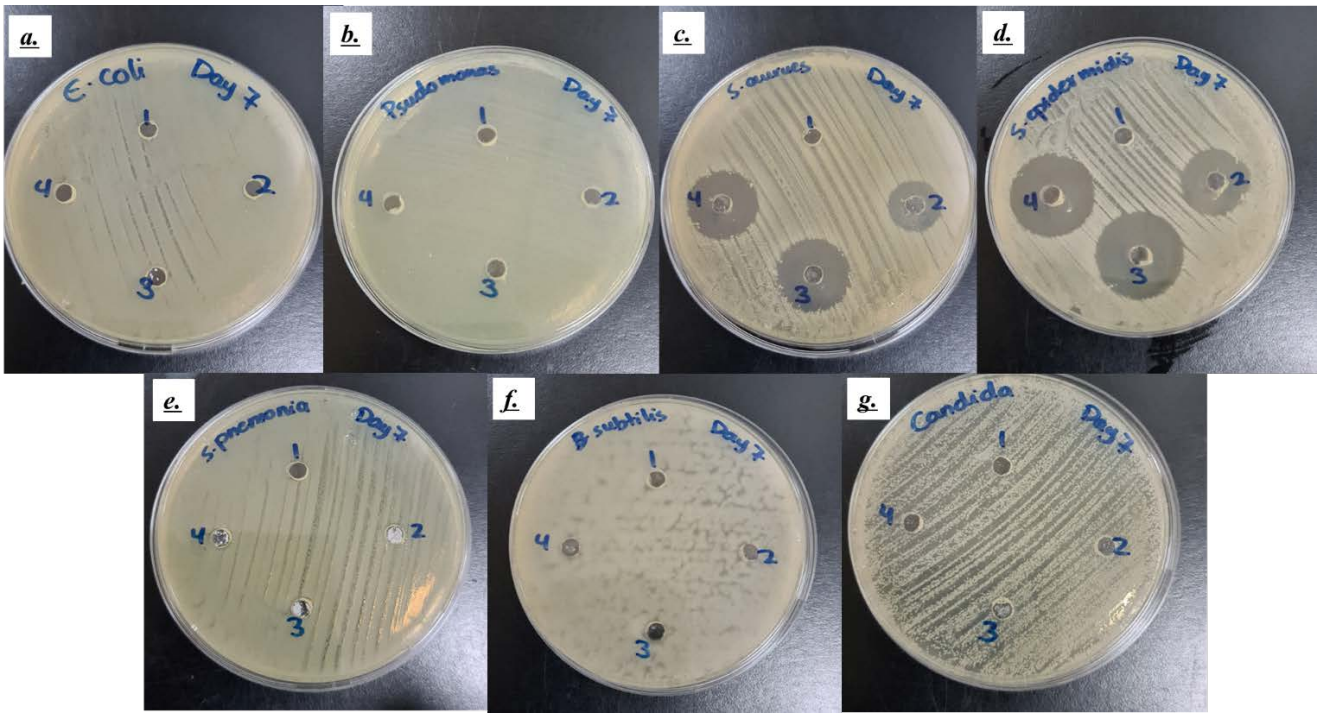

Figure 2. Antimicrobial activity of the *Streptomyces* strain CSK1 against various organisms (a) *Escherichia coli* (ATCC ® 25922), (b) *Pseudomonas aeruginosa* (ATCC® 55638), (c) *Staphylococcus aureus* (ATCC® 29213), (d) *Staphylococcus epidermidis* (ATCC® 14990), (e) *Streptococcus pneumoniae* (ATCC® 6301), (f) *Bacillus subtilis* (ATCC® 6051), (g) *Candida albicans* (ATCC® 66027).

3.2. Optimal conditions for antimicrobial activity production

The Optimal physical and chemical conditions for production of the active inhibitory metabolites by *Streptomyces* CSK1 strain against *S. aureus* were investigated. The isolate was grown in three types of culture media and was tested for antimicrobial activity against *S. aureus*. Figure 3 showed that all three culture media broths were suitable for antimicrobial production. The average zone of inhibition in all three culture media broths ranged from 13.5 mm and 16 mm. Results indicated that there was no statistical significant difference ($P \geq 0.05$) between the antimicrobial activity in the three types of culture media after performing the statistical analysis. The culture medium used for further analysis was the inorganic salts starch media broth (ISP4).

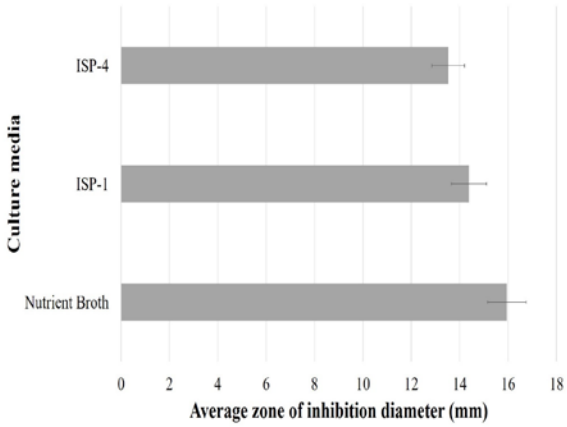

Figure 3. *Streptomyces* strain CSK1 antimicrobial activity against *S. aureus*. The isolate was cultured in three types of broth media: nutrient broth (NB), tryptone-yeast extract broth (ISP-1), and inorganic salts starch broth (ISP-4).

The carbon sources that affect the production of the inhibitory compounds was also tested. Inorganic salts starch media broth was used as a basal media and the starch was substituted with different carbon sources. No carbon source added to the basal media was considered the negative control and starch was used as the positive control. Results in Figure 4 showed that starch, fructose, and glucose produced the highest antimicrobial activity (average zone of inhibition >19 mm) while sorbitol produced the lowest antimicrobial activity (average zone of inhibition < 8 mm) against *S. aureus*. There was no significant difference ($P \geq 0.05$) between the carbon sources that produced high antimicrobial activity. The carbon source used for further analysis was starch.

The nitrogen sources were tested next for antimicrobial activity. Inorganic salts starch broth was used as a basal media and ammonium sulfate was substituted with

different nitrogen sources. The nitrogen sources selected for the test were from different organic and inorganic nitrogen sources and amino acids. No nitrogen source added to the basal media broth was considered the negative control and ammonium sulfate was considered the positive control. The nitrogen source affected the production of inhibitory compounds against *S. aureus* as illustrated in Figure 5. The highest antimicrobial activity was when ammonium sulfate or yeast extract were used as nitrogen sources (average zone of inhibition > 16 mm). There was almost no antimicrobial activity in the negative control (average zone of inhibition > 4 mm). There was no significant difference ($P \geq 0.05$) between the nitrogen sources that produced the highest antimicrobial activity. The ammonium sulfate was used for the analysis of the physical conditions of the inhibitory compounds production.

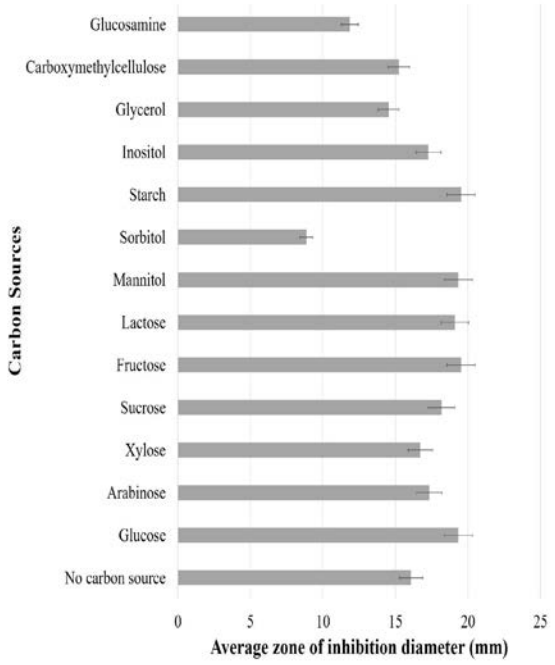

Figure 4. *Streptomyces* strain CSK1 antimicrobial activity against *S. aureus* using different carbon sources added to the optimal culture media (ISP4 as a basal media). No carbon was added to the negative control, and starch was added as the positive control.

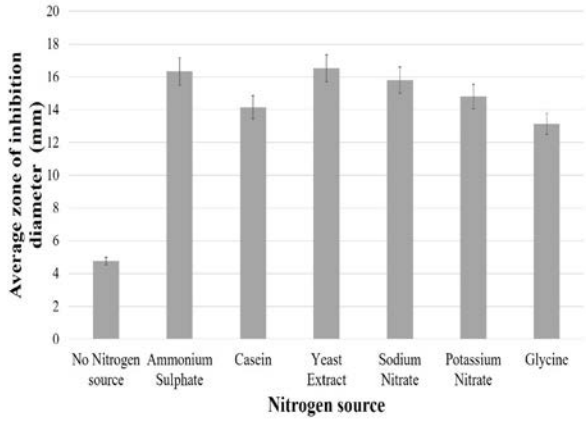

Figure 5. *Streptomyces* strain CSK1 antimicrobial activity against *S. aureus* using different nitrogen sources added to the optimal culture media (ISP4 as a basal media) and the optimal carbon source (Starch). No nitrogen source was added to the negative control while ammonium sulphate was considered as the positive control.

The antimicrobial activity against *S. aureus* were tested at various temperatures and pH values ranged from 25 °C to 37 °C and 5.0 to 9.0, respectively. The controls of the temperature and pH were 28 °C and 7.2. The highest antimicrobial activity against *S. aureus* was observed at temperatures 28 °C and 31 °C, while the lowest was at temperature 34 °C (Figure 6). As for the pH, the antimicrobial activity against *S. aureus* was higher in alkaline conditions rather than acidic conditions (Figure 7). The highest antimicrobial activity was observed in pH 7.2. There was no antimicrobial activity when the initial pH of the broth was adjusted to pH 5.0. There was a significant difference ($P \leq 0.05$) between the results, so the temperature and pH used for further analysis and extraction of metabolites was 28 °C and pH 7.2.

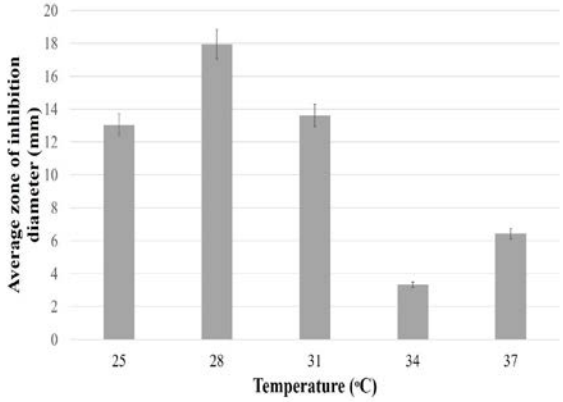

Figure 6. *Streptomyces* strain CSK1 antimicrobial activity against *S. aureus* cultured in the optimal culture media (ISP4, starch and ammonium sulphate) at different temperatures.

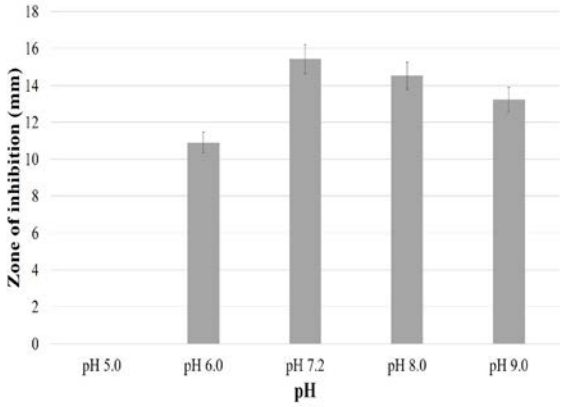

Figure 7. *Streptomyces* strain CSK1 antimicrobial activity against *S. aureus* cultured in the optimal culture media (ISP4, starch and ammonium sulphate, 28 °C) at different pH values.

GC-MS analysis of the ethyl acetate crude extract

After optimizing the conditions, *Streptomyces* strain CSK1 was grown in a larger scale. The metabolites were extracted using ethyl acetate as an organic solvent. The crude extract was collected and analyzed using Gas Chromatography Mass Spectrometry (GC-MS) for identification of metabolites. The samples were filtered using 0.25 µm filters to remove any solid particulates. The GC chromatogram (Figure 8) revealed three major products that appeared after 9.46, 11.60, and 17.93 minutes. The GC profile was completed in 43 minutes, and

no products were identified after 20 minutes. The peak at 9.46 showed a mass spectrum that match the identified compound 3,6-dimethyl-1,4-dioxane-2,5-dione commonly known as lactide. The major peak appeared at 11.6 minutes corresponds to the presence of lactic acid. Finally, the third product appears after 17.93 minutes, which showed a mass profile that corresponds to the presence of n-butyl lactate derivative. The GC peak areas indicated that lactic acid is the major product that is present in 5 times the abundance of the two other products where the relative ratio between the three compounds is 1: 5 : 1. Figure 9 shows the mass spectrum associated to the 11.6 min GC peak with all major ion fragments identified and presented within the figure.

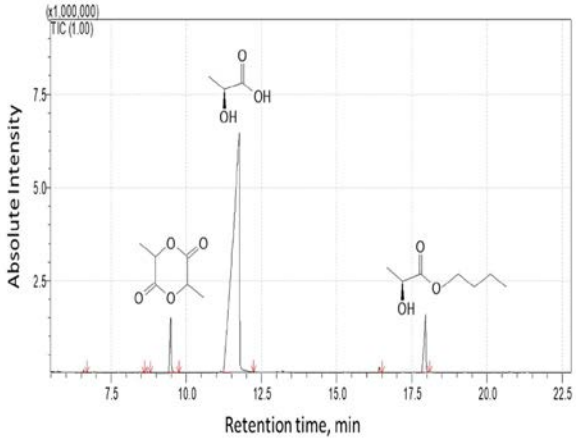

Figure 8. GC chromatogram of the ethyl acetate crude extract. The chromatogram shows three compounds were found in the ethyl acetate crude extract: Lactide appeared at minute 9.46, lactic acid appeared at 11.60 minutes and the last one is butyl lactate at 17.93 minute.

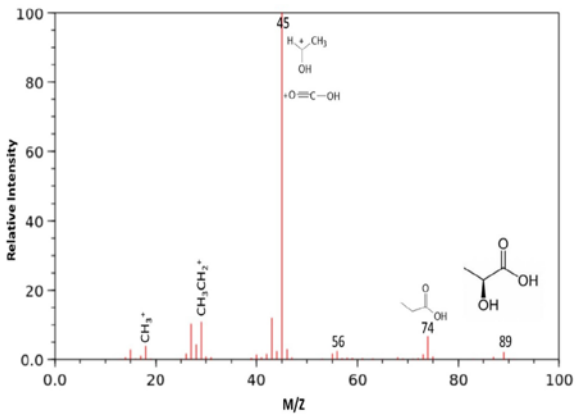

Figure 9. Mass spectrum of the 11.60 min GC peak along with all identified fragments.

3.3. Antimicrobial Activity analysis of the ethyl acetate crude extract

The ethyl acetate crude extract was used for antimicrobial activity analysis against *S. aureus*. The crude extract was dissolved in 1.0 mL distilled water and filter sterilized through 0.22 µm syringe filters. The concentration of the ethyl acetate crude extract was 50.3 mg/mL. Antimicrobial activity of the ethyl acetate crude extract was carried out using well diffusion method. The crude extract was tested for antimicrobial activity against *S. aureus*. The controls used in this test were pure lactic acid as a positive control, pure ethyl acetate and ISP4 broth as negative controls. The antimicrobial activity of the ethyl acetate crude extract as illustrated in Table 2 resulted in a 21 mm zone of inhibition diameter, while the positive control generated a 36 mm zone of inhibition against *S. aureus* (Figure 10).The minimum inhibitory concentration (MIC) of the crude extract was 0.79 mg/mL, which is the lowest concentration to prevent the bacterial growth, and the minimum bactericidal concentration (MBC) is 6.29 mg/mL, which is the lowest concentration to kill the bacteria.

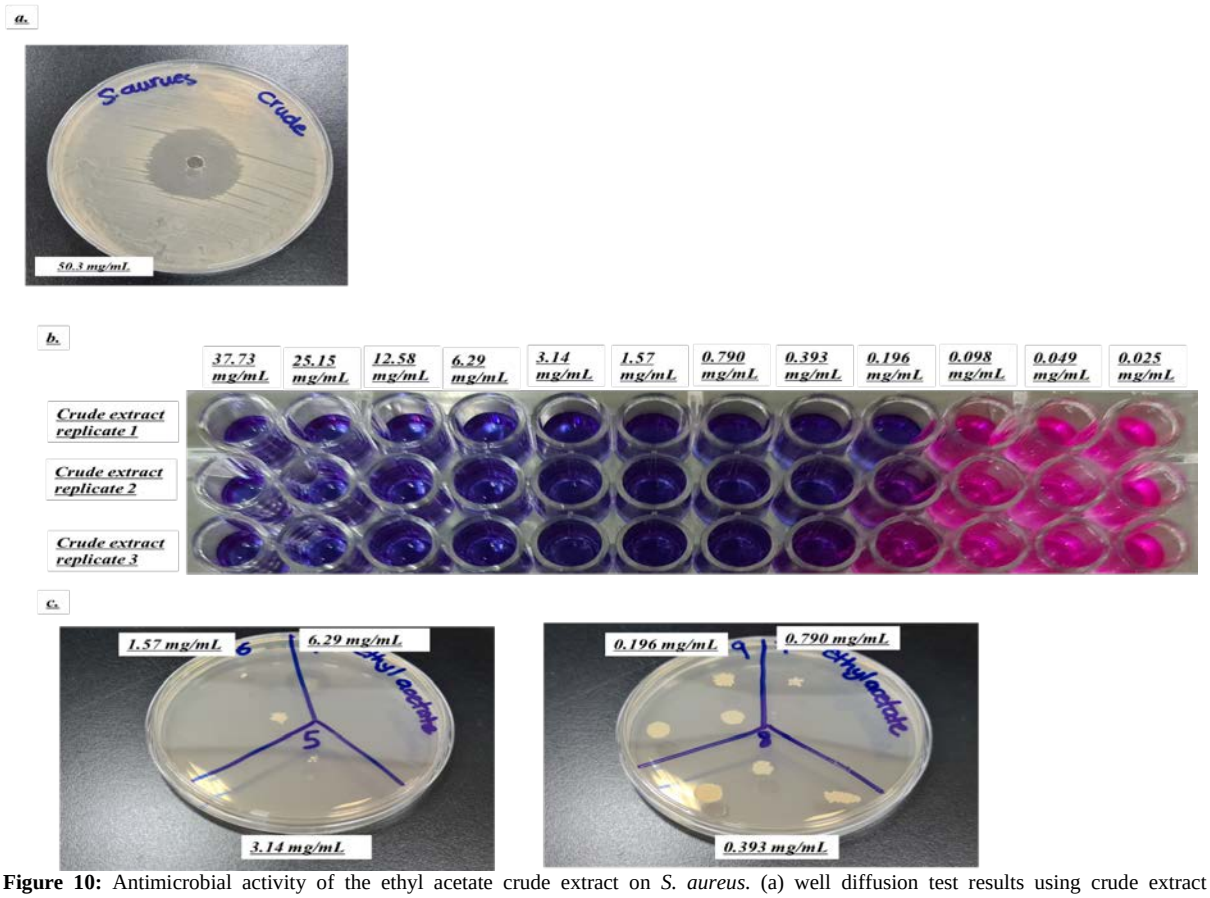

Figure 10: Antimicrobial activity of the ethyl acetate crude extract on *S. aureus*. (a) well diffusion test results using crude extract concentration of 50.3 mg/mL. (b) Minimum Inhibitory Concentration (MIC) test was performed in a 96 well plate, the crude extract was serially diluted with Mueller Hinton broth starting from well one at 37.73 mg/mL followed by two-fold dilutions from 25.15 to 0.025 mg/mL in wells 2 through 12 performed in triplicates (columns A, B, and C). Positive control of tested *S. aureus* growth and negative control presenting only Mueller Hinton medium were assessed in columns G and H (data not shown). (c) Minimum bactericidal concentration (MBC) assessed using 10 µL of crude extract with concentrations ranging from 0.196 to 6.29 mg/mL.

Table 2. Antimicrobial activity of the ethyl acetate crude extract against *S. aureus*.

	Antimicrobial activity (Zone of inhibition in mm)				MIC	MBC
	Crude extract	Control				
		Lactic acid	ISP4 broth	Ethyl acetate		
S. aureus	23.3 ± 2.08	39.7 ± 3.5	0	0	0.79 mg/mL ± 0.00	6.29 mg/mL ± 0.00

4. Discussion

Streptomyces are important microorganisms for the biotechnology industry. They are known producers of many natural products that are beneficial to the human health. Genetic analysis showed that not all secondary metabolites can be activated in a lab setting (Del Carratore *et al.*, 2022). One of the strategies that are used to activate the cryptic metabolic pathways of the *Streptomyces* is through the optimization of the cultural conditions (Maithani *et al*., 2022). In our study, *Streptomyces* strain CSK1 that has been recovered previously from the UAE soil showed an inhibitory activity against the Gram-positive bacteria. The antimicrobial activity was optimized to achieve the highest antimicrobial activity against the tested organism and then proceeded to identify the crude extract through GC-MS.

Streptomyces are known producers of many natural products. The diversity of natural products produced by *Streptomyces* are due to environment and their natural habitat conditions (Donald *et al*., 2022). One of the methods that is used to optimize the production of secondary metabolites and discover new metabolites is the modification of the contents and types of culture conditions. It is important to optimize the culture media components as it was found in some studies that the optimization of the culture media increases the production of secondary metabolites (Kalaiyarasi *et al*., 2020; Norouzi *et al*., 2019). In this study, the cultural conditions that were optimized are culture media broth, carbon sources, nitrogen sources, temperature, and pH. The culture media composition contributes to the activation of the silent metabolic pathways for secondary metabolites production (Antoraz *et al*., 2015). In this study, three different culture media broths were used to check and optimize the production of antimicrobial activity against *S. aureus*. The optimal culture medium that was chosen for further analysis was the inorganic salts starch broth or ISP4 (ISSB). Similarly, Sholkamy *et al*. (2020) demonstrated that the best culture medium for producing an antibacterial and antinematicidal from a *Streptomyces* strain was inorganic salts starch broth. Carbon and nitrogen sources affect the production of secondary metabolites in *Streptomyces*. Some carbon sources are found to suppress the secondary metabolites production such as glucose and

glycerol. *Streptomyces* are found in soil where the carbon sources available are the polysaccharides, so they secrete enzymes to break them down and they do not affect the production of secondary metabolites. Nitrogen sources are important in the production of primary metabolites and secondary metabolites (Romero-Rodríguez *et al*., 2018). In other studies, ISP4 broth media was also utilized for the synthesis of antimicrobial compounds, but after the carbon and nitrogen sources were optimized, the starch and ammonium sulphate were replaced with maltose and casein, respectively (Sebak *et al*., 2021).

In our study, the optimal culture medium that produced high activity against *S. aureus* is the inorganic salts starch media broth (ISP4) that is supplied with starch as a carbon source and ammonium sulphate as a nitrogen source. Previous studies found that using starch as a carbon source yielded the best antibacterial secondary metabolites, which is consistent with our findings (Da Silva *et al*., 2012; Mary *et al*., 2021; Rakesh *et al*., 2014). However, Leulmi *et al*. (2019) reported that starch was not a suitable carbon source for their microorganism's secondary metabolites production since it suppressed their product completely. This shows the importance of the optimization of the carbon sources and their effects on the production of secondary metabolites. The highest antimicrobial activity against *S. aureus* was achieved in our study when ammonium sulphate or yeast extract was used as a nitrogen source, which is consistent with the optimal nitrogen sources for secondary metabolites production in previous studies by Ahmad *et al*. (2017) and Al Farraj *et al*. (2020).

Environmental factors such as pH and temperature were found to affect secondary metabolites production, therefore an optimization step is required (Al-Ansari et al., 2020). In the present study, pH 7.2 and 28 ºC provided the highest levels of antibacterial activity against *S. aureus*. Chandrakar and Gupta (2019) and Khattab *et al*. (2016) reported various results for their optimal temperature and initial pH optimization analysis. Temperatures ranged between 25 and 30 ºC, and initial pH in between 7.0 and 7.5, achieved the highest antimicrobial activity depending on the environment and habitat from which the *Streptomyces* isolates were obtained.

The chromatography profile of the crude extract showed three compounds produced by *Streptomyces* strain CSK1. The compounds are lactic acid, butyl lactate and lactide. The lactic acid was the most abundant compound in the crude extract. *Streptomyces lacticiproducens sp. nov.* was also a lactic acid producer (Zhu et al., 2011), while this strain produced lactic acid in the cell free broth when cultured on Gause synthetic medium containing similar ingredients to inorganic salts starch broth (Zhu *et al*., 2014). Christensen *et al*., 2021 confirmed that bacteria producing lactic acid have antibacterial activity against *S. aureus*. Butyl lactate is a derivative of lactic acid. It is a lactate ester that is used in the food, cosmetic and pharmaceutical industry. It has been used as a food additive as it is considered safe by the Food and Drug administration. Butyl lactate has antimicrobial activity against various organisms that causes food spoilage, which is important to extend the shelf life of food while avoiding the use of toxic chemicals for disinfecting food (Kavčič *et al*., 2014). Lactide is an important compound for the medical industry. It is a cyclic ester that is used to synthesize polylactic acid (PLA). PLA is used to produce bio-based polymeric materials and medical products such as prostheses and membranes in surgery and prosthetics. It is also used in drug delivery and hydrogels due to its biodegradation and safety to human body. Lactide is an expensive dimer due to its difficult and time-consuming synthesis process (Cunha *et al*., 2022).

Using *Streptomyces* bacteria to produce compounds that are important for the industry will certainly reduce the use of harsh chemicals and their high costs. Further exploration of conditions affecting the production of lactic acid, butyl lactate and lactide are necessary for the food and pharmaceutical industries.

5. Conclusions

Using a one strain many compounds (OSMAC) strategy, specifically media optimization on *Streptomyces* strain CSK1 to enhance antibacterial activity against *S. aureus*, the produced compounds (lactic acid, butyl lactate and lactide) may have valuable significance in food and pharmaceutical industries.

Acknowledgments

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