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Effect of pH, Carbon and Nitrogen Sources on Antibiotic Production by *Actinomycetes* Isolates from River Tana and Lake Elementaita, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The escalating concern over antibiotic resistance and its profound impact on public health have underscored the urgent need to explore alternative reservoirs of antimicrobial agents. In this regard, *Actinomycetes* have emerged as a compelling area of investigation due to their remarkable capacity to produce bioactive compounds. Therefore, this study sought to investigate the influence of pH and various carbon and nitrogen sources on the antibacterial activity of *Actinomycetes* isolates collected from Lake Elementaita and River Tana. By examining the effects of these factors, we aimed to gain insights into the optimization of growth conditions and nutrient availability to enhance the production of bioactive compounds with potent antibacterial properties. The *Actinomycetes* isolates used in this

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study were from Lake Elementaita and River Tana, known for their diverse ecological characteristics and potential as sources of bioactive compounds. The isolates were subjected to morphological, biochemical, and molecular techniques to ensure accurate identification. To assess the antibacterial activity of the *Actinomycetes* isolates, they were tested against *E. coli* using the agar well diffusion method. The independent variables examined in this study were pH levels (4, 7, and 9) as well as different carbon sources (fructose and sucrose) and nitrogen sources (urea and sodium nitrate). The diameter of the inhibition zones served as the dependent variable. The data collected on the effects of pH and nutrients on the inhibition zones of *Actinomycetes* isolates were subjected to statistical analysis. One-way ANOVA was performed to assess significant differences in antibacterial activity among the isolates under different carbon and nitrogen preference. Mean values were compared using the LSD test at a significance level (α) of 0.05. Furthermore, the Kruskal-Wallis test was utilized to analyze the pH preferences of the *Actinomycetes* isolates at a significance level (α) of 0.05. The results showed that pH significantly influenced the bioactivity of the *Actinomycetes* isolates, with pH 7 exhibiting the highest inhibition zones against *E. coli.* The isolates displayed varied antibacterial activities depending on the carbon and nitrogen sources provided. Sucrose was the most preferred carbon source, followed by fructose, while urea was the preferred nitrogen source, followed by sodium nitrate. The study concluded that pH and nutrient availability play crucial roles in determining the antibacterial activity of *Actinomycetes* isolates. Other than contributing to our in-depth understanding of the factors influencing the antimicrobial potential of *Actinomycetes*, the results of this study highlight the importance of optimizing growth conditions and nutrient availability to enhance the production of bioactive compounds with potent antibacterial properties. Further investigations and exploration of *Actinomycetes* from diverse environments are recommended to discover new bioactive molecules for combating antibiotic resistance.

Keywords: Actinomycetes; antibacterial activity; pH; carbon; sources.

1. INTRODUCTION

The increasing prevalence of bacterial infections and the emergence of antibiotic-resistant strains have raised significant concerns for global health. Antibiotic resistance refers to the ability of bacteria to withstand the effects of antibiotics, rendering them ineffective in treating infections [1]. This phenomenon poses a grave threat to public health, leading to higher mortality and morbidity rates, particularly among vulnerable populations such as children, the elderly, and individuals with weakened immune systems [2][21]. The impact of antibiotic-resistant bacterial infections is felt worldwide, with Africa experiencing an estimated 4,150,000 deaths annually attributed to antibiotic resistance [3]. In Kenya, bacterial infectious diseases contribute to a substantial mortality rate, estimated at approximately 26%. Antibiotic-resistant bacterial strains are a significant factor contributing to the mortality burden in the country [4]. Healthcare facilities, including Kenyatta National Hospital, have reported a considerable number of fatalities caused by infections from antibiotic-resistant bacteria [5]. These alarming statistics highlight the urgent need to address antibiotic resistance and explore alternative sources of antimicrobial agents.

Actinomycetes, a group of bacteria found in diverse environment, have attracted considerable attention due to their ability to produce bioactive compounds, including antibiotics [5] [6] [8]. *Actinomycetes* have been a rich source of novel antibiotics, contributing to the development of numerous life-saving drugs. The *Actinomycetes* isolated in various regions of the world have revealed many secondary metabolites of polyketide, cyclo dipeptides, alkaloids and terpenes that have antibiotic activity against pathogenic bacteria [7] [27] [28]. This has underscored the importance of exploring new *Actinomycetes* strains and optimizing conditions for antibiotic production. [8] [10]. Nutritional sources such as carbon, minerals, nitrogen and factors like pH, incubation period and temperature profoundly affect antibiotic activity of antibiotics synthesized by bacteria [8] [29]. In most *Streptomyces* species, antimicrobial synthesis starts after seven days of incubation and at its highest at ten days. The optimum pH for production antibiotic with the highest activity ranges from 6 to10 with the highest level at pH of 8 [9].

In most bacteria that produce antibiotic, polysaccharides and oligosaccharides are better than glucose as a carbon source because

glucose is rapidly utilized for cellular material synthesis and acts as an inhibitor of secondary metabolite production [10] [14]. In contrast, oligosaccharides would be less rapidly utilized hence available during antibiotic synthesis [10]. *Streptomyces* strains produce antibiotics with the highest activity when grown in the media containing glucose, then sucrose, mannose, fructose, mannitol, rhamnose and xylose, respectively [28]. No antibiotics activity is revealed when *Streptomyces* grow in media containing raffinose, lactose, maltose and galactose as only source carbon [9] [15].

Streptomyces species prefer sodium nitrate, peptone and yeast extract as source of nitrogen for the effective growth and synthesis of high activity antibiotics [9]. The antibiotic produced by *S. cuspidosporus* when growing in media fortified with fructose, sucrose and glucose was found to have better antibacterial activity than the one in a media containing complex carbon sources such as polysaccharides [11] [30,31]. The organisms also preferred ammonium sulphate, sodium nitrate and beef extract as prime nitrogen sources in biosynthesis of antibiotic with highest activity. For the same organism pH 9 was the optimum for the biosynthesis of antibiotic with the highest activity [11].

A research to understand role of nitrogen and carbon on the antibiotic activity of *Streptomyces* from estuarine fish *Chanos chanos*, revealed that the antibacterial agent with high activity was obtained in ISP-2 media [8].This media contains yeast extract, malt and glucose. The media was reported to increase growth, pigment production and biosynthesis of antibiotic with high activity [17]. A study by Abdelghani reported that 1% concentration of malt produced antibiotic with high activity in *S. albovinaceus.* In growth media containing 0.835% of yeast extract and 0.55% glucose increased the biosynthesis of high activity antibiotic [8].

A co-culture containing *Streptomyces rimosus*-OG95 and *Streptomyces xinghaiensis*-OY62 synthezed an effective antibiotic in media supplemented with potassium nitrate and casein but could not produce the same effective antibiotics in a media singly supported by casein or potassium nitrate [12]. The organisms produced clear inhibition zone of about 38.3 mm against *Enterococcus faecalis*. The same organism was not able to produce antibiotic while growing in media containing peptone, malt extract, potassium nitrate and ammonium nitrate with effective activity against the test organisms [12]. This organisms was able to produce antibiotic with high activity against *E. faecalis*, *C. jejuni, B. subtilis* and *P. aeruginosa* in media fortified with glucose, galactose, maltose, sucrose, glycerol and starch [12].

This study aimed at investigating the effects of pH, sucrose, fructose, urea and sodium nitrate on antibacterial activity of six *Actinomycetes* isolated from River Tana and Lake Elementaita. The study will help to determine the optimum conditions for the growth and production of antibiotics from these *Actinomycetes*. Understanding these preferences and optimizing growth conditions can enhance antibiotic production and potentially lead to the discovery of new antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Sample Collection Site

The samples for this study were collected from Lake Elementaita and River Tana, two important ecosystems in the region. The selection of these sites was based on their ecological significance and relevance to human activities such as agriculture, livestock farming, and fishing. Lake Elementaita, located at GPS coordinates 0°26'59.99" N 36°14'60.00" E, is a freshwater lake surrounded by diverse land uses. The activities in the area include charcoal burning, salt harvesting, agriculture, and livestock farming. These human interventions may have an impact on the microbial communities present in the lake [13]. River Tana, on the other hand, serves as a vital water source for various purposes, including crop irrigation, fishing, and livestock farming. The sampling points along the river were Kathungu (-0.28028, 38.11425), Kamarandi (-0.37323, 37.93167), Kamanyaki (-0.35923, 37.96761), and Kamaindi (-0.4354, 37.96627). These points were selected to represent different locations along the river with varying land uses and human activities.

At each sampling point, environmental samples were collected using a systematic approach. These samples included soil, water, and sediment samples, which were carefully collected to ensure representative coverage of the study area. The six *Actinomycetes* were isolated from samples taken from these points and identified through morphological, biochemical and molecular techniques [21].

2.2 Data Collection

2.2.1 Effect of pH, sucrose, fructose, urea and sodium nitrate on antibacterial properties of *Actinomycetes* isolates

The test for suitable pH for the maximum activity of antibacterial agents was investigated by growing the microorganism isolates in 10 ml nutrient broth tubes of varying pH (4, 7 and 9) at 28 ˚C for 10 days [20] [22]. The culture was centrifuged for 10 minutes at 2500 rpm and supernatant filtered, extracted using ethyl acetate (5 ml) to extract the metabolites. The resultant metabolite was tested for bioactivity against *E.coli* using agar well diffusion criteria. The Muller-Hinton agar plates were evenly inoculated with *E. coli*. Then by means of sterile wet swab Muller-Hinton agar (MHA) plates were inoculated by even streaking of the plate surface [14]. The agar wells (eight millimeters) were made in the inoculated agar using a sterile cork borer and applied with 0.2ml of pure culture of the isolates. The experiments were replicated thrice for every pH (4, 7 and 9. The plate were incubated for 24 hours at 37˚C. Then resultant zones of inhibition measured using a ruler [12].

The optimum fructose, sucrose, urea and sodium nitrate for effective antibacterial activity was determined by culturing the isolates of interest in 10 ml of nutrient broth fortified with 30 mg and 50 mg of carbon sources: sucrose and fructose and nitrogen sources: urea and sodium nitrate at 28 ˚C for 10 days. The culture were centrifuged for 10 minutes at 2500 rpm and supernatant filtered, extracted using ethyl acetate (5 ml) to extract the metabolites and their antibacterial activity against *E.coli* determined using the procedure above [14].

2.3 Data Analysis

The data collected on the effects of pH and nutrients on the inhibition zones of *Actinomycetes* isolates were subjected to statistical analysis. One-way ANOVA (analysis of variance) was performed to evaluate the significant differences in antibacterial activity among the isolates under different nutrient conditions. The mean values of the inhibition zones were compared using the LSD (Least Significant Difference) test at $\alpha = 0.05$ to determine the specific pairwise differences between the groups. Kruskal-Wallis test was used to analyze data on the effect of different pH on the inhibition zones of *Actinomycetes* isolates. Furthermore, the carbon and nitrogen preferences of the *Actinomycetes* isolates were analyzed using the Kruskal-Wallis test. The results of the Kruskal-Wallis test were further analyzed using the Dunn's post hoc test with the Bonferroni correction for multiple comparisons to determine the specific differences between the groups in R studio version 4.2.3.

3. RESULTS

3.1 Effect of pH on the Antibacterial Activity of *Actinomycetes* Isolates

The antibacterial activity of *Actinomycetes* isolates at pH 4 was assessed. Among the isolates, LEL2201 demonstrated the highest mean inhibition zone of 6.0 mm (*Mdn* = 6.0 mm), indicating significant antibacterial activity. Isolate RT2202 exhibited a mean inhibition zone of 5.0 mm (*Mdn* = 5.0 mm), while RT2207 showed a mean inhibition zone of 5.0 mm (*Mdn* = 5.0 mm). These values were significantly different from the control groups (H (7) = 16.403, p = 0.022; Table 1).

The antibacterial activity of *Actinomycetes* isolates at pH 7 was examined. Among the isolates, LEL2201 demonstrated the highest mean inhibition zone of 8.0 mm (*Mdn* = 8.0 mm), indicating significant antibacterial activity. Isolate RT2204 exhibited a mean inhibition zone of 4.0 mm (*Mdn* = 4.0 mm), while RT2205 showed a mean inhibition zone of 2.0 mm (*Mdn* = 2.0 mm). These values were not significantly different from each other (H (7) = 13.707, p = 0.057; Table 1). The antibacterial activity of *Actinomycetes* isolates at pH 9 was investigated. Among the isolates, LEL2201 displayed the highest mean inhibition zone of 12.0 mm (*Mdn* = 12.0 mm), indicating significant antibacterial activity. Isolates RT2205 and RT2207 both showed a mean inhibition zone of 3.0 mm (*Mdn* = 3.0 mm), while RT2202 exhibited a mean inhibition zone of 2.0 mm (*Mdn* = 2.0 mm). These values were not significantly different from each other (H (7) = 16.679, p = 0.0282; Table 1).

3.2 Effect of Sucrose, Fructose, Urea and Sodium Nitrate on Antibacterial Activity of the Actinomycetes Isolates against E. coli

Significant differences were observed in the effect of fructose at both 30 mg and 50 mg concentrations on the inhibition zones of the *Actinomycetes* isolates against *E. coli* (30 mg: *F*

Table 1. Effect of pH on the Antibacterial Activity of *Actinomycetes* Isolates isolated from Lake Elementaita and River Tana in Kenya

PH Level	Utilize	N	Mean	Med	Min	Max	Kruskal-Wallis Test
PH4	RT2201	3	5.33	4.0 [c]	4.00	8.00	*H* (7) =16.403
	RT2202	3	4.33	5.0 [b]	2.00	6.00	*p* = 0.022
	RT2204	3	6.67	6.0 [b]	4.00	10.00	
	RT2205	3	5.00	3.0 [c]	2.00	10.00	
	RT2207	3	4.33	5.0 [b]	2.00	6.00	
	LEL2201	3	9.00	10.0 [a]	6.00	11.00	
	Tetracycline	3	0.00	0.0 [e]	0.00	0.00	
	Saline	3	1.73	1.7 [d]	1.60	1.90	
PH7	RT2201	3	3.33	3.0b	2.00	5.00	*H* (7) =13.707
	RT2202	3	2.83	3.0b	1.50	4.00	*p* = 0.057
	RT2204	3	6.33	4.0b	3.00	12.00	
	RT2205	3	5.67	2.0bc	1.00	14.00	
	RT2207	3	2.67	3.0b	2.00	3.00	
	LEL2201	3	7.00	8.0a	4.00	9.00	
	Tetracycline	3	0.00	0.0d	0.00	0.00	
	Saline	3	2.03	2.0bc	1.80	2.30	
PH9	RT2201	3	4.00	4.0b	4.00	4.00	*H* (7) =16.679
	RT2202	3	4.00	3.0c	3.00	6.00	*p* = 0.0282
	RT2204	3	6.17	5.0b	4.50	9.00	
	RT2205	3	4.50	3.0c	1.50	9.00	
	RT2207	3	4.50	2.0c	1.50	10.00	
	LEL2201	3	10.67	12.0a	5.00	15.00	
	Tetracycline	3	0.00	0.0d	0.00	0.00	
	Saline	3	2.03	1.9cd	1.40	2.80	

The figures followed by the same letter in columns are not significantly different at α = 0.05.

(7, 16) = 37.11; *p*<.0001; 50 mg: *F* (7, 16) = 77.71; *p*<.0001). The RT2202 displayed the highest activity with an inhibition zone of 12.83 mm at 30 mg fructose and 13.5 mm at 50 mg fructose. In contrast, RT2205 exhibited the lowest activity with inhibition zones of 2.15 mm and 2.20 mm at 30 mg and 50 mg fructose, respectively (Table 2). Similarly, there was a significant effect of sucrose at both 30 mg and 50 mg concentrations on the inhibition zones of the *Actinomycetes* isolates (30 mg: *F* (7, 16) = 45.55; p<.0001; 50 mg: *F* (7, 16) = 27.63; p<.0001). The LEL2201 displayed the highest activity with an inhibition zone of 12.67 mm at 30 mg sucrose and 10.33 mm at 50 mg sucrose. In contrast, RT2202 exhibited the lowest activity with inhibition zones of 1.33 mm and 1.99 mm at 30 mg and 50 mg sucrose, respectively (Table 2). Furthermore, urea at both 30 mg and 50 mg concentrations had a significant effect on the inhibition zones of the *Actinomycetes* isolates (30 mg: *F* (7, 16) = 51.49; p<.0001; 50 mg: *F* (7, 16) = 166.46; p<.0001). RT2201 exhibited the highest activity with inhibition zones of 14.44 mm and 15.0 mm at 30 mg and 50 mg urea, respectively. In contrast, RT2204 displayed the lowest activity with inhibition zones of 2.32 mm and 2.47 mm at 30 mg and 50 mg urea, respectively (Table 2). Similarly, there was a significant effect of sodium nitrate at both 30 mg and 50 mg concentrations on the inhibition zones of the isolates (30 mg: *F* (7, 16) = 13.03; p<.0001; 50 mg: *F* (7, 16) = 21.40; p<.0001). LEL2201 exhibited the highest activity with inhibition zones of 3.42 mm and 2.59 mm at 30 mg and 50 mg sodium nitrate, respectively. In contrast, RT2201, RT2202, RT2205, and RT2207 showed the lowest activity with inhibition zones of 1.9 mm at both 30 mg and 50 mg sodium nitrate (Table 2).

3.3 Fructose, Sucrose, Urea and Sodium Nitrate preference by *Actinomycetes* for the Biosynthesis of Antibiotic with Activity against *E. coli*

The carbon and nitrogen preferences of selected *Actinomycetes* isolates for the biosynthesis of antibiotics with activity against *E. coli* were significantly different ($p < 0.05$) ($H = 35.7816$, N = 48, $p < 0.0001$; Table 3). Among the tested carbon sources, the isolates showed a preference for sucrose as the principal carbon

Table 2. Effect of nutrients on zones of inhibition of selected *Actinomycetes* isolated from R. Tana and L. Elementaita against *E. coli*

Isolates	N	Fructose		Sucrose		Urea		NaNO3	
		30 mg	50 mg	30 mg	50 mg	30 mg	50 mg	30 mg	50 mg
RT2201	24	3.48^{c}	10.2^{a}	4.17^{c}	4.67^{bc}	14.4^{a}	15.0^{a}	1.99^{b}	1.99^{c}
RT2202	24	12.83^{a}	13.5^{a}	1.33^{de}	3.83^{c}	3.78^{c}	3.33^{b}	1.99^{b}	1.99^{c}
RT2204	24	4.35^{bc}	2.80^{bc}	4.50^{c}	5.83^{b}	2.32^{d}	1.90^{cd}	2.65^{ab}	2.47^{bc}
RT2205	24	2.15d	2.20^{c}	6.50^{b}	1.83^{d}	3.1^{cd}	2.32^{d}	2.15^{b}	1.99^{c}
RT2207	24	5.40^{b}	3.10^{b}	4.17^{c}	6.00^{b}	2.7^{cd}	2.70^{bc}	1.99^{b}	1.99^{c}
LEL2201	24	6.07^{b}	10.6^{a}	12.67^{a}	10.3^{a}	10.6^{b}	12.3^{a}	3.42^{a}	2.59^{b}
Saline	24	0.00^{e}	0.00^{c}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{c}	0.0^{d}
Tetracycline	24	2.15^{cd}	2.90^{b}	2.00^{d}	2.00^{d}	2.00^{d}	2.00^{d}	2.99^{a}	2.99^{a}
LSD (*p<0.05*)		1.451	1.36	1.74	1.83	1.44	1.26	1.36	1.24
Cv (%)		16.20	12.76	22.76	24.48	14.5	7.99	23.26	17.1

The figures in the column followed by same letter are not significantly different at α = 0.05

Table 3. Carbon and nitrogen preference by *Actinomycetes* isolates showing most isolates preferred sucrose and least preferred sodium nitrate

Utilize	N	Mean	Median	Minimum	Maximum
Fructose	48	4.36	2.25^{b}	0.00	15.00
Sucrose	48	4.36	4.00^{a}	0.00	15.00
Urea	48	4.08	2.00^{b}	0.00	18.00
sodium nitrate	48	1.23	1.00^{c}	0.00	4.00
Kruskal-Wallis Test			H = 35.7816	N = 48	p <.0001

The figures followed by the same letter in columns are not significantly different at α = 0.05.

source, with a median inhibition zone of 4 mm, indicating its effectiveness in the synthesis of antibiotics with the highest activity against *E. coli.* Fructose followed with a median inhibition zone of 2.25 mm. In terms of nitrogen sources, the *Actinomycetes* isolates preferred urea as the primary nitrogen source, with a median inhibition zone of 2 mm, for the biosynthesis of antibiotics with the highest activity against *E. coli*. On the other hand, sodium nitrate was the least preferred nitrogen source, with a median inhibition zone of 1 mm.

4. DISCUSSION

4.1 Effect of pH on the Antibacterial Activity of *Actinomycetes* Isolates against *E. coli*

Bacterial infections are a growing concern worldwide, with antibiotic-resistant strains causing significant mortality and morbidity, especially among vulnerable populations such as children, the elderly, and immune-compromised individuals [2] [3]. To combat this issue, researchers are exploring alternative sources of antimicrobial agents, and *Actinomycetes* have emerged as promising candidates. *Actinomycetes* are a group of bacteria known for their ability to produce bioactive compounds, including antibiotics, which can effectively combat pathogenic bacteria [5] [6]. In this study, we investigated the impact of pH on the bioactivity of *Actinomycetes* isolates against *E. coli*, a common pathogenic bacterium. We found that pH had a significant effect on the antibacterial activity of the isolates. Different pH levels influenced the production of metabolites with varying inhibitory effects on *E. coli*. Interestingly, certain isolates displayed higher inhibition zones at pH 7 compared to pH 4 and pH 9. This suggests that these *Actinomycetes* isolates have adapted to grow and produce metabolites under specific pH conditions. They may have developed metabolic characteristics that enable them to thrive and effectively combat bacteria in their preferred pH range [23]. Indeed, *Actinobacteria* have been shown to possess remarkable adaptability, allowing them to not only thrive in typical environments but also withstand extreme conditions. These extremophilic environments are characterized by factors such as acidic or alkaline pH levels, wide temperature ranges, salinity, high levels of radiation, limited moisture, and nutrient scarcity. The physiological and metabolic flexibility of *Actinobacteria* enables their survival and growth in the face of hostile and unfavorable

circumstances [16] [25]. Their unique capabilities equip them to endure and flourish in conditions that would pose significant challenges or even prove fatal for many other organisms. The present findings are consistent with the previous research conducted by Ripa *et al.* [12], which demonstrated the impact of pH on the bioactivity of antibiotics produced by *Actinomycetes* and other bacterial species. It has been consistently observed that pH levels ranging from 6 to 10 are optimal for achieving maximum antibacterial activity, with pH 8 often being the most favorable. These results also align with a recent study by Lertcanawanichakul and Sahabuddeen [15], who reported that *Streptomyces* sp. KB1 (TISTR 2304) exhibited optimal biosynthesis of bioactive compounds at a pH level close to 7, despite a suitable BCAs production pH range of 5 to 9. Thus, the preference for pH 7 observed in our study reinforces the significance of pH in influencing the bioactivity of *Actinomycetes* and supports the notion that specific pH conditions can enhance the production of bioactive compounds with potent antibacterial properties. The observed higher antibacterial activity at pH 7 could be attributed to the isolates' preference for aerobic conditions and their sensitivity to acidic and alkaline pH levels, which can affect secondary metabolism [16]. The significance of pH in influencing the bioactivity of *Actinomycetes* highlights the importance of optimizing growth conditions to enhance the production of bioactive compounds with potent antibacterial properties.

4.2 Effect of Sucrose, Fructose, Urea and Sodium Nitrate on Antibacterial Activity of the *Actinomycetes* Isolates against *E. coli*

Actinomycetes are renowned for their diverse metabolic capabilities and their ability to utilize a wide range of carbon and nitrogen sources for growth and the production of secondary metabolites due to their possession of enzymes that are able to metabolize urea, fructose, sucrose and sodium nitrate allowing them to grow and produce various metabolites [17][24][26]. In our study, we observed that the utilization of fructose led to increased antibacterial activity in isolate RT2201, while there was no significant change in activity with increasing concentrations of urea, sucrose, and sodium nitrate. This finding suggests that fructose may play a role in promoting the production of bioactive compounds with antibacterial properties in this particular isolate. In contrast, the antibacterial properties of isolates RT2202, RT2204, and RT2207 were not affected by changes in the concentrations of these nutrients, indicating a reduced impact of nutrient availability on secondary metabolite synthesis in these isolates. These results are consistent with the findings of previous studies that have investigated the influence of different sugars on the production of bioactive compounds in *Actinomycetes*. For example, researchers have reported that the presence of glucose and other monosaccharides can strongly decrease the production of certain bioactive compounds, such as oleandomycin, avilamycin, nystatin, spiramycin, and neomycin [18]. This phenomenon has been attributed to carbon catabolized repression, which is a regulatory mechanism that inhibits the synthesis of secondary metabolites in the presence of easily metabolizable carbon sources [19] [20]. In comparison to our study, where fructose was found to enhance antibacterial activity in one isolate, the previous studies focused on the inhibitory effects of glucose and other monosaccharides on the production of bioactive compounds. This highlights the diverse responses of *Actinomycetes* to different carbon sources and the complexity of metabolic regulation in these organisms. While glucose and other monosaccharides have been reported to repress secondary metabolite synthesis in certain *Actinomycetes* strains [15], our findings suggest that fructose may have a stimulating effect on the production of bioactive compounds in isolate RT2201. This observation is consistent with the findings of Selvin *et al.* [19], who reported that optimal production of antimicrobial compounds in *Streptomyces* strains was achieved using medium supplemented with fructose or other sugars such as glucose and glycerol. These variations in response to different sugars underscore the importance of understanding the specific metabolic characteristics and regulatory mechanisms of individual *Actinomycetes* isolates in order to optimize the production of bioactive compounds with desired antibacterial properties.

Sucrose was the preferred carbon source, followed by urea, fructose, and sodium nitrate, in the biosynthesis of effective antibiotics against *E. coli* by the *Actinomycetes* isolates. The preference for urea as a nitrogenous source over sodium nitrate may be attributed to the ability of *Actinomycetes* to produce urease, which breaks down urea into carbon dioxide and ammonia [13]. These observations align with the findings of [11] where different nitrogen sources resulted

in varying levels of inhibition zones in *Streptomyces cuspidosporus*. Additionally, Ripa *et al.* [9] reported that *Streptomyces* isolates preferred sodium nitrate, yeast extract, and peptone as nitrogen sources for the synthesis of highly active antibiotics against test organisms. *Actinomycetes* exhibit a strict preference for utilizing various sugars for growth and secondary metabolite biosynthesis. The preferential utilization of sucrose over fructose may be attributed to the slower hydrolysis of sucrose compared to fructose, resulting in slower organism growth and better carbon availability during antibiotic synthesis [32][29]. Furthermore, Fructose-1,6-bisphosphate, a product of fructose metabolism, has been shown to inhibit expandase enzymes involved in antibiotic biosynthesis, further supporting the preference for sucrose [20] [33]. These findings regarding the effects of sucrose and fructose on the antibacterial activity of *Actinomycetes* are consistent with previous studies [9] reported that *Actinomycetes* extracts from sucrose-enriched media exhibited larger zones of inhibition compared to fructose-enriched media. Similarly, Adeyemo *et al.* [1] demonstrated that *Actinomycetes* cultures fortified with various sugars, including sucrose and glucose, displayed high antibacterial activity against test organisms.

5. CONCLUSION AND RECOMMENDATION

The study concluded that pH and nutrient availability play crucial roles in determining the antibacterial activity of *Actinomycetes* isolates. There was a significant ($p<0.05$) difference in the effect of fructose, sucrose, urea and sodium nitrate at 30 and 50 mg to the bioactivity of metabolites of *Actinomycetes* isolates against *E. coli,* however the isolates preferred sucrose to others. Other than contributing to our in-depth understanding of the factors influencing the antimicrobial potential of *Actinomycetes*, the results of this study highlight the importance of optimizing growth conditions and nutrient availability to enhance the production of bioactive compounds with potent antibacterial properties. Further investigations and exploration of *Actinomycetes* from diverse environments are recommended to discover new bioactive molecules for combating antibiotic resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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