Production and Escalation of L-Lysine via Bacterial Fermentation Utilizing Streptococcus Sp

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Abstract

Background and Aims

The importance of L-lysine as an essential amino acid in the nutrition of human beings has made it desirable supplement of the diet in recent years. It can be produced in different ways among them fermentation is the most economical and practical means of producing lysine. In this method low temperature, low pressure and low-cost carbon sources are used and a biological form of lysine (L-lysine) is produced.

Methods

In the present study, the production of L-lysine was achieved through fermentation developed from locally isolated bacterial strains. In total, twenty-nine (29) bacterial strains were isolated and tested using paper chromatographic technique. Six different parameters for optimization were scrutinized for improved bacterial growth and significant yield of lysine was obtained using selected strains.

Key Results

For *Streptococcus sp.* molasses media with vitamins (w) formed 24.4 g/L, 40º C generated 24.4 g/L, addition of 1mM solution of metal ion (Mg) yielded 20.4 g/L, pH 6.5 delivered 6 g/L, fermentation period of 96 hours engendered 24.4 g/L, and 0.3 mL of inoculum results in 9.2 g/L of lysine.

Conclusions

Laboratory scale production of L-lysine was carried out using 1 L Erlenmeyer flask. For *Streptococcus sp.* 23.4 g/L of lysine was produced after optimized conditions.

Literature Review

In primeval eons, bacterial fermentation had been the avocation of assiduous deliberation regarding rampant perspective, in addition to the conformation of their lysine production response as being documented in conventional literature. Under mentioned literature review will subsidize towards a glance of attempt made around the universe in the discipline of microbiology and fermentation biotechnology:

Ezemba *et al.,* 2016 manifested the assembly of lysine by *Microbacterium lacticum* via immersed cultivation by many hydro-carbons, sweeten plus nitrogen cradles. Consequence for variable deliberation of carbons plus nitrogen cradles onto the L-lysine growth revealed that 4 % D-glucose and 1 % ammonium sulfate correspondingly improved L-lysine assembly.

Ekwealor and Obeta, 2005 evinced the lysine assembly through *Bacillus megaterium*. Toting of 0.01unit/mL penicillin in the direction of the fermentative agent, instantaneously when inoculated, enthused escalation plus substantially improved lysine accretion.

Husssain *et al.,* 2015 worked on the optimized cultivation media intended for L-lysine assembly using *Corynebacterium glutamicum.* Glucose was considered as the finest cradle of carbon along with ammonium sulfate as superlative nitrogen cradle was then improved.

Irshad *et al*., 2015 evaluated the bulk assembly plus retrieval of lysine using bacterial cultivation via *Brevibacterium flavum*. Soft creamy clean quartzes of lysine (25g) achieved using 95 % ethanol. Great attentiveness of L-lysine as quartzes formed having 62.5% pureness plus strenuous cultivated chowder having 8 % L-lysine could be securely expended in rooster consignments in the form of a cradle for L-lysine.

Nasab *et al*., 2007 described the fermentative assembly of L-lysine by *C. glutamicum* using various carbon sources. Outcomes of fermentative trials displayed that the supreme production resembled to treacle is 48 g/L with the inoculation time of 96hours.

Rastegari *et al*., 2013 worked on the enhancement in the assembly of lysine through above-countenance of Asparto-kinase within *C. glutamicum*. The fallouts of L-lysine test as given by Chin-nard technique indicated that L-lysine fabrication improved almost double paralleled with the parental type, which end up as amplified replica figures like *lys-C* genetic factor in the mutant species.

Reza *et al.,* 2017 revealed the widespread valuation of the lysine assembly method using cultivation of sugar-cane treacle. The finest lodge plan displays full produce return (0.31g lysine g-1 D-glucose) plus yield (1.99gL-1h-1), attaining 26.5 % gain proceeding asset having a reimbursement time of 3.8years, lessening aquatic plus vigor ingestion, as well as having a little possible ecological influence (PEI) directory.

Theodora and Bustard, 2005 deliberated the fermentative assembly of lysine via *Corynebacterium glutamicum* using trans-membrane passage plus metabolic fluidity investigation. Digested fluidity investigation, which remains reflected to stay an actual influential instrument giving treasured evidence about holdups around the assembly of required digesters, is too enclosed as a relative to L-lysine exudation in combination through the importance of conveyance instruments.

Xafenias *et al.,* 2017 described the improvement of an-aerobic L-lysine assembly under *Corynebacterium glutamicum* electro-fermentations. Current report shows for the 1st time that cathode conductor situations joint through carbon dioxide plus AQ2s acting as a red-ox facilitator could ominously increase equally the produces as well as the piles of L-lysine assembly by *C. glutamicum*.

Introduction

Amino acids are synthesized through microorganisms from the last 50 years. The most extensive formed amino acid (roughly 900,000 tons each year) occurs as L-glutamic acid, trailed by L-lysine (420,000 tons each year) as well as DL-methionine (350,000 tons each year) whiles the rest of the amino acids trail behind (Tryfona and Bustard, 2005). L-lysine is considered as the chief restraining amino acid that every

single known cereal morsel has, therefore it takes a greater aptitude for increasing the protein content of cereal-based foods mostly around developing countries due to their huge reliance on cereal crops. It is also used for production of antibodies, enzymes and hormones inside the human body as well synthesis of antihypertensive agents and neutralizer for analgesic (Sadoul et al., 2008). Amino acid produced via fermentation has currently touched in a phase where it is frolicking an indispensible part for the cradle of usual amino acids at several manufacturing levels.

Micro-organisms that are already stated to produce L-lysine contain, '*Bacillus megaterium* (Ekwealor & Obeta, 2005), *Brevibacterium linens*, *Streptomyces albulus IFO* (Shih *et al*., 2006), *Brevibacterium flavum*, *M. methylophilis* (Ishikawa et al., 2008), *B. lactofermentum* (Tosaka et al., 1979), *B. subtilis* and *Bacillus laterosporus* (Umerie *et al*., 2000'). As per nitrogen cradles, numerous inorganics plus organic salts as well as compounds like ammonium salts along with other parallel compounds, urea, pure proteolytic organic affluences like peptone plus casein hydrolysate plus yeast extract plus corn steep liquor plus soybean protein hydrolysate, plus numerous other extricates of vegetal as well as animal tissues may possibly be used (Nasab et al., 2007). The final outcome is usually staged as a salt like lysine-HCl (lysine mono-chloridrate) (Junior et al., 2016) Nonetheless; it can similarly be staged as L-lysine liquid articulations or in granulated configuration. The present work is planned to maximize yields of free lysine obtained in a culture broth by using bacterial strain recover from various soil and water samples.

Materials And Methods

Isolation of bacteria

Techniques of microbial isolation undergo drastically changed throughout the past 50 years, as of a labor perspective through increasing mechanization, as well as in concern to the technology embroiled, and hence promptness and accuracy. The isolation of bacterial strains was done stepwise as:
Preliminary screening test

Final screening test

Preliminary screening test

Preliminary screening was encompassed to restraint the presence of L-lysine by the amassed bacteria. The test consists of following parameters:
Collection of samples

Isolation medium

Preparation of medium plates

Serial dilution method

Medium for sub-culturing of bacteria

Streak plate method

Screening medium for L-lysine production

Cultivation

Detection of L-lysine

Collection of samples

Fifteen different samples were collected from the adjacent areas of Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories complex Lahore during 2016-2017. The sample contains soil, soil with bird feces, soil with sanitary leakage, bird feces, sewage water, and waste water, soil with vegetation, tap water as well as drain water. All the samples were labeled and weighed as 5g for soil samples and 5mL for water samples in test tubes and petri plates respectively.

Isolation medium

A modified medium for the isolation of lysine producing bacteria (e.g., *Corynebacterium glutamicum*) was employed having the composition as described by Nasab et al., (2007). Quantities in solution consumed were: Glucose 5 g, Peptone 2.5 g, Yeast extracts 2.5 g, NaCl 0.6 g, $MgSO_4.7H_2O$ 0.7 g, $MnSO_4.H_2O$ 0.02 g, K_2HPO_4 0.2 g, KH_2PO_4 0.2 g, $CaCO_3$ 2 g, $(NH_4)_2SO_4$ 3 g, Urea 1.2 g, Agar 3.4g. All these ingredients were accurately weighed and dissolved with the assistance of steady rate of stirring in 500 mL Pyrex beaker using distilled water. More distilled water was supplemented to the relevant mixture and the final volume was made to 250 mL. To facilitate the process of dis-solvation of agar, the beaker containing the medium was placed on the hot plate and was supplied with high temperature until the apparent light beige solution was formed. The pH was adjusted to 7.0 by using conc. HCl plus pure NaOH solution. Ultimately, the medium was dispensed in the 500 mL conical flask and then sterilized at 121°C temperature and 15 lb. pressure for 15 minutes.

Preparation of medium plates

The petri plates inside which medium had to be dispensed were subjected to autoclave for 15 minutes at 121°C temperature and 15 lb. pressure followed by thorough washing. However, petri plates could also be sterilized by heating them in oven overnight at 180°C. The 15 mL of medium was allocated to every petri plate and their lids were positioned on them. Subsequently the plates were preserved with the cling film and were stored in the uncontaminated location at room temperature until consumption. Still, it was recommended to employ the prepared petri plates within the time extent of 2-3 days to attain the correct affirmative consequences.

Serial dilution method

A serial dilution is basically a chain of sequential dilutions tapped to reduce an intense culture of cells into a more exploitable concentration. Each dilution can reduce the attentiveness of bacteria by an

identifiable amount. So, by scheming the total dilution ended the entire chain, it is feasible to know how much bacteria you commenced with. 1 g or 1 mL of each sample was added to 9 mL sterile distilled water and a number of six-fold dilutions were prepared in the same diluent. Aliquots 0.1 mL of 10-6 diluted sample suspension was added to the agar plates prepared from the isolation medium and distributed evenly over the surface with the aid of sterile glass spreading rod. Following incubation at 37^{o}C for 24 hours, those plates which contained the desired bacterial well isolated colonies were selected as the source of culture to be evaluated for the production of L-lysine.

Medium for sub-culturing of bacteria

Single colonies of the desired bacteria were then cultured on the nutrient agar medium. The medium was prepared with the American Public Health Association (APHA) standards and the ingredients were utilized according to Cruick-Shank *et al*., (1975). The amount used (g/L) were: (Nutrient broth 8, Agar 15, Distilled water to raise volume up to 1 L). 8g powdered nutrient broth was accurately weighed and dissolved with the assistance of steady rate of stirring in 1L Pyrex beaker using 1L distilled water. Eventually, 15g agar was supplemented to the relevant mixture and the final volume was made to 1L. To facilitate the process of dis-solvation of agar, the beaker containing the medium was placed on the hot plate and was supplied with high temperature until the apparent light beige solution was formed. Ultimately, the medium was dispensed in the 1L conical flask and was autoclaved for 15 minutes at 121°C temperature and 15 lb. pressure. Plates were poured as described previously.

Streak plate method

The streaking is completed using a disinfected tool, like a cotton swab and normally an inoculation loop or needle. The inoculation loop or needle is first sterilized via passing it over a flame. Once the loop is chill, it is immersed into an inoculum like a broth or a petri plate containing the desired bacterial colony. The inoculation loop is subsequently dragged through the surface of culture plate containing agar back and forth wearing a zigzag motion till approximately 30% of culture plate has been obscured. The loop afterwards is re-sterilized as well as the plate is twisted 90 degrees. Beginning in the earlier streaked sector, the loop is hauled through these two to three stints continuing the zigzag configuration. The procedure is thenceforth repeated once more ensuing cautious to not tad the formerly streaked areas. Each spell the loop gathers rarer and rarer bacteria until it assembles just lone bacterial cells that have ability to grow into a cluster. The plate must show the heaviest progression in the first unit. The second unit will have less progression and a few sequestered colonies, while the ultimate unit will have the minimum amount of progression and many quarantined colonies.

Screening medium for L-lysine production

For the production of L-lysine, isolation medium with the exception of agar was prepared. All those ingredients were accurately weighed and dissolved to make the final volume up to 250 mL with distilled water in a 500 mL Pyrex beaker. The pH was adjusted to 7.1 by using conc. HCl plus pure NaOH solution. Ultimately, 10 mL of the medium was dispensed in the test tubes and then sterilized at 121°C temperature and 15 lb. pressure for 15 minutes.

Cultivation

Each test tube was inoculated with a loop-full of desired bacteria from each pure culture plate and incubated with shaking in a horizontal shaking water bath at 100 rpm maintained at 37^{o}C for 48 and 72 hours respectively.

Detection of L-lysine

For the detection of L-lysine, paper chromatographic technique of Momose and Takagi (1978) was employed in the culture broth. This was run on a Whatman filter paper No. 1. Sample spots were applied using a sterile capillary tube, then heat-fixed on a chromatogram following a relevant sequence (Figure -I). It was cogitated as the stationary phase. The solvent systems applied included n-butanol: acetic acid: water (1: 2: 4, v/v). It was also contemplated as the mobile phase. Basically, amino acids have no color. So, all of these measures need to be conceded out "blind", plus the results were perceived when a revealing mediator (e.g., ninhydrin) was applied on the subsequent chromatogram. Hence, the spots were visualized by spraying with a solution of 0.5% ninhydrin in butanol. For each spot, calculated the Rf value (Rf means relative to front) by using the following formula: Rf value = distance moved by spot / distance moved by solvent front.

Final screening test

Final screening test comprise of subsequent parameters:
Purification and maintenance of culture

Production medium

Cultivation

Identification of L-lysine

Purification and maintenance of culture

Organisms flaunting positive L-lysine production by fermentation were further purified by reiterated cultivation plus sub-culturing using streak plate method and maintained at 4^{o}C on agar slants prepared by using nutrient agar medium.

Production medium

The composition and pH of the production medium was similar to that of the screening medium for lysine production. Aliquots 20 mL of this solution were decanted in 50 mL of Erlenmeyer flasks and sterilized by autoclaving for 15 min at 121°C temperature and 15 lb. pressure.

Cultivation

Each flask was inoculated with two loop-full of organism from each slant culture and incubated within a horizontal shaking water bath at 100 rpm maintained at 37^{o}C for 24 hours. The cultivation was executed

for those selected bacteria that revealed ameliorate results in the preliminary screening tests.

Identification of L-lysine

Ascending Thin Layer Chromatography (TLC) described by Nasab et al., (2007) was used for the detection of L-lysine in the culture broth. Spots on the TLC plate were concocted, following the same pattern of assembling chromatogram for paper chromatographic technique illustrated in the detection of L-lysine during preliminary screening. The solvent systems applied were also conceived as mentioned earlier. When the sample was put on the plate, the respective solvent or solvent amalgam (renowned as the mobile phase) strained up the plate by means of capillary action. Since dissimilar analytes ascend the respective TLC plate at altered rates, separation was achieved. L-Lysine didn't confer a strong positive response with ninhydrin and the color of spots emerged when heated at 60^{o}C on a hot plate. For each spot appear, reckoned the Rf value by means of the formula.

Naming of bacteria

Naming of PCSIR-NL-42 was primarily based on the taxonomic comparison. The characteristic morphological, cultural, bio-chemical, physiological and chemical properties were taken into consideration (Bergy's manual of Systematic Bacteriology – Vol. 2 1986; Bergy's manual of determinative Bacteriology, 9th Ed. 1994; Goodfellow and Schaal, 1979).

Escalation of L-lysine production

In comparison, escalation means trying to accomplish the highest or supreme result or outcome deprived of the regard to cost and expenditure. Different parameters studied for escalation included different substrate ranges, different temperature ranges, different metal ions concentration (mM), different pH ranges, different incubation periods and different inoculum sizes.

Results

Production and escalation of L-lysine via bacterial fermentation utilizing *Streptococcus sp.* were conceded in Food and Biotechnology Research Centre (FBRC), Lahore. The rampant investigation was consummate and deliberated scrupulously.

Isolation of Bacterial Strains

For the isolation of bacteria fifteen different samples were collected from the adjacent areas of Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories complex Lahore. A modified medium was employed having the composition as described by Nasab et al., (2007). In total, twenty-nine (29) bacterial strains were seperated and tested for the accumulation of L-lysine (Table - I). The screening test for L-lysine producing bacterial strains was carried out with the media containing carbohydrate and nitrogen source as chief ingredients supplemented with inorganic salts.

For the detection of L-lysine, paper chromatographic technique of Momose and Takagi (1978) was employed in the culture broth. The solvent systems applied included n-butanol: acetic acid: water (1: 2: 4, v/v). The spots were visualized by spraying with a solution of 0.5% ninhydrin in butanol. The intensity of spots designated as high (+ + +), medium (+ +), and low (+) which ultimately indicated the formation of L-lysine by each isolate.

The strains showing positive results were then subjected to purification into the same medium to evaluate their L-lysine producing capability and to identify the desired bacteria. Organisms were cultivated in Erlenmeyer flask containing screening medium at 37^{o}C for 24 hours in a horizontal shaking water bath at 100 rpm.

The qualitative estimation of L-lysine and any other amino acid so produced was checked by ascending Thin Layer Chromatography (TLC) described by Nasab et al., (2007). Rf values were measured and compared with that of authentic amino acids. Table –II shows the Rf values obtained from the authentic amino acids.

The quantitative estimation of L-lysine produces by selected strains was governed by a reliable method as mentioned by Shakori, *et al.*, 2012. Table – III shows the results of the quantitative estimation of L-lysine by designated bacterial strains.

Naming of bacterial strains

The most significant task of bacteriology was to identify the pathogens commencing the clinical sample so that apposite treatment can be established. There are several procedures to identify the altered type of bacteria. Results of taxonomic comparison, i.e., characteristic morphological, cultural, biochemical and physical properties of the selected strains are given in Table –IV.

Escalation of L-lysine production from Streptococcus sp.

Effect of different substrates on L-lysine production was revealed in Table –V while their relationship in terms of lysine concentration to substrate ranges were prearranged in Figure –II. The ardent stimulatory effect on lysine production was reached using molasses media with vitamins (w) i.e., 24.4 g/L.

Influence of different temperatures on L-lysine production was mentioned in Table –VI while their relationship in terms of lysine concentration to temperature ranges were given in Figure –III. The potent stimulatory effect on lysine production was achieved at 40º C i.e., 24.4 g/L.

Upshot of different metal ions on L-lysine production was declared in Table –VII while their relationship in terms of lysine concentration to metal ions concentration was assumed in Figure – IV. The powerful stimulatory effect on lysine production was succeeded by adding 1mM solution of Mg i.e., 20.4 g/L.

Consequence of different pH ranges on L-lysine production was avowed in Table –VIII while their relationship in terms of lysine concentration to various pH ranges was snapped in Figure –V. The most

ardent stimulatory effect on lysine production was comprehended at pH 6.5 i.e., 6 g/L.

Outcome of different incubation periods on L-lysine production was said in Table –IX while their relationship in terms of lysine concentration to incubation periods was specified in Figure – VI. The intense stimulatory effect on lysine production was succeeded after 96 hours i.e., 24.4 g/L.

Result of different inoculum sizes on L-lysine production was revealed in Table –X while their relationship in terms of lysine concentration to inoculum sizes was accorded in Figure –VII. The fiercest stimulatory effect on lysine production was attained using 0.3 mL of inoculum i.e., 9.2 g/L.

Laboratory scale production of L-lysine was carried out using 1 L Erlenmeyer flask. Optimized conditions for each bacterium were maintained correspondingly. For *Streptococcus sp.* the parameters for optimization i.e., suitable substrate: (w) molasses media with vitamins- optimum temperature: 40ºC- metal ion: Mg- optimum pH: 6.5- incubation period: 96 hours- inoculum size: 0.3ml/L results in 23.4 g/L of lysine.

Discussion

The L-lysine production by fermentation was first reported by Casida and Beldwin in 1956, using a two-step method. In the first step, di-amino-pimelic acid (DAP) was accumulated in the medium containing glycerol and corn steep liquor by a lysine requiring strain of *Escherichia coli*. Conversion of DAP to lysine was then accomplished with DAP-decarboxylase of *Aerobacter aerogens*. Subsequently, amino acid production by microorganisms continued to attract the attention of various workers throughout the world.

In the present study, the sewage and soil samples collected from different areas of Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories complex Lahore indicated potential for the lysine producing bacteria. In total, twenty-nine (29) bacterial strains were separated and tested for the accumulation of L-lysine: 96.6% of total isolates. The soil samples were observed more potential than the sewage samples. The screening technique adapted in the present study was that described by Nasab et al., (2007) with slight modification in the composition of screening medium. The cultivation and purification of all isolates on a maintenance or storage medium was carried out as it was certainly justified in the preliminary stage because their potential to synthesize the amino acid was determined.

For the detection of L-lysine, paper chromatographic technique of Momose and Takagi (1978) was employed in the culture broth. The solvent systems applied included n-butanol: acetic acid: water (1: 2: 4, v/v). The spots were visualized by spraying with a solution of 0.5% ninhydrin in butanol. Results given in Table-I indicate the formation of L-lysine by each isolate which is according to the intensity of spots. Out of twenty-nine (29) strains, three (3) were found to produce high (+ + +), ten (10) as medium (+ +) and sixteen (16) as low (+) intensity producing L-lysine intensively.

The high intensity strains showing positive results were then subjected to purification into the same medium to evaluate their L-lysine producing capability and to identify the desired bacteria. Organisms

were cultivated in Erlenmeyer flask containing screening medium at 37^{o}C for 24 hours in a horizontal shaking water bath at 100 rpm. The qualitative estimation of L-lysine and any other amino acid so produced was checked by ascending Thin Layer Chromatography (TLC) described by Nasab et al., (2007). The broth of each cultivated strain was applied on aluminium T.L.C. plates, 0.2 mm thick and up flow was made at 25^{o}C. Since the free amino acids have been reported as marked hydrophilic compounds, the separation efficiency was noted with solvent systems, chloroform - methanol - 17% ammonium hydroxide (2:2:1 v/v), n-butanol – acetic acid – water (4:1:1 v/v) and Phenol – water (3:1 v/v) (Brenner et al., 1969). However, in the present study, the solvent system n-butanol - acetic acid - water (1: 2: 4, v/v) provided best separation and was thus used throughout the whole study.

Apart from L-lysine, different amino acid like L-glutamic acid was also identified. Rf values were measured and compared with that of authentic amino acids. Table –II shows the Rf values obtained from the authentic amino acids. The quantitative estimation of L-lysine produces by selected strains was governed by a reliable method as mentioned by Shakori, *et al.*, 2012. Table – III shows the results of the quantitative estimation of L-lysine by designated bacterial strains.

A morphological screening test which was comprised of Gram's staining, pleomorphism, and capsule staining was first carried out. Strains identified as Gram-positive were then subjected to detailed morphological, cultural, bio-chemical and physiological studies. Results of taxonomic comparison, i.e., characteristic morphological, cultural, biochemical and physical properties of the selected strains are given in Table –IV.

The process intensification (especially, the media composition, addition of certain growth factors and cultural conditions) is indispensible for the scale up and commercial production of amino acids. Jyothi et al., 2005 studies the characterization of glutamic-acid assembly commencing cassava arrowroot plant remains by *Brevibacterium divaricatum.* By using 0.7 % ammonium-nitrate inside the mixture, maximum glutamate returns of 3.86 %, grounded upon the mass of the remains stayed, attained at 38^{0}C with a pH as 7.0. Also, maximum glutamate production remained chronicled using 5 % inoculum mass with shakeup speed as 180 rpm. According to the present study, effect of different substrates (Table –V & Figure –II) on lysine production is reached using molasses media with vitamins (w) i.e. 24.4 g/L. Influence of different temperatures (Table –VI & Figure –III) is achieved at 40ºC i.e. 24.4 g/L. Upshot of different metal ions (Table –VII & Figure –IV) is succeeded by adding 1mM solution of Mg i.e. 20.4 g/L. Consequence of different pH ranges (Table –VIII & Figure –V) is comprehended at pH 6.5 i.e. 6 g/L. Outcome of different incubation periods (Table –IX & Figure –VI) is succeeded after 96 hours i.e. 24.4 g/L. Result of different inoculum sizes (Table –X & Figure –VII) is attained using 0.3 mL of inoculum i.e. 9.2 g/L. Similarly, Ezemba et al., 2016 manifests the assembly of lysine by *Microbacterium lacticum* via immersed cultivation by many hydro-carbons, sweeten plus nitrogen cradles. Improving the fermented circumstances for *M. lacticum* under immersed media offered a met return as 2.99 mg/mL of L-lysine within the liquid fermentation following 96 hours.

From the results of the laboratory scale study, conclusion can be drawn that locally available cane molasses is a useful carbon source along with commercially available glucose. The quantity of L-lysine produced by such bacteria as well as the end products was quite comparable. Thus, keeping in view, the high cost of other carbon sources, commercially available glucose justified its use as a carbon source for L-lysine fermentation. Hence, a considerably less expensive L-lysine fermentation could be carried out using these raw materials.

Declarations

Ethics approval and consent to participate: not applicable

Consent for publication: not applicable

Availability of data and materials: not applicable

Competing interests: not applicable

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Author's contribution: SS did the main work for this research, MS helped in collecting of samples, ZH worked in applying stats to the results, NM helped in making graphs, ZR helped in compiling the information, SA helped in performing the identification.

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Tables

TABLE – I: SCREENING OF BACTERIAL STRAINS AS L-LYSINE PRODUCERS.

SERIAL No.	LAB – ID (PCSIR-NL-No.)	SOURCE	SAMPLE No.	INTENSITY OF SPOTS RECORDED
1.	37	soil with vegetation	1	+ + +
2.	38	soil with bird faeces	2	+
3.	39	Soil	1	+
4.	40	bird faeces	3	+
5.	41	drain water	5	+
6.	42	soil with bird faeces	7	+ + +
7.	43	sewage water	6	+ +
8.	44	waste water	6	+ +
9.	45	soil with sanitary leakage	5	+ + +
10.	46	waste water	2	+
11.	47	drain water	4	+
12.	48	sewage water	8	+
13.	49	Soil	11	+ +
14.	50	soil with vegetation	8	+
15.	51	drain water	3	+ +
16.	52	waste water	12	+
17.	53	bird feces	10	+
18.	54	soil with sanitary leakage	4	+ +
19.	55	soil with vegetation	11	+
20.	56	sewage water	7	+
21.	57	Soil	9	+
22.	58	bird faeces	10	+ +
23.	59	drain water	14	+ +
24.	60	waste water	12	+
25.	61	sewage water	9	+ +
26.	62	soil with vegetation	13	+ +

27.	63	tap water	15	+
28.	64	soil with sanitary leakage	14	+ +
29.	65	sewage water	13	+

(PCSIR-NL-No.) - Pakistan Council of Scientific and Industrial Research (PCSIR)

laboratories complex Lahore-54000, Pakistan.

(+ + +) - High L-lysine producing strains.

(+ +) - Medium L-lysine producing strains.

(+) - Low L-lysine producing strains.

TABLE – II: Rf VALUES OF THE STANDARD AMINO ACIDS.

SERIAL No.	AMINO ACIDS	ABBREVIATIONS	Rf VALUES
1.	Lysine	Lys	0.14
2.	Glutamic acid	Glu	0.25

Chromatographic plates - Aluminium T.L.C. Plates (20 X 20cm).

Solvent system - n-butanol -acetic acid -water (1: 2: 4, v/v).

Solvent migration - 17cm at 25^{o}C

Duration - 8 hours.

By following all the statistical parameters pragmatic on the quantitative estimation for L-lysine production and intensification, it was clinched that the mean value of lysine produce was trailed by various alphabetical letters in same column which remain portentously different at a level of ‘$p \leq$’ according to Duncan’s new multiple range test (5 %) using ANOVA, while ‘±’ indicates standard error calculated from the Microsoft Excel 2016 formula sheet. However, LSD means least significant difference. Significance level, error mean square and degree of freedom were also mentioned accordingly.

TABLE – III: QUANTITATIVE ESTIMATION OF L-LYSINE.

SERIAL No.	STRAIN (PCSIR-NL-No.)	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	37	13.3±1.27bc	2.203
2.	41	10.5±2.27bcde	3.946
3.	42	20.1±3.49a	6.057
4.	43	10.4±5.06bcde	8.772
5.	44	8.1±2.55bcde	4.424
6.	45	15.4±0.87ab	1.514
7.	46	12±3.02bcd	5.245
8.	47	6.7±2.41cde	4.174
9.	48	7.8±1.10cde	1.908
10.	49	3.9±0.43e	0.750
11.	50	3.5±0.67e	1.171
12.	51	6.8±1.04cde	1.814
13.	52	5.8±1.55de	2.690
14.	53	4.3±0.45e	0.793
15.	54	9.3±2.18bcde	3.780
16.	55	6.1±0.35cde	0.611

Significance level = .05

Error mean square = 14.14

Degree of freedom = 32

LSD .05 = 6.337

TABLE – IV: IDENTIFICATION OF BACTERIAL STRAIN

Strain (PCSIR-NL-NO.)	Morphological Characteristics				
	Gram Stain	Cell Form		Pleomorphism	Capsule Stain
		Young Cultures (12 To 24 Hours Old)	Old Cultures (72 To 120 Hours Old)		
42	Positive (+) Violet	Rounded Cocci, Mostly Single, Some Occurs in Pairs.	Composed Largely or Entirely of Coccoid Cells.	+	+

Biochemical Characteristics				
Indole Test	Methyl Red Test	Catalase Test	Starch Hydrolysis Test	Casein Hydrolysis Test
-	+	-	-	-

Cultural Characteristics
Small, Smooth, Entire, Circular, Convex, Pale Yellow in Colour.

Physiological Characteristics				
Optimum Temperature	Oxygen Requirements	Growth After Heat ($80^{o}c$)	Growth in NaCl	
			5%	10%
27-45^{o}C	FA	-	+	-

A –Aerobes; FA -Facultative Anaerobes

TABLE – V: EFFECT OF DIFFERENT SUBSTRATES ON L-LYSINE PRODUCTION BY*Streptococcus sp.*

SERIAL No.	SUBSTRATE RANGES	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	Glucose media with vitamins (g)	22.8±0.02b	0.028
2.	Glucose media without vitamins (G)	10±0.04g	0.056
3.	Molasses media with vitamins (w)	24.4±0.03a	0.042
4.	Molasses media for *Bacillus* without shaking (b)	12±0.02f	0.028
5.	Molasses media for*Bacillus* with shaking (B)	21.2±0c	0
6.	Molasses media without vitamins (m)	12.8±0.01e	0.014
7.	Simple glucose media (o)	16.8±0.01d	0.014

Significance level = .05

Error mean square = 0.001

Degree of freedom = 7

LSD .05 = 0.07

TABLE – VI: EFFECT OF DIFFERENT TEMPERATURES ON L-LYSINE PRODUCTION by*Streptococcus sp.*

SERIAL No.	TEMPERATURE RANGES	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	25ºC	6.8±0.01f	0.041
2.	27ºC	16.8±0.01c	0.041
3.	30ºC	18±0.02b	0.028
4.	35ºC	18±0.03b	0.042
5.	37ºC	10.4±0.01e	0.014
6.	40ºC	24.4±0.02a	0.028
7.	45º C	11.2±0.01d	0.014

Significance level = .05

Error mean square = 6E-04

Degree of freedom = 7

LSD .05 = 0.05

TABLE – VII: EFFECT OF DIFFERENT METAL IONS ON L-LYSINE PRODUCTION BY*Streptococcus sp.*

SERIAL No.	METAL IONS (mM)	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	Ca	12.4±0.01c	0.014
2.	Na	11.6±0.02d	0.028
3.	K	10.8±0.02e	0.028
4.	Mn	9.6±0.02f	0.028
5.	Fe	18±0.01b	0.014
6.	Mg	20.4±0.08a	0.113
7.	Cu	2.8±0.01g	0.014

Significance level = .05

Error mean square = 0.002

Degree of freedom = 7

LSD .05 = 0.112

TABLE – VIII:EFFECT OF DIFFERENT pH RANGES ON L-LYSINE PRODUCTION BY*Streptococcus sp.*

SERIAL No.	pH RANGES	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	5.5	4±0.02b	0.028
2.	6	2.4±0.03e	0.042
3.	6.5	6±0.05a	0.070
4.	7	2.8±0.02d	0.028
5.	7.5	2.8±0.01d	0.014
6.	8	2.4±0.02e	0.028
7.	8.5	3.2±0.02c	0.028

Significance level = .05

Error mean square = 0.001

Degree of freedom = 7

LSD .05 = 0.09

TABLE – IX:EFFECT OF DIFFERENT INCUBATION PERIODS ON L-LYSINE PRODUCTION BY*Streptococcus sp.*

SERIAL No.	INCUBATION PERIOD (hours)	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	24	4±0.02g	0.028
2.	48	2.8±0.03i	0.042
3.	72	3.2±0.01h	0.014
4.	96	24.4±0.05a	0.070
5.	120	11.2±0.03c	0.042
6.	144	1.6±0.07i	0.098
7.	168	7.2±0.03f	0.042
8.	192	9.2±0.04e	0.056
9.	216	13.2±0.06b	0.084
10.	240	10.8±0.03d	0.042

Significance level = .05

Error mean square = 0.02

Degree of freedom = 10

LSD .05 = 0.351

TABLE – X:EFFECT OF DIFFERENT INOCULUM SIZES ON L-LYSINE PRODUCTION BY*Streptococcus sp.*

SERIAL No.	INOCULUM SIZES (ml/L)	L-LYSINE PRODUCED (g/L).	STANDARD DEVIATION
1.	0.1	6.8±0.01c	0.014
2.	0.2	7.2±0.03b	0.042
3.	0.3	9.2±0.04a	0.056
4.	0.4	5.2±0.05e	0.070
5.	0.5	6±0.06d	0.084
6.	0.6	4.4±0.03g	0.042
7.	0.7	4.8±0.01f	0.014
8.	0.8	3.2±0.03h	0.042
9.	0.9	2.8±0.02i	0.028
10.	1	1.6±0.03j	0.042

Significance level = .05

Error mean square = 0.002

Degree of freedom = 10

LSD .05 = 0.108

Figures

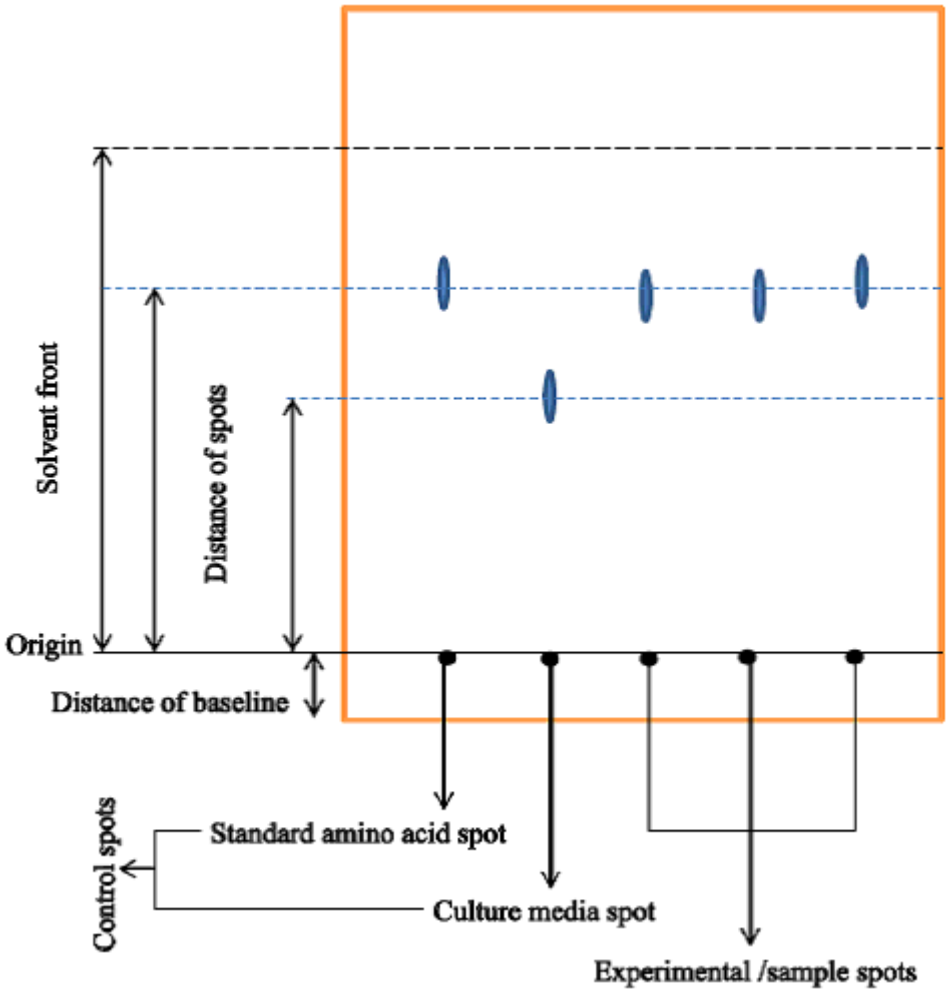

Figure 1

CHROMATOGRAM / TLC PLATE

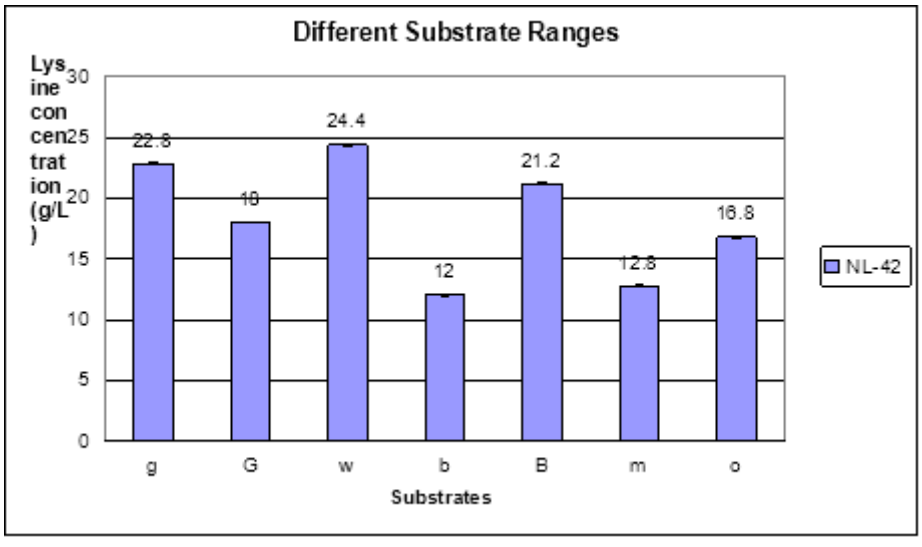

Figure 2

GRAPHICAL REPRESENTATION OF DIFFERENT SUBSTRATES ON L-LYSINE PRODUCTION BY Streptococcus sp.

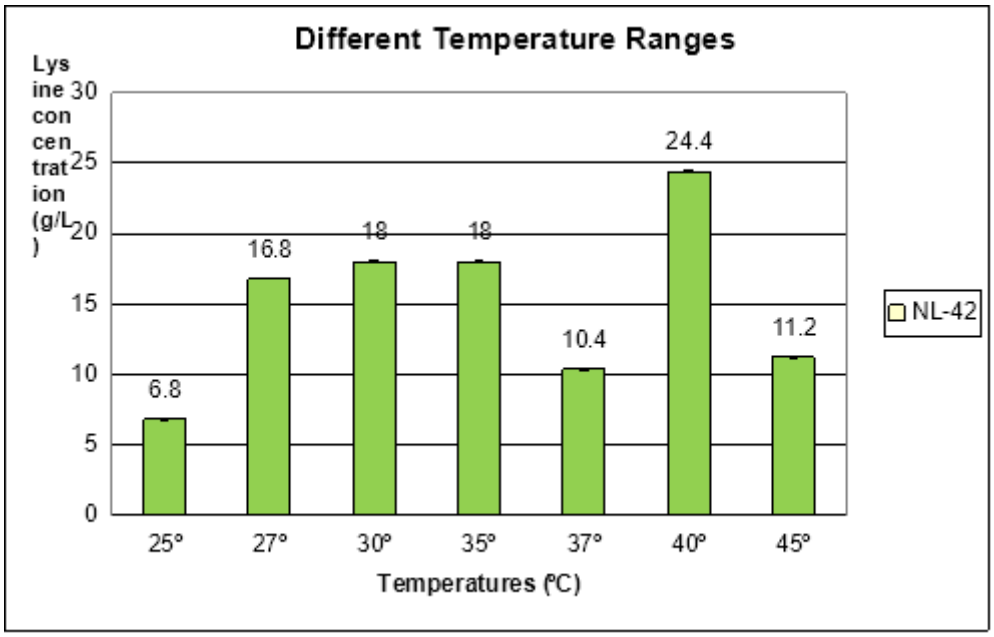

Figure 3

GRAPHICAL REPRESENTATION OF DIFFERENT TEMPERATURES ON L-LYSINE PRODUCTION BY Streptococcus sp.

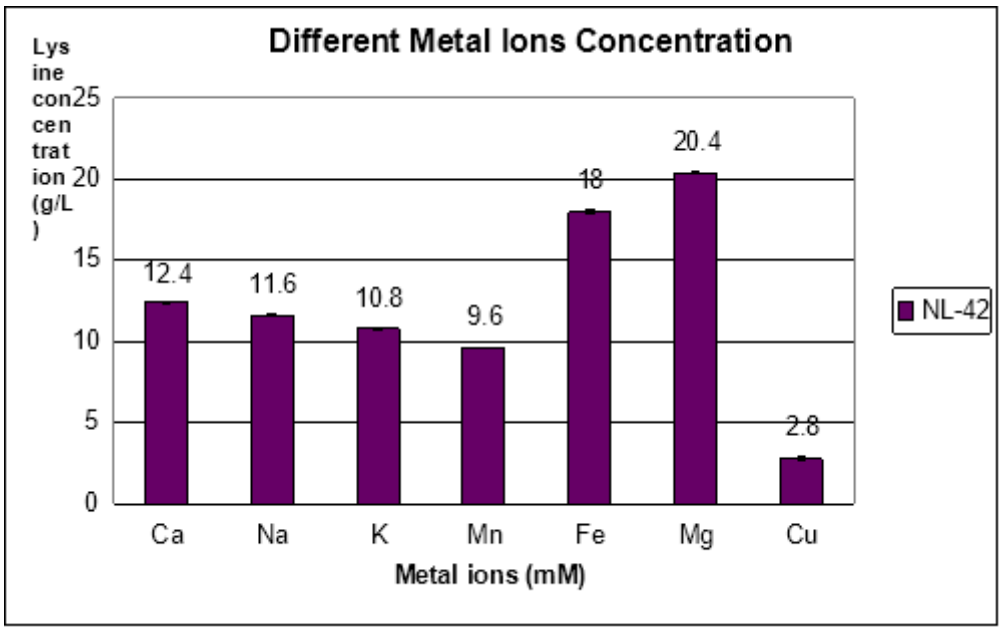

Figure 4

GRAPHICAL REPRESENTATION OF DIFFERENT METAL IONS ON L-LYSINE PRODUCTION BY Streptococcus sp.

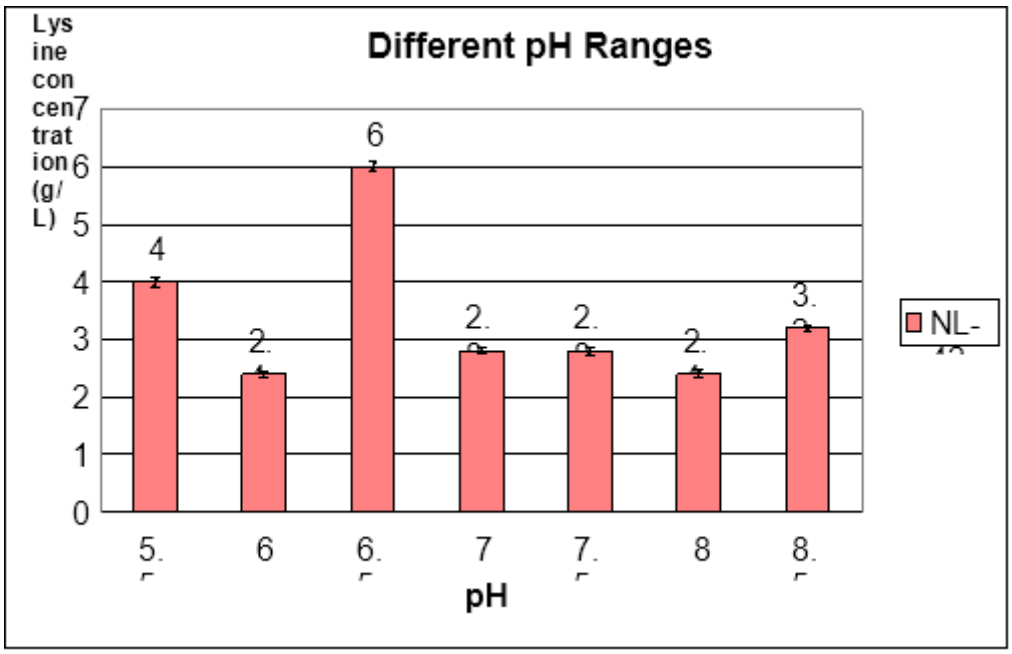

Figure 5

GRAPHICAL REPRESENTATION OF DIFFERENT pH RANGES ON L-LYSINE PRODUCTION BY Streptococcus sp.

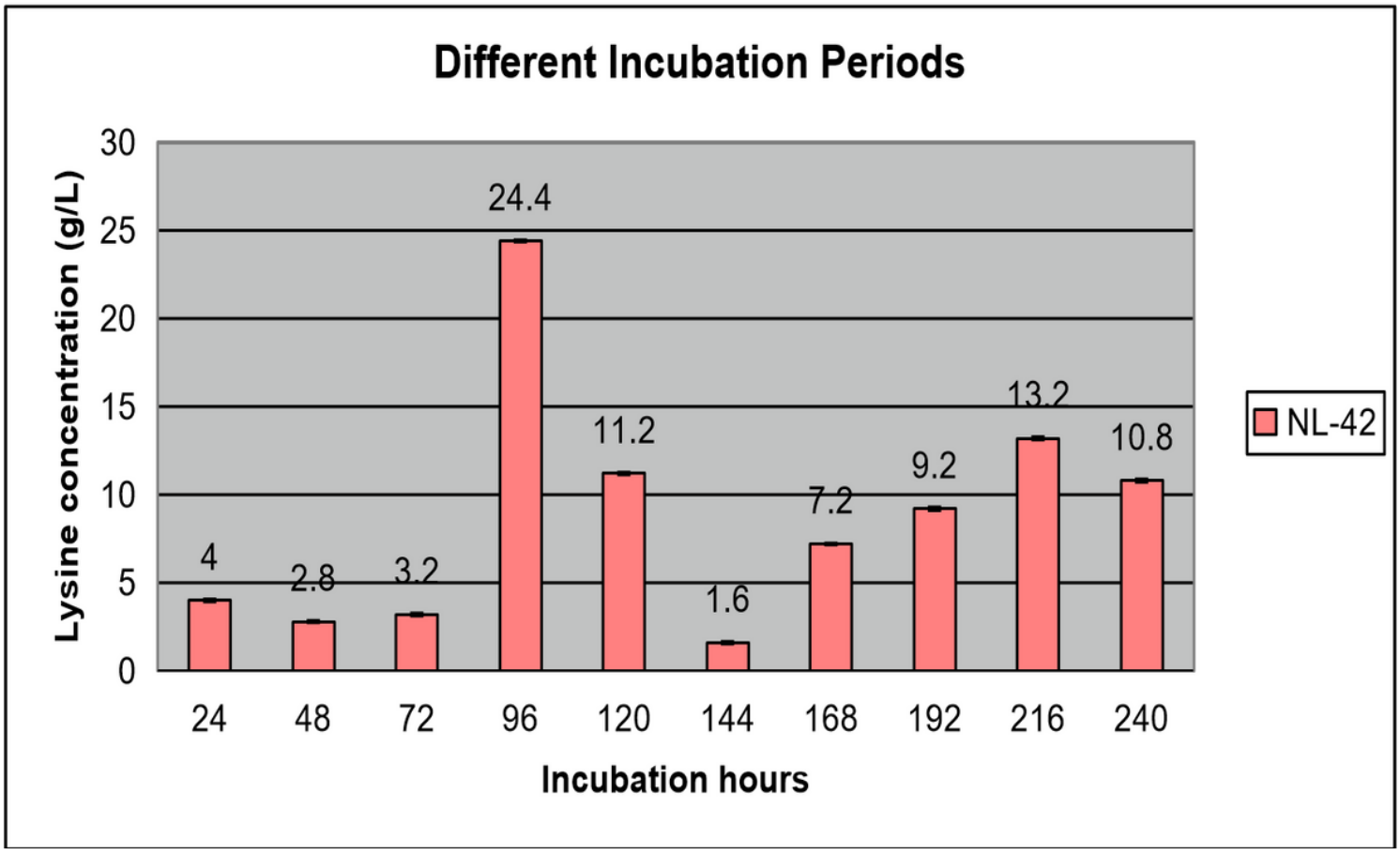

Figure 6

GRAPHICAL REPRESENTATION OF DIFFERENT INCUBATION PERIODS ON L-LYSINE PRODUCTION BY Streptococcus sp.

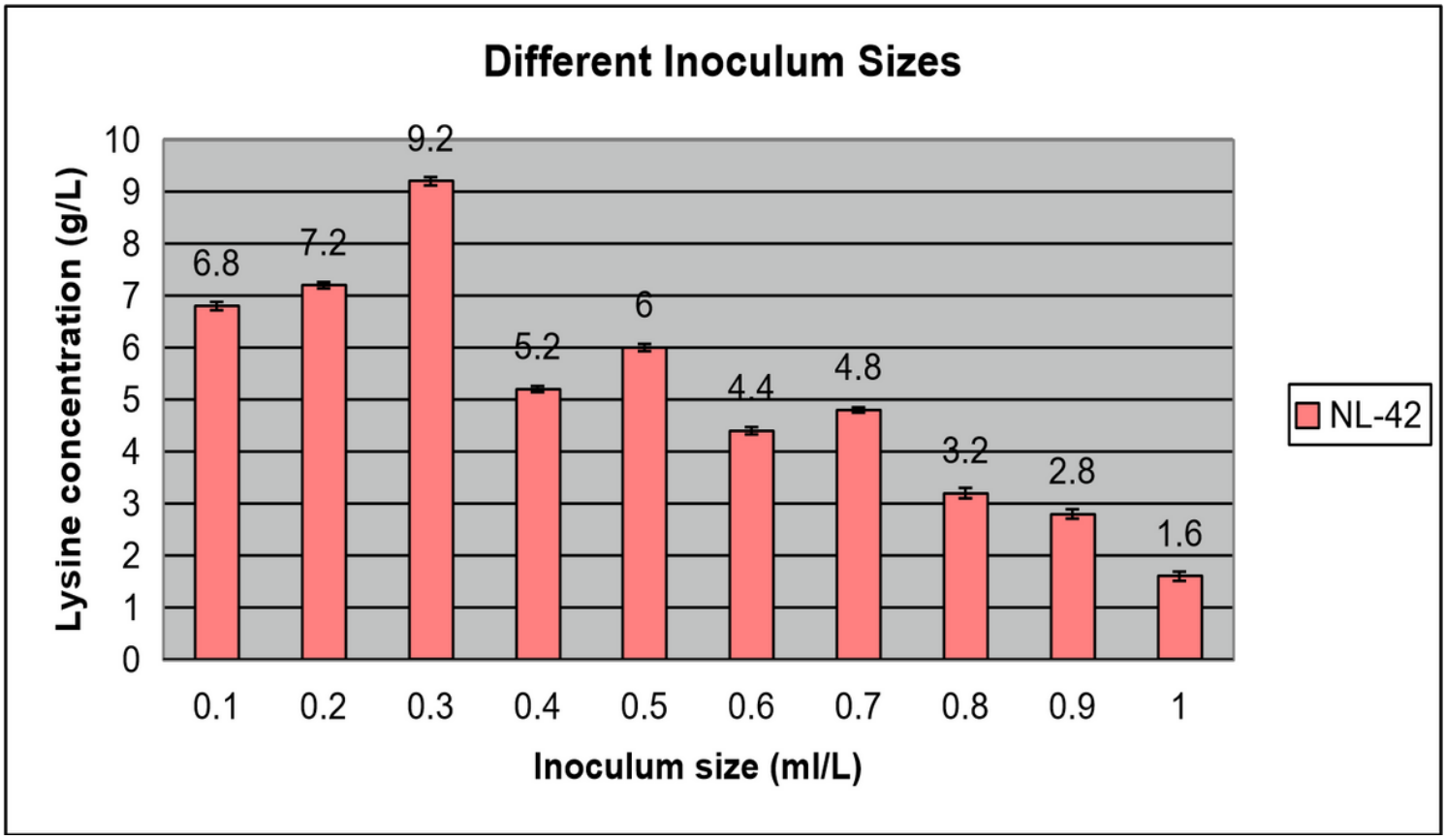

Figure 7

GRAPHICAL REPRESENTATION OF DIFFERENT INOCULUM SIZES ON L-LYSINE PRODUCTION BY Streptococcus sp.