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Comparative Study and the Optimum Conditions On the Use of Certain Bacterial Strains For the Production of Some Amino Acids

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Abstract

This study aims to use some strains (*Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* for the production of some amino acids (lysine, methionine, threonine, arginine, tyrosine, aspartic acid, glutamic acid, alanine, and proline) on synthetic fermentation media under different conditions (incubation periods; incubation temperatures; pH values; media volume and different sources of carbon and nitrogen) on the course of fermentation reactions of amino acids, and then quantitative analysis of amino acids by HPLC for choose the best conditions for increasing production of amino acids. The results showed that in the best conditions for three strains, the highest production of amino acids was recorded for *Brevibacterium linens* the pH (7.5), incubation temperature (30°C), medium volume (50 ml), and incubation period (48 hr), while for *Corynebacterium glutamicum* observed on pH (7), incubation temperature (30°C), medium volume (50 ml) and incubation period (48 hr), however, for *Lactobacillus bulgaricus* observed on pH 6.5, incubation temperature 37°C, medium volume (50 ml) and incubation period (48 hr). The best carbon and nitrogen sources were recorded for glucose and ammonium sulfate for three tested strains.

Keywords: Amino acids, *Corynebacterium glutamicum*, *Brevibacterium linens*, and *Lactobacillus bulgaricus batch fermentation*

1-Introduction

The amino acids are the basic categories of nutrients in both human and animal diets and have mainly been utilized in cosmetic, pharmaceutical, and food fortification [1-4].

Amino acids can be produced using different ways such as enzymatic methods, protein hydrolysis, chemical synthesis, fermentation, recombinant DNA technology, and protoplast fusion [5, 6]. For the production of amino acids in these, fermentation techniques were the most practical and economical methods [7]. *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* are widely used to produce amino acids such as L-glutamate and lysine at the industrial scale [8]. ***C. glutamicum*** can utilize a variety of carbohydrates and organic acids as a source of carbon and energy for producing numerous amino acids and rapid growth of microorganisms [9,10].

Due to biotechnology advancements, amino acids with a million-ton production titer, are the third most significant fermentation products after ethanol and antibiotics [11]. In particular, large-scale production of the L-glutamate family of amino acids (L-GFAAs), which includes L-glutamate, L-arginine, L-citrulline, L-proline, L-hydroxy proline (HYP) and 5-aminolevulinic acid (5-ALA), has undergone rapid developments due to the world rapidly rising demand [1, 2]. For instance, L-glutamate, the first commercialized amino acid [12], occupies more than 4 million tons of the global markets per year [13]. Furthermore, L-arginine is semi-essential amino acid with a demanding capacity of 1200 tons per year that is widely employed in the industrial and medicinal industries [14,15].

The greater needs for the manufacture of these compounds are put forth by the tremendous demand. From the standpoint of environmentally friendly biotechnology and sustainable developments, fermentation approaches have been widely used for the fermentation-based synthesis of amino acids. [16-18].

C. glutamicum is a gram-positive and facultative anaerobic bacterium used for the industrial production of amino acids, like L-glutamate and L-lysine, under aerobic conditions. Given that L-

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glutamic acid is produced industrially on a global scale annually of more than 2,500,000 tons as a favor enhancer, C.glutamicum currently holds the biggest market share for amino acids [1]. Other amino acids, including L-threonine and L-tryptophan, are industrially produced by *C. glutamicum* through microbial fermentation. The worldwide production of amino acids has doubled to nearly 5,000,000 tons in the past decade [19].

Corynebacterium glutamicum, a generally recognized as a safe strain that accumulated considerable amounts of L-glutamate, was discovered by Japanese scientists in 1956, offering a practical process in the fermentative production of amino acids [20] and other high-value chemicals [21], [22]and [23]. *Corynebacterium glutamicum* and *Brevibacterium linens* have been successfully engineered to produce L-arginine, in addition to GFAAs, which are used in the processing of L-glutamate [24], L-proline [25] and [26], L-citrulline [27] and [28] HYP and 5-ALA [29].

As a starter in the production of various fermented dairy products, *Lactobacillus bulgaricus* is used most frequently. Its metabolic activity is not only able to make lactic acid but is also protein hydrolysis and amino acid biosynthesis, which creates peptides and amino acids for bacterial growth as well as metabolites that contribute to flavor formation in fermented products [30]. The amino acid catabolism system of *Lactobacillus bulgaricus* functions to balance the bacterium's requirement for amino acids [31] and [32].

Brevibacterium linens require biotin for cell growth. Biotin is necessary for fatty acid synthesis. When biotin levels in the culture media were low, it was hypothesized that the cell membrane permeability would increase. Likewise, the addition of detergent, penicillin, ethambutol, or cerulenin changes the permeability of the cell membrane and cell wall [33]. *Brevibacterium linens* is particularly useful in the industrial synthesis of vitamins and amino acids [8]. Its capacity to create significant amounts of bacteriocins Important physiological functions include its ability to produce high levels [34] and its metabolism of aromatic acids metabolism are among its crucial physiological processes [35].

This study aims to use of some strains (*Corynebacterium glutamicum*, *Lactobacillus bulgaricus,* and *Brevibacterium linens* for the production of some amino acids (lysine, methionine, threonine, arginine, tyrosine, aspartic acid, glutamic acid, alanine, and proline) on synthetic fermentation media under different conditions as well as, incubation periods; incubation temperatures; pH values; media volume and various sources of carbon and nitrogen and then qualitative and quantitative analysis of amino acids by HPLC, for choose the best conditions for the growth of the used microorganisms and increasing of amino acids productivity.

2-MATERIALS AND METHODS

2.1 Materials

2.1.1 Microbial Strains:

Three microbial strains were used in this investigation. *Corynebacterium glutamicum* ATCC 13032, *Lactobacillus delbrueckii ss. bulgaricus* ATCC 7995 and *Brevibacterium linens* ATCC 14929 were obtained from the Microbiological Resources Center (MIRCEN), Cairo, Faculty of Agriculture Ain Shams University.

2.1.2 Culture Media:

Peptone yeast extract agar (PYA 1%) medium has the following composition of (g/L) bactopeptone 10, yeast extracts 10, NaCL 5, and agar-agar 20 [36].

2.1.3 Seed culture media:

Seed culture media has the composition of (g/L) glucose 20, $MgSO_4.7H_2O$ 0.25, KH_2PO_4 1, peptone water 10, K_2HPO_4 1, NaCl 2.5, $MnSO_4.H_2O$, 0.1, yeast extract 10 and distilled water 1L [7,38].

2.1.4 Basal media:

Basal media consist of (g/L) glucose 20, $(NH_4)_2SO_4$ 10, KH_2PO_4 1, K_2HPO_4 0.02, $MnSO_4.H_2O$ 0.002, $MgSO_4.7H_2O$ 0.4, $FeSO_4.7H_2O$ 0.002 and distilled water 1L [39] with some modification.

2.2 Chemicals Used:

All Chemicals used in this study were purchased from El-Gamhouria Trading Chemicals and Drugs Company, Egypt.

2.3 Methods

All the experimental work of this study was carried out at the Food Science and Technology Department, Faculty of Agriculture, the Regional Center for Mycology and Biotechnology (RCMB), at Al-Azhar University, except HPLC was achieved at Food Industries and Nutrition Division, National Research Center, Dokki, Giza, Egypt.

2.3.1 Biotechnological experiments:

2.3.1.1 Preparation of Culture method:

These ingredients of culture were completed to 1000 ml of distilled water. The pH of the medium was adjusted to 7.2, 6.25, and 7.4 for *Corynebacterium glutamicum*, *Lactobacillus delbrueckii ss. bulgaricus* and *Brevibacterium linens*, respectively, then autoclaved at 121°C for 15 min. The Petri dishes were inoculated with the tested microbial strains and incubated at 37ºC for two days. The suspended glucose solution was sterilized separately and mixed with other nutrients before inoculation.

Seed medium (50 ml) was inoculated in 250 ml Erlenmeyer flask by loop having cells of strains from

24 hrs. Old complete agar plates were incubated with shaking at 150 rpm in a gyratory shaking incubator at 30°C for 24 hr.

Basal medium (50 ml) was inoculated in 250 ml Erlenmeyer flask with 2% (v/v) of standard inoculum (seed culture) containing 4.8×10^6, 4.3×10^6 and 5.5×10^6 viable cells/ml for *Corynebacterium glutamicum*, *Lactobacillus delbrueckii ss. bulgaricus* and *Brevibacterium linens*, respectively, incubated in a gyratory shaking incubator at 150 rpm and 30°C for 48 hrs. Samples were taken on the second day and centrifuged at 8000×g for 5 minutes. The supernatants were examined for amino acids and residual sugar, and the same procedures were followed as discussed [40] with some modifications.

2.3.1.2 Preparation of microbial inoculates:

The microbial strains were periodically transferred and kept on yeast extract agar, stored in a refrigerator at 4°C, and reactivated at intervals every 15 days until use with some modification.

The microbial cultures used in this investigation were inoculated on Petri-dishes containing yeast extract agar and incubated for one day at 37˚C for colony formation.

A serial dilution is essential 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 that ended the entire chain, it is feasible to know how much bacteria you commenced with. 1 mL of each sample was added to 9 mL of sterile distilled water, and a number of six-fold dilutions were prepared in the same diluent. Aliquots of 0.1 mL of 10-6 diluted sample suspension ere added to the agar plates prepared from the yeast extract agar and distributed evenly over the surface with a sterile glass spreading rod. Following incubation at 37ºC for 24 hr, those plates which contained the desired bacterial well-isolated colonies were selected as the source of culture to be evaluated for the production of amino acids. [37]

2.3.2 Production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* bacteria under different conditions:

2.3.2.1 Incubation periods:

For the detection of a suitable incubation period for maximizing the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens*. These bacteria were allowed to grow on a growth medium adjusted at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum*, and pH 7.4 for *Brevibacterium linens* and incubated for 24, 48, and 72 hr under the same conditions (30°C and 150 rpm) [42, 5, and 43].

2.3.2.2 Incubation temperatures:

For the detection of a suitable incubation temperature for maximizing the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens*, the bacteria were allowed to grow on a growth medium and incubated at different temperature degrees covering the range from 28, 30 and 37°C at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum* and pH 7.4 for *Brevibacterium linens* and incubated for 2 days and 150 rpm [44].

2.3.2.3 pH values:

The effect of pH values (6.5, 7, and 7.5) of the culture medium on amino acid production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* was studied. The medium was initially adjusted before sterilization, using concentrated ammonia (NaOH 1N) and (HCL 6N) to the tested pH values by (3505 pH Meter system - JENWAY).

The *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* bacterias were allowed to grow on a growth medium for 2 days at 30°C and 150 rpm [45].

2.3.2.4 Media volumes:

The effect of media volumes (50, 75, and 100 ml) of the culture medium on amino acids production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* were investigated, the bacteria were allowed to grow on growth medium adjusted at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum* and pH 7.4 for *Brevibacterium linens* and incubated for 2 days under the same conditions (30°C and 150 rpm) [46].

2.3.2.5 Carbon sources:

The effect of sources of carbon (glucose, sucrose, and d-fructose) of the culture medium on amino acid production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* was studied. The bacteria were allowed to grow on a growth medium adjusted at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum*, and pH 7.4 for *Brevibacterium linens* and incubated for 2 days under the same conditions (30°C and 150 rpm) [47].

2.3.2.6 Nitrogen sources:

The effect of nitrogen sources (ammonium sulfate, ammonium nitrate, and urea) of the culture medium on amino acids production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* was investigated. The bacteria were allowed to grow on a growth medium adjusted at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum*, and pH 7.4 for

Brevibacterium linens and incubated for 2 days under the same conditions (30°C and 150 rpm) [48,49].

2.3.3 Methods analysis:-

Samples were taken on the second day and centrifuged at 8000×g for 5 minutes. The supernatants were examined for amino acids and residual sugar, and the same procedures were followed as discussed by [40] with some modifications.

2.3.3.1 Determination quantitative of amino acids produced by HPLC: -

The analysis of free amino acids has been demonstrated using the Pico–Tag method after deproteinization and precipitation with 5–sulfosalicylic acid (SSA), followed by centrifugation to remove the precipitated protein. The supernatant was taken for free amino acids analysis. The amino acid composition of experimental samples was determined using HPLC – Pico – Tag method described by [50, 51].

2.3.3.2 Determination of residual sugar:

Residual sugar was determined by using the dinitrosalicylic acid (DNS) method. The fermentation broth was centrifuged and then used supernatant for the estimation of residual sugar [41].

2.4 Statistical Analysis:

The SPSS statistical package program was used to analyze the data obtained from three replicates of the tested samples to determine residual sugar only, were analyzed by (ANOVA), and to assess differences among the means were compared using the Duncan's Multiple Range tests [52]. The chosen significant level of 0.05.

3-Results and Discussion: -

3.1Effect of different conditions on the production of amino acids: -

3.1.1 Effect of incubation periods: -

This experiment was performed at different incubation periods (24, 48, and 72 hr) on a growth medium adjusted at pH 6.25 for *Lactobacillus bulgaricus*, pH 7.2 for *Corynebacterium glutamicum* and pH 7.4 for *Brevibacterium linens* under the same conditions (30°C and 150 rpm) to choose the best incubation period for production of the highest yield from amino acids. Data is illustrated in Table (1). The obtained data in Table (1) it could be observed that the highest yield production of total amino acids for *Corynebacterium glutamicum*, *Lactobacillus bulgaricus,* and *Brevibacterium linens* was recorded after incubation periods 48 hr (5.846, 6.196, and 7.627 g/L respectively), compared with basal media (0.371 g/L), which presented approximately more than two times than those obtained at 24 hr of the incubation period (2.051, 2.510 and 2.832 g/L, respectively). After that, when the incubation period increased, the amount of amino acids decreased after 72 hr, which recorded the lowest content of amino acids (4.649, 4.410, and 6.075 g/L).

Residual sugar in the broth was found at minimum at 48 hr incubation (5.40, 2.94, and 4.04 g/L, respectively) compared with basal media (19.52 g/L). These results were following the data obtained by [53,54], these outhers were found optimized incubation time of 48 hr optimum fermentation period for enhanced amino acids production.

3.1.2 Effect of incubation temperatures on the production of amino acids:

For the detection of a suitable incubation temperature for maximizing amino acids production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens*, which was allowed to grow on growth medium adjusted at pH 7.20, 6.25, and 7.4 and incubated for 2 days at different temperature degrees covering (28, 30 and 37°C) and the results presented in Table (2), the results showed that the optimum temperature capable of promoting amino acids production at 30°C for *Corynebacterium glutamicum* and *Brevibacterium linens* (5.846 and 7.627 g/L, respectively) while *Lactobacillus bulgaricus* at 37°C (6.321 g/L) of promoting amino acids production as compared with basal media (0.371 g/L),

Residual sugar in the broth was found to be minimum at incubation temperatures of 30°C for *Lactobacillus bulgaricus and Brevibacterium linens (* 2.94, 4.04 g/L, respectively) and 28°C for *Corynebacterium glutamicum* (2.67 g/L) as compared with basal media (19.52 g/L). These obtained results are approximate by outhers [44 and 55].

Table (1): Effect of incubation periods on amino acids production (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Lysine	**0.010**	**ND**	**0.038**	**0.008**	**ND**	**0.024**	**0.008**	**ND**	**0.067**	**0.050**
Methionine	**0.015**	**0.067**	**0.189**	**0.099**	**0.077**	**0.121**	**0.091**	**0.077**	**0.334**	**0.165**
Threonine	**ND**	**0.085**	**0.308**	**0.279**	**0.097**	**0.368**	**0.288**	**0.179**	**0.579**	**0.278**
Arginine	**ND**	**0.303**	**0.713**	**0.604**	**0.163**	**0.473**	**0.354**	**0.173**	**0.545**	**0.365**
Tyrosine	**0.156**	**0.592**	**1.202**	**0.985**	**0.987**	**1.865**	**0.887**	**1.087**	**2.544**	**2.258**
Aspartic acid	**0.112**	**0.906**	**2.800**	**2.205**	**1.056**	**2.885**	**2.505**	**1.156**	**2.961**	**2.605**
Glutamic acid	**ND**	**ND**	**0.090**	**0.075**	**0.022**	**0.116**	**0.050**	**0.052**	**0.154**	**0.076**

Alanine	**0.078**	**0.098**	**0.398**	**0.299**	**0.108**	**0.302**	**0.199**	**0.108**	**0.352**	**0.199**
Proline	**ND**	**ND**	**0.108**	**0.095**	**ND**	**0.042**	**0.028**	**ND**	**0.091**	**0.078**
Total	**0.371**	**2.051**	**5.846**	**4.649**	**2.510**	**6.196**	**4.410**	**2.832**	**7.627**	**6.075**
Residual sugar	**19.52^{a} ±0.98**	**3.29^{g} ±0.13**	**0.54^{h} ±0.02**	**0.69^{h} ±0.03**	**7.72^{d} ±0.32**	**2.94^{g} ±0.15**	**5.87^{e} ±0.27**	**10.83^{b} ±0.44**	**4.04^{f} ±0.16**	**8.54^{c} ±0.27**

- ND: not-detect-

The obtained results represent the mean of triplicate determination. Standard error; the means within the same row having different superscript are significantly varied (P ≤0.05) for residual sugar.

Table (2): Effect of incubation temperatures on amino acids production (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		28°C	**30°C**	**37°C**	**28°C**	**30°C**	**37°C**	**28°C**	**30°C**	**37°C**
Lysine	0.010	0.016	0.038	ND	0.015	0.024	0.035	0.015	0.067	0.038
Methionine	0.015	0.089	0.189	0.055	0.067	0.121	0.151	0.202	0.334	0.081
Threonine	ND	0.189	0.308	0.157	0.169	0.368	0.287	0.259	0.579	0.266
Arginine	ND	0.575	0.713	0.550	0.262	0.473	0.525	0.277	0.545	0.228
Tyrosine	0.156	1.005	1.202	0.757	1.105	1.865	1.957	1.565	2.544	1.117
Aspartic acid	0.112	2.001	2.800	1.985	2.00	2.885	2.909	2.256	2.961	2.056
Glutamic acid	ND	0.075	0.090	0.055	0.055	0.116	0.095	0.051	0.154	0.065
Alanine	0.078	0.186	0.398	0.187	0.096	0.302	0.297	0.188	0.352	0.207
Proline	ND	0.055	0.108	0.055	0.035	0.042	0.065	0.055	0.091	0.066
Total	**0.371**	**4.191**	**5.846**	**3.801**	**3.804**	**6.196**	**6.321**	**4.868**	**7.627**	**4.124**
Residual sugar	19.52^{a} ±0.98	2.67^{f} ±0.10	0.54^{g} ±0.02	3.19^{e} ±0.14	8.82^{d} ±0.41	2.94^{f} ±0.15	8.12^{d} ±0.42	10.94^{b} ±0.55	4.04^{e} ±0.16	9.51^{c} ±0.38

- ND: not-detect
- The obtained results - ND: not-detect
- The obtained results represent the mean of triplicate determination. Standard error;
the means within the same row having different superscript are significantly varied (P ≤0.05) for residual sugar.

3.1.3 Effect of pH values on the production of amino acids:

The effect of different pH values on the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* were studied. For this purpose, the growth medium was adjusted at pH values (6.5, 7, and 7.5) and incubated for 2 days at 30ºC. The results were listed in Table (3). It could be concluded from the results presented in Table (3) the optimum initial pH value capable of promoting amino acids production was found at pH value (7) for *Corynebacterium glutamicum*, (6.100 g/L), at pH value (6.5) for *Lactobacillus bulgaricus* (6.295 g/L) and pH value (7.5) for *Brevibacterium linens* (7.129 g/L) as compared with basal media (0.371 g/L).

On the other hand, the residual minimum sugar in the broth was found at pH value (7) for *Corynebacterium glutamicum* (1.40g/L), at pH value (6.5) for *Lactobacillus bulgaricus* (4.34 g/L), and pH value (7.5) for *Brevibacterium linens* (3.37g/L) as compared with basal media (19.52 g/L).

The other Present rehearses [56, 43 and 57] were showed that the suitable pH for amino acids production from bacterial strains was 6.5-7.5.

For the biosynthesis of microbial primary and secondary metabolites, the medium's pH was considered an extremely significant element. The pH impacts the cell membrane permeability characteristics, which result in either ions uptake or loss in nutrient media during microbial growth. Thence, pH was regarded as a decisive and crucial element influencing culture media for microbial growth and product yields. The pH of the medium was controlled using basic substances such as calcium carbonate, potassium, and sodium hydroxide, and inorganic acids [45].

3.1.4 Effect of media volumes on the production of amino acids:

This experiment was performed to detect the suitable media volume needed for the production of the highest yield of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* at conditions 150 rpm, 30ºC and 2 days at pH 7.2, 6,25 and 7.4. The bacteria were cultivated on different medium volumes of 50, 75, and 100 ml.

Data were illustrated graphically in Table (4) and showed that the medium volume (50 ml) was the best medium volume for *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* in the production of amino acids (5.846, 6.196 and 7.627 g/L respectively) as compared with basal media (0.371 g/L)..

Also, the residual minimum sugar in the broth was found at medium volume (50 ml) for strains *Lactobacillus bulgaricus and Brevibacterium linens* (2.94 and 4.04 g/L respectively) while at medium volume (100 ml) for *Corynebacterium glutamicum (*2.14 g/L) as compared with basal media (19.52 g/L). Present results were in line with [40], [10] and [58] were observed that the best medium volume was 50 ml broth volume for the production of amino acids.

Table 3): Effect of pH values on production of amino acids (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		6.5	**7**	**7.5**	**6.5**	**7**	**7.5**	**6.5**	**7**	**7.5**
Lysine	0.010	ND	0.39	0.021	0.022	0.015	0.011	0.022	0.035	0.061
Methionine	0.015	0.066	0.197	0.095	0.106	0.057	0.061	1.06	0.257	0.298
Threonine	ND	0.189	0.315	0.296	0.289	0.200	0.196	0.209	0.350	0.498
Arginine	ND	0.450	0.709	0.699	0.478	0.159	0.109	0.378	0.355	0.459
Tyrosine	0.156	0.805	1.305	1.005	1.951	0.775	0.585	1.951	2.057	2.456
Aspartic acid	0.112	2.006	2.950	2.581	2.956	1.450	1.750	2.450	2.550	2.768
Glutamic acid	ND	0.065	0.088	0.056	0.150	0.078	0.026	0.094	0.107	0.166
Alanine	0.078	0.205	0.380	0.300	0.305	0.186	0.096	0.205	0.297	0.336
Proline	ND	ND	0.117	0.101	0.038	0.017	0.010	0.028	0.046	0.087
Total	**0.371**	**3.786**	**6.100**	**5.154**	**6.295**	**2.937**	**2.844**	**5.443**	**6.024**	**7.129**
Residual sugar	19.52^{a} ±0.98	2.92^{g} ±0.14	1.40^{b} ±0.08	3.07^{f} ±0.15	4.34^{e} ±0.14	8.25^{b} ±0.08	8.30^{b} ±0.15	7.80^{bc} ±0.25	6.83^{d} ±0.35	3.37^{f} ±0.12

- ND: not-detect
- The obtained results represent the mean of triplicate determination.

Standard error; the means within the same row having different superscript are significantly varied ($P \leq 0.05$) for residual sugar.

Table (4): Effect of media volumes on the production of amino acids (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		50 ml	**75 ml**	**100 ml**	**50 ml**	**75 ml**	**100 ml**	**50 ml**	**75 ml**	**100 ml**
Lysine	0.010	0.038	0.002	0.001	0.024	0.012	0.0011	0.067	0.052	0.021
Methionine	0.015	0.189	0.115	0.106	0.121	0.105	0.051	0.334	0.255	0.171
Threonine	ND	0.308	0.268	0.229	0.368	0.275	0.209	0.579	0.507	0.456
Arginine	ND	0.713	0.656	0.505	0.473	0.376	0.330	0.545	0.506	0.389
Tyrosine	0.156	1.202	0.857	0.689	1.865	1.557	0.869	2.544	2.227	1.875
Aspartic acid	0.112	2.800	2.554	2.256	2.885	2.474	2.206	2.961	2.524	2.287
Glutamic acid	ND	0.090	0.019	0.056	0.116	0.089	0.057	0.154	0.095	0.067
Alanine	0.078	0.398	0.297	0.268	0.302	0.265	0.205	0.352	0.285	0.209
Proline	ND	0.108	0.075	0.056	0.042	0.038	0.006	0.091	0.078	0.009
Total	**0.371**	**5.846**	**4.841**	**4.166**	**6.196**	**5.191**	**3.934**	**7.627**	**6.529**	**5.484**
Residual sugar	19.52^{a} ±0.98	0.54^{e} ±0.02	0.59^{e} ±0.03	2.14^{d} ±0.10	2.94^{d} ±0.15	4.34^{c} ±0.13	6.36^{b} ±0.26	4.04^{c} ±0.16	6.94^{b} ±0.20	7.16^{b} ±0.23

- ND: not-detect
- The obtained results represent the mean of triplicate determination. Standard error; the means within the same row having different superscripts are significantly varied ($P \leq 0.05$) for residual sugar.

3.1.5 Effect of carbon sources on the production of amino acids:

The Effect of carbon sources **(**glucose, sucrose, and fructose) on the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* at conditions 150 rpm, 30ºC and 2 days at pH 7.20, 6.25 and 7.4 were studies, and the result was listed in Table (5). The presented result in Table (5) showed that glucose was the best carbon source for the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens*, the content of the amino acids it was recorded (5.846, 6.196 and 7.627 g/L respectively) in the culture medium with glucose [47, 59, 60 and 61] they observed the highest output of amino acids in a medium containing glucose as carbon source compared to other carbon sources.

In the same way, the residual minimum sugar in the broth was recorded for use the glucose as a carbon source for the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* (5.40, 2.94 and 4.04 g/L respectively).

3.1.6 Effect of nitrogen sources on the production of amino acids:

The effect of nitrogen sources, including ammonium sulfate, ammonium nitrate, and urea, on amino acids production by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* at cultivation conditions 150 rpm, 30ºC and 2 days at pH 7.20, 6.25 and 7.4 were examined, and the obtained results are accounted in the Table (6). From the obtained data in Table (6), it was shown that the ammonium sulfate was the best nitrogen source for the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens*, the total amino acids content recorded (5.846, 6.196 and 7.627 g/L respectively) in the culture medium by

use ammonium sulfate as nitrogen sources for the production of amino acids. Where amino acids yield was highest compared with basal media was resulted in decreased production of amino acids, and residual sugar was increased [40, 62].

On the other hand, the minimum residual sugar in the broth was recorded for the use of ammonium sulfate as nitrogen sources for the production of amino acids by strains *Lactobacillus bulgaricus and Brevibacterium linens* (2.94 and 4.04 g/L, respectively). In comparison, it was recorded by using Ammonium ammonium nitrate as nitrogen source for *Corynebacterium glutamicum (*4.47 g/L) as compared with basal media (19.52 g/L).

3.1.7 The best conditions in the production of amino acids by *Corynebacterium glutamicum*, *Lactobacillus bulgaricus,* and *Brevibacterium linens*:

The effect of using the optimum conditions for the use of *Corynebacterium glutamicum* (Incubation periods 48 hr, incubation temperature 30°C, pH 7, medium volume 50 ml, glucose as carbon source, and ammonium sulfate as nitrogen sources), for *Lactobacillus bulgaricus (* Incubation periods 48 hr, incubation temperature 37°C, pH 6.5, medium volume 50 ml, glucose as carbon source and ammonium sulfate as nitrogen sources) and *Brevibacterium linens* (Incubation periods 48 hr, incubation temperature 30°C, pH 7.5, medium volume 50 ml, glucose as carbon source and ammonium sulphate as nitrogen sources in the production of amino acids were studied and the result was presented in Table (7).

Table (5): Effect of different carbon sources on amino acids production (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
Lysine	0.010	0.038	0.016	0.022	0.024	0.011	0.015	0.067	0.031	0.035
Methionine	0.015	0.189	0.101	0.135	0.121	0.078	0.096	0.334	0.206	0.275
Threonine	ND	0.308	0.206	0.225	0.368	0.225	0.287	0.579	0.426	0.485
Arginine	ND	0.713	0.585	0.606	0.473	0.307	0.389	0.545	0.405	0.474
Tyrosine	0.156	1.202	0.865	1.007	1.865	1.383	1.677	2.544	1.863	2.187
Aspartic acid	0.112	2.800	2.008	2.380	2.885	2.205	2.458	2.961	2.303	2.667
Glutamic acid	ND	0.09	0.058	0.07	0.116	0.072	0.096	0.154	0.092	0.116
Alanine	0.078	0.398	0.256	0.303	0.302	0.201	0.250	0.352	0.217	0.286
Proline	ND	0.108	0.075	0.092	0.042	0.017	0.033	0.091	0.051	0.073
Total	**0.371**	**5.846**	**4.170**	**4.840**	**6.196**	**4.499**	**5.301**	**7.627**	**5.594**	**6.598**
Residual sugar	19.52[a] ±0.98	0.54[f] ±0.02	10.05[b] ±0.004	9.60[b] ±0.03	2.94[e] ±0.15	4.04[d] ±0.17	4.26[d] ±0.18	4.04[d] ±0.16	4.94[c] ±0.21	5.02[c] ±0.24

- ND: not-detect
- The obtained results represent the mean of triplicate determination. Standard error; the means within the same row having different superscripts are significantly varied (P ≤0.05) for residual sugar.

Table (6): Effect of nitrogen sources on amino acids production (g/L):

Amino Acids (g/L)	Basal media	*Corynebacterium glutamicum*			*Lactobacillus bulgaricus*			*Brevibacterium linens*		
		Ammonium sulfate	Ammonium nitrate	Urea	Ammonium sulfate	Ammonium nitrate	Urea	Ammonium sulfate	Ammonium nitrate	Urea
Lysine	0.010	0.038	0.020	0.012	0.024	0.017	0.011	0.067	0.052	0.021
Methionine	0.015	0.189	0.131	0.063	0.121	0.085	0.047	0.334	0.290	0.246
Threonine	ND	0.308	0.206	0.165	0.368	0.298	0.185	0.579	0.478	0.396
Arginine	ND	0.713	0.655	0.606	0.473	0.396	0.306	0.545	0.486	0.408
Tyrosine	0.156	1.202	1.007	0.557	1.865	1.557	0.766	2.544	2.255	1.669
Aspartic acid	0.112	2.800	2.404	2.358	2.885	2.504	2.276	2.961	2.554	2.276
Glutamic acid	ND	0.090	0.072	0.057	0.116	0.088	0.067	0.154	0.101	0.077
Alanine	0.078	0.398	0.313	0.253	0.302	0.253	0.183	0.352	0.293	0.208
Proline	ND	0.108	0.089	0.052	0.042	0.028	0.018	0.091	0.068	0.029
Total	**0.371**	**5.846**	**4.897**	**4.123**	**6.196**	**5.226**	**3.859**	**7.627**	**6.577**	**3.859**
Residual sugar	19.52[a] ±0.98	0.54[h] ±0.02	4.47[f] ±0.18	10.46[c] ±0.06	2.94[g] ±0.15	8.41[de] ±0.40	9.03[d] ±0.40	4.04[f] ±0.16	12.08[b] ±0.51	10.88[c] ±0.44

- ND: not-detect
- The obtained results represent the mean of triplicate determination. Standard error; the means within the same row having different superscripts are significantly varied (P ≤0.05) for residual sugar.

From the obtained result in Table (7) it could be observed that the highest yield production of total amino acids for *Corynebacterium glutamicum*, *Lactobacillus bulgaricus,* and *Brevibacterium linens* was recorded (5.846, 6.196, and 7.627 g/L, respectively as compared with basal media was the amount of amino acids (0.371 g/L).

From the same Table (7) showed that the highest production of amino acids for *Corynebacterium glutamicum* was recorded in aspartic acid (2.800 g/L), tyrosine (1.202 g/L), and arginine (0.713 g/L) followed by alanine, threonine, methionine and proline (0.398, 0.308, 0.189, and 0.108 g/L, respectively) while the lowest production of amino acids it was Glutamic acid and Lysine (0.090 and 0.038 g/L respectively).

Also, From Table (7) shows that the highest production of amino acids for *Lactobacillus bulgaricus* was recorded in aspartic acid and tyrosine (2.885 and 1.865 g/L, respectively), followed by arginine, threonine, alanine, methionine, and glutamic acid (0.473, 0.368, 0.302, and 0.121 g/L, respectively), while the lowest production of amino acids it was Proline and Lysine (0.042 and 0.024 g/L respectively).

In the same way, Table (7) showed that the highest production of amino acids for *Brevibacterium linens* was recorded in aspartic acid and tyrosine (2.961 and 2.544 g/L, respectively), followed by threonine, arginine, alanine, methionine, and glutamic acid (0.579, 0.545, 0.352, and 0.334 g/L, respectively) while the lowest production of amino acids it was proline and lysine (0.091 and 0.067 g/L respectively).

Residual sugar in the broth was found minimum for the use of *Lactobacillus bulgaricus (*2.94 g/L), followed by *Brevibacterium linens* (4.04 g/L) and *Corynebacterium glutamicum* (5.846 g/L) compared with basal media (19.52 g/L).

A similar result was also found in a study by [63] and [64] found that the production of amino acids for *Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens* were excreted in broth and accumulated in the medium [65], isolated different amino acids from the bacterial culture broth.

Table (7): The best conditions in the production of amino acids by *Corynebacterium glutamicum, Lactobacillus bulgaricus,* and *Brevibacterium linens* (g/L):

Amino Acids (g/L)	Basal media	Microbial strains		
		Corynebacterium glutamicum	*Lactobacillus bulgaricus*	*Brevibacterium linens*
Lysine	0.010	0.038	0.024	0.067
Methionine	0.015	0.189	0.121	0.334
Threonine	ND	0.308	0.368	0.579
Arginine	ND	0.713	0.473	0.545
Tyrosine	0.156	1.202	1.865	2.544
Aspartic acid	0.112	2.800	2.885	2.961
Glutamic acid	ND	0.090	0.116	0.154
Alanine	0.078	0.398	0.302	0.352
Proline	ND	0.108	0.042	0.091
Total	**0.371**	**5.846**	**6.196**	**7.627**
Residual sugar	19.52^{a}±0.98	0.54^{d}±0.02	2.94^{c}±0.15	4.04^{b}±0.16

- ND: not-detect
- The obtained results represent the mean of triplicate determination. Standard error; the means within the same row having different superscripts are significantly varied (P ≤0.05) for residual sugar.

Conclusion

In This research aimed to choose the optimum conditions (pH values, media volumes, incubation periods, incubation temperatures, and different sources of carbon and nitrogen) on growth of the used microorganisms (*Corynebacterium glutamicum*, *Lactobacillus bulgaricus*, and *Brevibacterium linens)* and production of amino acids (lysine, methionine, threonine, arginine, tyrosine, aspartic acid, glutamic acid, alanine, and proline) and then quantitative analysis of amino acids by HPLC. The results showed that the optimum conditions for production of amino acids was recorded in *Brevibacterium linens* the pH (7.5), incubation temperature (30°C), medium volume (50 ml), and incubation period (48 hr), while for *Corynebacterium glutamicum* observed on pH (7), incubation temperature (30°C), medium volume (50 ml) and incubation period (48 hr), however, for *Lactobacillus bulgaricus* observed on pH 6.5, incubation temperature 37°C, medium volume (50 ml) and incubation period (48 hr). The best carbon and nitrogen sources were recorded for glucose and ammonium sulfate for three tested strains.

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Consent for Publication

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Conflict of Interests

The authors declare no conflict of interest, financial or otherwise

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