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SCREENING OF LYSINE PRODUCTION BY *Escherichia coli* AND *Klebsiella* ISOLATES AT DIFFERENT CARBON SOURCES

Nagina GILANI[1], Tahira MAQBOOL[2], Muhammad MOHIBULLAH[3], Ume HABIBA[4], Tauseef ANWAR[5], Huma QURESHI[6]

[1] Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.
[2] Govt. Fatima Jinnah Post Graduate College for Women, Muzaffarabad, Azad Jammu and Kashmir.
[3] Department of Environmental Science, Gomal University, Dera Ismail Khan, Pakistan.
[4] Department of Plant Breeding & Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan.
[5] Department of Botany, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.
[6] Department of Botany, University of Chakwal, Chakwal, Pakistan.

Corresponding author:
Huma Qureshi
huma.qureshi@uoc.edu.pk

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Abstract

Lysine is an essential amino acid that is not biologically manufactured in the body. Different chemical methods for lysine production are expensive and give low yields. The present study was conducted with the purpose to evaluate the biochemical production of lysine by different carbon sources using bacterial isolates. Three carbon sources namely glucose, sucrose, and fructose were used to evaluate the biochemical production of lysine by *Escherichia coli* and *Klebsiella* spp. isolates. Optimum incubation periods were between 48-96 hours. An extensive amount of lysine was produced by all of these isolates in L6 fermentation medium. Maximum lysine was produced by *Klebsiella* isolate K1 6.48 g/L after 96 hours of incubation by using glucose as carbon source followed by 6.0 g/L by *Klebsiella* isolates K3 after 72 hours of incubation when sucrose was used as a carbon source at 37 ˚C. Highest amount of lysine was produced at 96 hours by *Klebsiella* isolates in addition to *E. coli*. From all three carbon sources using *Klebsiella* isolates and *E. coli*, glucose showed better lysine production.

Keywords: Bacterial Isolates. Fermentation Media. Fermentative Production of Lysine.

1. Introduction

Lysine has been documented as one of the most scarce essential amino acids in the food supply of human beings as well as meat-producing animals because it is not produced biologically in the body (Leinonen et al. 2019). Lysine is nutritionally crucial for humans and animals as it could not be synthesized within living bodies but can be added supplementary to food and feed materials to increase the quality of protein in the body (Ekwealor and Obeta 2005). In addition to its role as feed supplement, lysine and some other amino acids (aspartic acid) are used in the pharmaceutical industry for diets formulation with balanced compositions along with amino acid infusion (Nadeem et al. 2001). Lysine is currently used in pharmaceutical, food, feed milling as well as cosmetics industries (Anastassiadis 2007).

Lysine can be manufactured by both chemical and biochemical method. From about fifty years, strains like B*revibacterium flavum, Brevibactirium lactofermentum,* and *Corynebacterium glutamicum* have been used for production of lysine in the industries (Rao et al. 2011). Since its early stages around 1960,

production of lysine by biotechnological methods has been significantly enhanced by strain upgrading, and more progress is anticipated in future (Vitorino and Bessa 2017). Developments in different methods of fermentation in addition to strain upgradation of microorganisms producing amino acids have enabled large production of lysine on industrial level (Leuchtenberger et al. 2005). The bacterial strain that produces amino acids is the decision-maker for industrial fermentation thus affecting the commercial and ecological performance of a biotechnological method on a higher level. *E. coli* is characteristically the major thermotolerant coliform in drinking water. *Klebsiella* is more commonly observed bacterial isolate in environment. In humans, the microorganisms generally do not cause problems as they are oftenly present in parts of the digestive tract. These bacterial strains are selected for screening for lysine production owing to their prevalence of in indigenous microbial flora.

Various carbon and nitrogen bases along with inorganic ions and trace elements (Fe, Mn), amino acids, vitamins, and complex organic compounds are present in common fermentation media for lysine production (Lim et al. 2019). The development of enhanced microbial strains along with multidimensional methods is expected to increase the economics of the process of fermentation. The conversion, the yield from the source of carbon, and productivity are utmost factors in the process of fermentation but production is strongly affected by the growth rate of strain, rate of utilization of glucose along with culture conditions. There are also some reports on the development of the fermentation process. Carbon sources mark the production of lysine to varying degrees in different microorganisms. Glucose, fructose, and sucrose are vital sources of carbon having a marked effect on the kinetics and stoichiometry of production of lysine by *C. glutamicum* (Kiefer et al. 2002). Many carbohydrates are applied independently or as a mixture for the production of lysine. Sugarcane molasses is a inexpensive source of carbon having sucrose, glucose, and fructose at a overall carbohydrate content of 50 to 60% (Nelofer et al. 2007). The present research was designed to investigate comparative production of lysine by carbon sources glucose, sucrose, and fructose using *Escherichia coli* and *Klebsiella* isolates.

2. Material and Methods

Bacterial strains of *E. coli* (E1-E5) were obtained from the water of Jhelum River (Lat.: 34.173°, Long.: 73.784°, Elevation: 943 m) while of *Klebsiella* (K1-K5) from Pakistan Institute of Medical Sciences (PIMS), Islamabad (Table 1). The pure cultures of these strains were prepared.

Table 1. Source of fermenting bacteria.

BACTERIAL STRAIN	SOURCE
E-1	River water
E-2	River water
E-3	River water
E-4	River water
E-5	River water
K-1	PIMS Islamabad
K-2	PIMS Islamabad
K-3	PIMS Islamabad
K-4	PIMS Islamabad
K-5	PIMS Islamabad

PIMS Islamabad = Pakistan Institute of Medical Sciences (PIMS) Islamabad.

Bacterial culture preparation

Ten grams of nutrient agar (Oxide CMO_3) was dissolved into distilled water, autoclaved, and poured into petri plates under aseptic conditions (TANZO E23 touch, Hirayama, Japan). Suggested quantity of nutrient agar was dissolved in distilled water to prepare slant. Bacterial strains were streaked on well-solidified nutrient agar plates to acquire the pure culture. Streaked plates were incubated at 37°C. After 24 hours smooth bacterial colonies were observed on a nutrient agar surface. This culture was used as stock culture. To standardize inoculum, single colonies which were well isolated from each other were inoculated into freshly prepared sterilized nutrient broth and incubated overnight at 37°C (Esco

Isotherm®–world-class laboratory incubator). A sample of 100 µL of broth culture was spotted in the center of the plate. The cells were placed over the entire surface of a plate with the help of a sterilized glass spreader.

Storage

Bacterial strains in the laboratory were preserved by mixing in 15% glycerol and placed in a refrigerator for permanent storage or fresh culture grown was kept temporary in a refrigerator at 4°C for 2-3 days. To prevent contamination of prepared nutrient agar, it was kept in an incubator at 37°C for 24 hours.

Selection of carbon source

The selected strains were grown in fermentation media with various sources of carbon. These carbon sources included commercially available glucose ($C_6H_{12}O_6$, an aldo reducing sugar), fructose ($C_6H_{12}O_6$, a keto reducing sugar), and sucrose ($C_{12}H_{22}O_{11}$, non-reducing sugar). All sources of carbon were sterilized individually and supplemented to a pre-sterilized fermentation medium in the same concentration.

Fermentation

For the production of amino acids, bacterial isolates were grown in a fermentation medium through various sources of carbon (glucose, fructose, sucrose) separately while keeping other constituents the same (Table 2) for 48-96 hours at 37±1°C. A three mL sample from each flask was removed and monitored every 24 hours. The fermented broth was centrifuged (Centrifuge (800) 6 Hole) at 1008 G-force and made cell free by filtration through membrane filters having pore size 0.45µm. The filtrate was then examined through paper chromatography for the detection of amino acid produced. The amino acids spots were cut into pieces and dipped into 3ml methanol to elute the entire color of the spots. Bacteria were grown in different fermentation media for a maximum of 96 hours at 37°C during which the sample was monitored after every 24 hours of incubation.

Table 2. Composition of fermentation media.

INGREDIENTS	QUANTITY (G/L)
Carbon Source*	10.0
$CaCO_3$	2.0
K_2HPO_4	0.4
KH_2PO_4	0.07
$Mg(SO_4)_2.7H_2O$	0.03
$(NH_4)_2SO_4$	3.0
Trypticase	0.75
pH	7.0

* Glucose, sucrose and fructose was used as carbon source to yield three types of media while keeping other constituents same.

Amino acids analysis

Quantitative analysis of amino acids was done through paper chromatography using a spectrophotometer (V-750 UV-Visible). The paper strips (Desaga NR-2045) were irrigated vertically for 18-20 hours with n-butanol: acetic acid: water (12:3:5) solvent system. The paper was removed from the chromatographic tank, carefully dried, and sprayed with 0.1% Ninhydrin-ethanol solution. The color of the spot of interest was eluted in 3 mL methanol and its optical density (O.D.) was observed at 550 nm. Upon drying at 70°C, the paper revealed colored spots of different amino acids. Lysine production was confirmed by calculating the retention factor (Rf) values of the respective spots as described by Hudaib et al. (2016).

$$Rf = \frac{\text{Distance traveled by amino acid}}{\text{Distance traveled by solvent system}}$$

The results were compared with the standard Rf of Lysine (0.131).

3. Results and Discussion

Carbon source not only acts as an energy source for microorganisms involved in fermentation but also have a vital role in the amino acids formation. Many carbohydrates such as maize, grain, potato, etc. are preferred as a carbon source for the process of microbial fermentation (Nkhata et al. 2018). The use of better microbial strains can improve the economics of fermentation. Many auxotrophic and regulatory mutants of microscopic organisms were known to show the production of lysine (Jie et al. 2015). The effect of carbon sources varies in different microorganisms for the production of lysine. Many important carbon sources like fructose, sucrose, and glucose are having a marked effect on the kinetics of the production of lysine by *C. glutamicum* (Lange et al. 2017). Only limited research is based on other substrates like sucrose or fructose. Lower lysine production was shown by using fructose as a carbon source similar to our study by using *Corynebacterium glutamicum* which may be due to lower activity of the pentose phosphate pathway (Siedler et al. 2013).

Table 3. Comparative Lysine production (grams/liter) by *Escherichia coli* and *Klebsiella* isolates using fructose, sucrose and glucose as carbon source.

CARBON SOURCE:		FRUCTOSE		SUCROSE		GLUCOSE	
STRAIN	Harvest Time (Hrs)	Harvest pH	Quantity (G/L)	Harvest pH	Quantity (G/L)	Harvest pH	Quantity (G/L)
Eco-1	48	6.76	0.06	6.09	__	6.19	0.09
	72	4.13	0.02	4.53	__	6.09	1.04
	96	4.51	0.03	4.11	__	4.99	1.09
Eco-2	48	6.34	0.07	6.92	0.01	4.76	__
	72	6.22	1.03	6.11	0.04	4.22	__
	96	5.76	5.20	6.00	0.04	4.14	__
Eco-3	48	6.84	0.09	6.24	0.01	6.90	0.02
	72	3.84	1.04	6.23	0.04	5.33	0.50
	96	3.60	2.09	6.00	0.07	3.67	1.08
Eco-4	48	6.11	1.09	6.65	0.06	4.67	1.09
	72	5.78	3.42	6.60	3.06	5.87	2.12
	96	6.33	4.07	6.11	3.09	3.56	5.09
Eco-5	48	6.00	0.04	6.88	0.05	4.55	0.02
	72	5.14	0.07	6.34	0.07	4.64	0.99
	96	4.99	1.09	6.10	0.07	4.23	2.04
K-1	48	5.98	0.04	6.91	0.01	5.14	3.09
	72	5.88	4.93	5.90	0.02	5.09	3.15
	96	5.13	3.04	4.74	1.07	5.01	6.45
K-2	48	6.09	0.05	6.85	0.01	4.66	0.45
	72	5.77	0.07	4.90	1.01	5.55	2.04
	96	5.43	1.10	4.54	2.05	4.19	3.99
K-3	48	6.87	0.28	6.88	2.98	4.59	2.89
	72	6.12	1.014	5.73	6.00	4.33	3.00
	96	5.43	3.03	4.34	3.06	3.34	3.44
K-4	48	6.44	0.03	6.77	0.01	6.32	2.59
	72	5.90	0.04	5.67	0.12	6.19	3.19
	96	4.88	1.05	4.69	0.39	6.04	3.89
K-5	48	6.33	0.09	6.99	0.29	5.13	1.77
	72	5.67	0.09	5.84	0.42	5.11	2.54
	96	4.99	1.08	4.57	1.04	3.64	3.00

__ indicates no yield at all.

In our study, maximum isolates of *E. coli* and *Klebsiella* showed production of lysine. Out of the total isolates of *E. coli* tested for the production of lysine, an isolate E-2 showed highest production of

lysine 5.20 g/L after 96 hours of incubation. The 5 isolates of *Klebsiella*, and isolate K-1 gave highest production of lysine 4.93 g/L after 96 hours of incubation. Comparatively, from isolates of *E. coli* and *Klebsiella*, E-2 showed maximum production of lysine when fructose was supplemented as carbon source (Table 3). Out of all isolates used for the production of lysine, nearly all isolates produced lysine except E-1 which produced lysine after 72 hours of incubation. From *E. coli* isolates E-4 showed maximum production of lysine 3.09 g/L after 96 hours of incubation. Among *Klebsiella* isolates K-3 gave the maximum production of lysine 6.00 g/L after 72 hours of incubation at the given fermentation conditions. When sucrose was used as a carbon source, *Klebsiella* isolates K-3 showed the maximum production of lysine from all of the isolates of *E. coli* and *Klebsiella* respectively (Table 3). Sucrose has yet not been studied much despite its useful effect on the production of amino acids like lysine. When fermentation media for the assemblage of lysine was supplemented with sucrose, it increased lysine production to 20-24 g/L after 72 hours (Hussain 2013). It was proved similar to our findings.

Glucose supported the production of the maximum amount of lysine. All of the isolates of *E. coli* and *Klebsiella* produced lysine except one strain of *E. coli* that is E-2 which did not produce lysine at all. In *E. coli* isolates maximum production shown by an isolate E-4, was 5.09 g/L after 96 hours of incubation under the given fermentation conditions. In contrast to *E. coli*, in *Klebsiella* isolates maximum production shown by K-1 was 6.45 g/L after 96 hours of incubation. So, when glucose was used as a carbon source, *Klebsiella* isolate K-1 showed the maximum production of lysine from all of the isolates of *E. coli* and *Klebseilla* (Table 3). Glucose is considered to be a good carbon source but it can vary with nutritional requirements of microorganisms (Sitanggang et al. 2010). It was reported that 45 g/L of lysine could be produced after 96 hours in a glucose medium (Shah et al. 2002). Maximum 60 g/L lysines were produced in glucose medium after 120 hours by *Corynebacterium spp.* (Nelofer et al. 2007). The lesser amount of lysine in the current study which might be owing to bacterial isolates i.e., *Escherichia coli* and *Klebsiella* as compared to other bacterial species in earlier studies.

4. Conclusions

While various sources of carbo were applied for the manufacture of lysine by fermentation, glucose was found to be the best carbon source. Similarly, from bacterial strains used for the fermentation *Klebsiella* showed better production of lysine as compared to *E. coli.* Lysine was the main amino acid fermented by all bacterial strains, in all three carbon sources. However, the response of the isolates varied with changing carbon sources. Therefore, in our case, it can be concluded that when simple carbon sources are used, better lysine yield could be produced at a lower cost by a biochemical process using *E. coli* and *Klebsiella* strains.

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References

ANASTASSIADIS, S. L-Lysine Fermentation. *Recent Patents on Biotechnology*. 2007, **1**, 11-24. https://doi.org/10.2174/187220807779813947

EKWEALOR, I.A. and OBETA, J.A.N. Studies on lysine production by *Bacillus megaterium*. *African Journal of Biotechnology*. 2005, **4**(7), 633-638. https://doi.org/10.5897/AJB2005.000-3115

HUDAIB, T., BROWN, S. and WILSON, D. Identification of free amino acids in several crude extracts of two legumes using thin-layer chromatography. *Journal of Planar Chromatography.*2016, **29**(2), 145–147. https://doi.org/10.1556/1006.2016.29.2.9

HUSSAIN, A. Enhanced production of l-lysine from bacterial species through metabolic and bioprocess engineering. Thesis of Doctor of Philosophy in the Subject of Biotechnology, 2013.

KIEFER, P., HEINZLE, E. and WITTMANN C. Influence of glucose, fructose and sucrose as carbon sources on kinetics and stoichiometry of lysine production by *Corynebacterium glutamicum*. *Journal of Industrial Microbiology and Biotechnology*. 2002, **28**(6), 338-343. https://doi.org/10.1038/sj/jim/7000252

LANGE, A., et al. Bio-based succinate from sucrose: High-resolution13C metabolicfluxanalysis and metabolic engineering of the rumen bacteriumBasfiasucciniciproducens. *Metabolic Engineering*. 2017, **44**, 198-212. https://doi.org/10.1016/j.ymben.2017.10.003

LEINONEN, I., et al. Lysine supply is a critical factor in achieving sustainable global protein economy. *Frontiers in Sustainable Food Systems*. 2019, **3**, 1-11. https://doi.org/10.3389/fsufs/2019.00027

LEUCHTENBERGER, W., HUTHMACHER, K. and DRAUZ, K. Biotechnological production of amino acids and derivatives: current status and prospects. *Applied Microbiology and Biotechnology*. 2005, **69**, 1-8. https://doi.org/10.1007/s00253-005-0155-y

LIM, Y.H., et al. Optimized medium via statistical approach enhanced threonine production by *Pediococcus pentosaceus* TL-3 isolated from Malaysian food. *Microbial Cell Fact*ories. 2019, **18**, 125. https://doi.org/10.1186/s12934-019-1173-2

JIE L.V., et al. Isolation and molecular identification of auxotrophic mutants to develop a genetic manipulation system for the *Haloarchaeon natrinema* sp. J7-2. *Archaea*. **2015**, 483194. https://doi.org/10.1155/2015/483194

NADEEM, S., et al. Enhanced L-Lysine Production by an Escherichia coli Mutant WARN 30522 after MNNG Treatment. *International Journal of Agriculture & Biology*. 2001, **3**(4), 448-450.

NELOFER, R., et al. L-lysine production by the homoserine auxotrophic mutant of *Corynebacterium glutamicum* in stirrer fermenter. *Pakistan Journal of Zoology*. 2007, **39**(3), 159-164.

NKHATA, S.G., et al. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition*. 2018, **6**(8), 2446-2458. https://doi.org/10.1002/fsn3.846

RAO, B.S., MURALIDHARARAO and SWAMY, A.V.N. Studies on continuous production kinetics of L-Lysine by immobilized *Corynebacterium glutamicum* 13032. *Middle-East Journal of Scientific Research*. 2011, **7**(2), 235-240.

SHAH, A.H., HAMEED, A. and KHAN, G.M. Fermentative Production of L-Lysine: Bacterial Fermentation-I. *Journal of Medical Sciences*. 2002, **2**, 152-157. https://doi.org/10.3923/jms.2002.152.157

SIEDLER, S., et al. Reductive whole-cell biotransformation with Corynebacterium glutamicum: improvement of NADPH generation from glucose by a cyclized pentose phosphate pathway using pfkA and gapA deletion mutants. *Applied Microbiology and Biotechnology.* 2013, **97**(1), 143-152. https://doi.org/10.1007/s00253-012-4314-7

SITANGGANG, A.B., et al. Fermentation Strategies: Nutritional Requirements. In: KRAUSE, J. and FLEISCHER, O. Industrial Fermentation: Food Processes, nutrient sources and production strategies, 2010.

VITORINO, L.C. and BESSA, L.A. Technological microbiology: development and applications. *Frontiers in Microbiology*. 2017, **8**, 827. https://doi.org/10.3389/fmicb.2017.00827

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