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Isolation and Screening of Amino Acids Producing Bacteria from Milk

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Abstract: Twenty eight strains from buffalo and cow milk were tested for amino acids production in M-1, M-II and L-6 media, fourteen being from each source. Generally, bacterial strains did not produce significant amount of all amino acids, but bacteria from both sources produced aspartic acid and alanine in molasses media (M-I and M-II). Maximum production of aspartic acid (1.9 g L^{-1}) was given by MB-7 and MB-12 in M-I medium after 72 h of fermentation. Some strains of both the sources also produced glutamic acid. Valine (maximum 1.4 g L^{-1} by MC-10) was produced in M-I medium by some bacterial strains isolated from cow's milk only. In L-6 medium, lysine (maximum 1.8 g L^{-1} by MB-6 and MC-13) was produced by all the isolates of both sources. The other amino acid which was produced in L-6 medium by majority of strains was isoleucine (maximum 2.1 g L^{-1} by MB-6). MB-5 isolated from buffalo milk was found as the best strain in L-6 medium and its produced glutamic acid, lysine and alanine as 4.5, 1.3 and 5.1 g L^{-1}, respectively. It was observed that bacterial strains isolated from buffalo milk were more productive than the strains obtained from cow milk. The strains MB-1, MB-5, MB-13 and MB-14 obtained from buffalo milk and MC-1, MC-7 and MC-13 from cow milk were selected as good amino acids producers strains. Thus aspartic acid, alanine, glutamic acid, lysine and isoleucine were the major amino acids produced from the bacterial strains of both the milk sources.

Key words: Bacteria of milk, fermentation, amino acids, valine, glutamic acid

Introduction

Milk, a liquid food secreted by the mammary glands of the female mammal for the nourishment of new borns, containing a wide range of dietary components of vital importance like water, carbohydrates, fats, proteins, minerals and vitamins (Webb ***et al.***, 1974). Milk secreted by the udders of healthy cows and buffaloes is sterile but by the time it is contaminated by several kinds of bacteria. It acquires a sizeable bacterial population from different sources like dust in air, coat of the cow, the person or the milkier, utensils, food, water, particles of manure and from the soil (Bryan, 1962).

Milk is excellent growth medium for different kind of microorganisms and whether milked by hand or machine, contains bacteria. It is desirable medium for microbiological growth and it is essential for it to be cooled as quickly as possible to keep bacterial population low (Misra and Kulla, 1989).

Many microorganisms such as ***Escherichia coli***, ***Enterobacter cloacae***, ***Ent. Aerogenes***, ***Ent. Gergoviae***, ***Citrobacter amalonaticus***, ***C. freundi***, ***Klebsiella oxytoca***, ***K. ozaenae***, ***K. pneumonia*** and some species of corynebacterium have excellent growth in raw milk (Ahmed and Salam, 1991).

Compared to chemical methods, the fermentative methods have the advantage of yielding optically active and biologically required L-form of amino acids directly (Yamada ***et al.***, 1972). In order to compete successfully with the chemical methods, fermentative production of amino acids must be improved in terms of the efficiency of the substrate to be converted into the desired amino acid (Amin ***et al.***, 1993).

Amino acids producing bacteria have been used commercially since 1950s and strains have been subsequently improved by regulatory mutants (Nadeem and Ahmad, 1999). The use of wild type bacteria for the production of amino acids such as L-glutamate, L-valine, L-alanine, L-glutamine and L-proline relies on either inherent metabolic regulations or stimulation of secretion by environmental factors. Many genera of bacteria are capable of amino acid production e.g. ***Corynebacterium***, ***Brevibacterium***, ***Bacillus***, ***Enterobacter***, ***Mycobacterium*** and ***Eschericia*** (Bona and Moser, 1988).

Essential amino acids are those which are not synthesized by the body of an organisms; therefore have to be supplied to it in food. Amino acids produced by these bacteria are used in food to minimize the deficiencies of essential amino acids. Traditionally amino acids are used as animal feed and human food additives and their use as animal feed may increase as other proteinaceous food stuffs became more expensive (Malumbers ***et al.***, 1995).

Amino acids also play a fundamental role in pharmaceutical fields. Many amino acids such as phenylalanine, cysteine, ornithine, valine, threonine, tryptophan, proline and hydroxyproline have been employed in the synthesis of several antibiotics e.g. bacitracin, cephalosporin, penicillin, gramicidin, tyrocidin and actinomycins (Meister, 1965).

The objective of this work includes the scanning and comparison of buffalo and cow milk for amino acid producing bacteria and screening these bacteria for the production of different amino acids.

Materials and Methods

The project was carried out at Biological Chemistry Division, NIAB, Faisalabad and completed in six months.

Isolation of bacterial strains

Milk samples were collected and diluted to ten, hundred and thousand times. In this way, a number of dilutions were made. The dilutions were filtered by sterile millipore per filtration pad to remove the dust particles and the filtrates were placed on nutrient agar plates incubated overnight at 37°C for the growth of bacteria. The colonies appeared were picked up, again streaked on nutrient agar plates and incubated at 37°C. Well separated colonies from each plate were cultured on nutrient agar slants for obtaining pure culture.

Production of amino acids through fermentation

Compositions of fermentation media used for the production of amino acids are given in Table 1. All the media were sterilized by autoclaving and then inoculated by fresh cultures

Table 1: Composition of fermentation media used for the production of amino acids by amino acid producing bacteria

Ingredients (%)	Medium M-I	Medium M-II	Medium L-6
Glucose	-	-	10.0
Trypticase	-	-	0.75
Molasses	10.00	10.0	-
$CaCO_3O$ 2.0	2.00	2.0	
KH_2PO_4O	0.05	0.05	0.07
K_2HPO_4O	0.05	0.05	0.4
$MgSO_4.7H_2O$	0.025	0.025	0.03
$(NH_4E)_2SO_4$	2.00	-	3.0
NH_4ENO_3	-	2.0	-
pH	7.20	7.2	7.0

Table 2: Absorbance of different concentrations of Isoleucine, Aspartic acid, Glutamic acid, Lysine and Alanine

Concentrations ($g\ L^{-1}$)	Absorbance (550 nm) Isoleucine	Aspartic acid	Glutamic acid	Lysine	Alanine
0.5	0.140	0.133	0.137	0.125	0.150
1.0	0.255	0.247	0.252	0.240	0.265
1.5	0.381	0.401	0.378	0.366	0.391
2.0	0.485	0.490	0.452	0.470	0.495
2.5	0.580	0.575	0.595	0.565	0.590

(over night growth) of bacteria. The inoculated media were incubated at 30-32°C in incubator shaker under continuous shaking conditions (150 rpm).

Harvesting

After every 24 h, samples were centrifuged and filtered through millipore filter having pore size 0.45 micron. The broths (filtrate) were analyzed qualitatively as well as quantitatively for every amino acid.

Qualitative analysis

Paper chromatography

Paper chromatography was carried out on Whatman and Desaga chromatographic paper No. 2045 by applying 10 µl sample with the help of micropipette. The paper was hanged vertically in chromatographic tank and irrigated for 18 h with n-butanol: acetic acid: water (4:1:5) solvent system; then dried and sprayed with 0.1% ninhydrin alcoholic solution. Coloured spots of amino acids were appeared on drying at 70°C for 10 min. The results were confirmed by comparing their Rf values with standard amino acids.

Paper electrophoresis

Some times two spots were too close to be distinguished. In this case, paper electrophoresis was applied. Whatman paper No. 3 was dipped on 0.05 M phosphate buffer and 10 µl of the sample from each filtered broth was spotted on the paper. The voltage of 1.5 KV was passed through the system from one hour. Paper was dried at 70°C for 15 min and sprayed with 0.1% ninhydrin solution. Colored spots of amino acids appeared after drying and results were confirmed by comparing their Rf values with standard amino acids.

Quantitative analysis

Quantitative analysis was performed by spectrophotometer. Colored spots of amino acids were eluted in 3 ml methanol and their absorbance was noted at 550 nm on spectrophotometer. The quantity of each amino acid was determined by comparing their absorbance with standard curve of specific amino acid (Mathews and Hold, 1990).

Standard curves of amino acids (Isoleucine, Aspartic acid, Glutamic acid, Lysine and Alanine)

Different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 g L^{-1}) of isoleucine, aspartic acid, glutamic acid, lysine and alanine were made in sterile distilled water. Chromatography was performed with 10 µl sample of each concentration of every amino acid. The chromatographs were eluted in methanol and absorbance was noted at 550 nm. Standard curves for each amino acid were obtained by plotting graph between absorbance and concentration.

Results

Twenty eight slants, including 14 from buffalo milk and 14 from cow milk, were studied for amino acids production in three different media (M-I, M-II and L-6). All the isolates produced different amino acids in different media.

Amino acids production by isolates from buffalo milk

Production of amino acids in medium M-1

All of the bacterial strains produced aspartic acid after 24 h. Maximum production of aspartic acid was observed by MB-7 and MB-11 strains, which produced aspartic acid in an amount of 1.3 g L^{-1}. After 48 h there was a rise in the production of aspartic acid. Maximum aspartic acid production was given by MB-7 which produced aspartic acid in an amount of 1.6 g L^{-1} after 48 hours. After 72 h maximum production of aspartic acid was observed by all the 14 strains. The maximum aspartic acid production was given by MB-7 and MB-12 (1.9 g L^{-1}). All the other strains produced aspartic acid in the range of 1.2-1.7 g L^{-1}. generally there was gradual increase in amino acid (aspartic acid) production from 24-72 h by each strain (Table 3).

Production of amino acids in medium M-II

Aspartic acid was the major amino acid that was produced by majority of strains in M-II after 24 h of incubation, except MB-1, MB-5 and MB-13 which produced both aspartic acid and alanine. Maximum aspartic acid production was shown by MB-2 which was 1.5 g L^{-1}. After 48 h there was generally decrease in aspartic acid production by all the strains except MB-1, MB-4 and MB-14 which produced increased amount of aspartic acid.

After 72 h, mix attitude was shown by strains (Table 3). Out of 14 strains, three strains MB-2, MB-4 and MB-7 showed more decline in aspartic acid production as compared with production after 48 h. Only two isolates MB-3 and MB-12 did not show aspartic acid production. MB-5 and MB-13 produced alanine in detectable quantity i.e. 0.9 and 1.2 g L^{-1} respectively, where as MB-12 produce 2.9 g L^{-1} glutamic acid after 72 h of fermentation.

Production of amino acids in medium L-6

After 24 h of fermentation all the isolates produced lysine in fermentation broth, while two amino acids, alanine and isoleucine were detected in fermentation broths of some strains (Table 3). After 48 h, there was an increase in amino acids production. Production of lysine was increased in all the isolates except MB-3 which give no amino acid production in L-6 medium after 48 h. Lysine was produced in the range of 1.0-1.5 g L^{-1} after 48 hours of fermentation. Six strains MB-1, MB-4, MB-5, MB-6, MB-13 and MB-14 produced alanine in an amount of 1.4, 1.7, 3.7, 0.7, 0.9 and 0.9 g L^{-1} after 48 h, respectively.

Table 3: Production of Amino acids by different bacterial strains isolated from buffalo milk in M-I, M-II and L-6 media

Strain	Time	Production in M-I Medium			Production in M-II Medium			Production in L-6 Medium		
No.	Hours	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity g L^{-1}
	24	6.34	Asp.	0.8	6.38	Asp., Ala.	0.5,0.6	8.02	Lys., Ala.	0.6, 0.7
MB-1	48	6.45	Asp.	1.2	6.74	Asp.	0.8	7.24	Lys., Ala.	1.2, 1.4
	72	6.30	Asp.	1.4	6.04	Asp.	1.2	7.30	Lys., Ala.	1.5, 1.5
	24	6.5	Asp.	1.0	6.9	Asp.	1.5	7.60	Lys.	0.9
MB-2	48	6.7	Asp.	1.2	6.2	Asp.	0.9	7.65	Lys., Ile.	1.1, 1.3
	72	6.9	Asp.	1.5	6.6	Asp.	0.7	7.60	Lys.	1.4
	24	6.6	Asp.	1.1	6.34	Asp.	1.1	7.29	Lys.	1.2
MB-3	48	7.8	Asp.	1.3	6.37	Asp.	1.0	7.50	-	-
	72	7.0	Asp.	1.5	8.8	-	-	7.41	-	-
	24	6.7	Asp.	1.2	6.8	Asp.	1.2	7.71	Lys.	0.8
MB-4	48	6.6	Asp.	1.5	6.4	Asp.	1.3	7.89	Lys., Ala.	1.0, 1.7
	72	6.9	Asp.	1.7	6.7	Asp.	1.0	7.80	Lys., Ala., Ile.	1.3, 1.7, 1.6
	24	6.85	Asp.	0.7	6.30	Asp., Ala.	0.7,0.5	7.99	Lys., Ala.,	0.6, 0.9
MB-5	48	6.60	Asp.	1.0	6.75	Asp.	0.6	7.36	Lys., Glu., Ala	1.1, 2.5, 3.7
	72	6.54	Asp.	1.2	6.05	Asp., Ala.	1.2,0.9	6.80	Lys., Glu., Ala.	1.3, 4.5, 5.1
	24	6.6	Asp.	1.2	6.2	Asp.	1.3	7.61	Lys.,	1.3
MB-6	48	6.8	Asp.	1.4	6.3	Asp.	1.0	7.58	Lys., Ala.	1.5, 0.7
	72	7.0	Asp.	1.6	6.6	Asp.	1.1	7.56	Lys., Ala. Ile.	1.8, 1.2, 2.1
	24	6.6	Asp.	1.3	6.2	Asp.	1.3	7.48	Lys.	0.9
MB-7	48	6.7	Asp.	1.6	6.8	Asp.	1.2	7.45	Lys.	1.2
	72	6.9	Asp.	1.9	6.4	Asp.	0.9	7.50	Lys., Ala. Ile.	1.5, 1.2, 1.3

Ala. = Alanine, Lys. = Lysine, Asp. = Aspartic acid, Glu. = Glutamic acid and Ile. = Isolecucine, MC = Strain from buffalo milk

Table 3: continued

Strain No.	Time Hours	Production in M-I Medium pH	Amino acid	Quantity g L^{-1}	Production in M-II Medium pH	Amino acid	Quantity g L^{-1}	Production in L-6 Medium pH	Amino acid	Quantity g L^{-1}
	24	6.7	Asp.	1.2	6.1	Asp.	1.2	7.53	Lys., Ile.	1.3, 1.1
MB-8	48	6.8	Asp.	1.3	6.2	Asp.	0.9	7.73	Lys., Ile.	1.4, 1.3
	72	6.5	Asp.	1.4	7.2	Asp.	0.9	7.58	Lys., Ala., Ile.	1.6, 2.6, 1.9
	24	6.6	Asp.	1.1	6.4	Asp.	1.2	7.11	Lys.	0.9
MB-9	48	7.6	Asp.	1.2	6.3	Asp.	0.9	7.66	Lys.	1.3
	72	7.3	Asp.	1.3	6.6	Asp.	0.9	7.52	Lys.	1.5
	24	6.7	Asp.	1.2	6.6	Asp.	1.3	7.66	Lys.	1.3
MB-10	48	6.8	Asp.	1.4	6.5	Asp.	0.9	7.90	Lys.	1.4
	72	6.5	Asp.	1.6	6.8	Asp.	1.4	7.62	Lys.	1.6
	24	6.8	Asp.	1.3	6.3	Asp.	1.0	7.15	Lys.	1.2
MB-11	48	6.9	Asp.	1.5	6.7	Asp.	0.7	7.12	Lys.	1.3
	72	7.2	Asp.	1.7	6.8	Asp.	1.1	6.05	Lys.	1.5
	24	6.5	Asp.	1.1	6.4	Asp.	1.3	7.42	Lys.	1.1
MB-12	48	6.7	Asp.	1.4	6.3	Asp.	0.7	7.72	Lys.	1.2
	72	6.9	Asp.	1.9	9.0	Glu.	2.9	7.56	Lys.	1.4
	24	7.45	Asp.	1.0	6.36	Asp., Ala.	1.0,1.2	8.08	Lys., Ala.	0.7, 1.1
MB-13	48	7.47	Asp.	1.3	6.42	Asp.	0.5	7.01	Lys., Ala.	1.3, 0.9
	72	7.44	Asp.	1.6	6.15	Asp.	0.9	6.84	Lys.	1.5
	24	6.53	Asp.	0.9	6.37	Asp.	0.9	7.99	Lys., Ala.	0.6, 1.1
MB-14	48	6.91	Asp.	1.2	6.14	Asp.	1.0	7.85	Lys., Ala.	1.1, 0.9
	72	6.77	Asp.	1.4	6.17	Asp.	1.1	7.35	Lys.	1.4

Ala. = Alanine, Lys. = Lysine, Asp. = Aspartic acid, Glu. = Glutamic acid and Ile. = Isolecucine, MC = Strain from buffalo milk

Table 4: Production of Amino acids by different bacterial strains isolated from Cow milk in M-I, M-II and L-6 media

Strain	Time	Production in M-I Medium			Production in M-II Medium			Production in L-6 Medium		
No.	Hours	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity gL^{-1}
	24	7.44	Asp., Ala.	1.8, 0.6	6.45	Asp.	0.7	7.52	Lys., Ile.	0.9, 0.8
MC-1	48	7.25	Asp., Ala.	1.4, 1.0	6.38	Asp.	0.8	7.00	Lys., Ile.	1.2, 0.8
	72	7.10	Asp., Ala.	1.3, 1.1	8.32	Asp.	1.0	6.62	Lys.	1.5
	24	7.57	-	-	6.39	-	-	7.34	Lys.	0.8
MC-2	48	7.59	-	-	6.64	Asp.	0.4	7.22	Lys., Ala.	1.0, 2.1
	72	7.40	Ala.	1.1	7.28	Asp.	0.5	7.08	Lys., Ala.	1.4, 2.2
	24	7.76	-	-	6.99	Asp.	Neg.	7.53	Lys., Ile.	0.9, 1.3
MC-3	48	7.74	-	-	6.37	-	-	7.55	Lys., Ile.	1.2, 1.2
	72	7.66	Ile.	0.7	6.97	-	-	7.23	Lys.	1.6
	24	6.88	-	-	6.30	-	-	7.45	Lys., Ile.	0.7, 0.9
MC-4	48	7.23	-	-	6.43	-	-	6.93	Lys., Ile.	1.1, 0.7
	72	7.92	-	-	6.51	-	-	6.55	Lys.	1.4
	24	7.68	Asp.	1.0	6.21	Asp.	Neg.	7.62	Lys., Ile.	0.5, 1.0
MC-5	48	8.52	Asp.	0.9	7.0	Asp.	0.5	7.09	Lys., Ile.	1.0, 0.9
	72	7.72	Asp.	0.8	6.87	Asp.	0.7	6.78	Lys.	1.2
	24	6.80	-	-	7.27	-	-	6.85	Lys., Ile.	0.7, 1.3
MC-6	48	7.52	-	-	6.56	-	-	6.63	Lys., Ile.	1.1, 1.4
	72	7.85	Val.	0.9	7.28	-	-	7.08	Lys.	1.4
	24	7.43	Asp., Ala.	0.9, 0.5	6.35	Asp.	0.8	7.36	Lys., Ile.	0.9, 0.7
MC-7	48	7.45	-	-	6.39	Asp.	1.4	7.18	Lys.	1.3
	72	7.70	Ala.	1.1	6.57	Asp.	1.5	6.91	Lys.	1.6

Ala. = Alanine, Lys. = Lysine, Asp. = Aspartic acid, Glu. = Glutamic acid and Ile. = Isolecucine, MC = Strain from buffalo milk

Table 4: continued

		Production in M-I Medium			Production in M-II Medium			Production in L-6 Medium		
Strain No.	Time Hours	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity g L^{-1}	pH	Amino acid	Quantity g L^{-1}
	24	6.60	-	-	6.46	Asp.	0.3	7.10	Lys., Ile.	0.7, 1.0
MC-8	48	7.63	-	-	8.18	-	-	6.65	Lys.	1.0
	72	7.85	-	-	8.87	-	-	6.30	Lys.	1.3
	24	6.69	-	-	6.11	Glu., Asp.	Neg.	7.26	Lys., Ile.	0.8,0.9
MC-9	48	6.75	-	-	6.37	-	-	6.97	Lys., Ala., Ile.	1.1,1.2, 1.1
	72	6.76	-	-	6.55	Glu.	1.0	6.54	Lys., Ala.	1.2, 2.4
	24	6.59	Val.	1.4	6.65	Asp.	0.2	7.66	-	-
MC-10	48	7.28	-	-	7.73	-	-	6.93	Ile.	1.4
	72	7.78	-	-	7.43	Asp.	0.7	6.42	-	-
	24	6.34	Val.	1.1	6.24	Asp.	Neg.	7.26	Lys., Ile.	1.0, 1.3
MC-11	48	6.88	-	-	7.58	-	-	6.81	Lys., Ile.	1.3,1.3
	72	7.78	-	-	7.40	-	-	6.51	Lys.	1.6
	24	6.54	Asp., Val.	1.7, 0.9	6.26	Asp. Glu.	Neg.	7.15	Lys., Ile., Ala.	1.1, 1.3, 1.2
MC-12	48	6.97	-	-	7.21	-	-	7.50	Lys., Ile., Ala.	1.5, 1.2, 1.2
	72	7.52	-	-	6.80	-	-	7.65	Lys., Ile.	1.7, 1.0
	24	6.68	Ala.	0.8	6.33	Asp.	Neg.	7.16	Lys.	1.2
MC-13	48	7.25	Glu., Ile.	0.9, 1.0	8.20	Glu.	1.2	7.55	Lys.	1.6
	72	7.58	-	-	8.13	Glu., Ala.	2.0, 1.0	7.35	Lys.	1.8
	24	7.38	Lys., Ala.	0.8, 1.0	6.68	Asp.	Neg.	7.08	Lys., Ala., Ile.	0.9, 1.0, 0.9
MC-14	48	7.64	-	-	6.90	Asp., Ala.	1.3, 1.0	7.14	Lys.	1.2
	72	7.32	-	-	8.13	Asp., Glu., Ala.	1.4, 0.9,1.3	7.30	Lys. Ala.	1.6, 1.1

Ala. = Alanine, Lys. = Lysine, Asp. = Aspartic acid, Glu. = Glutamic acid and Ile. = Isolecucine, MC = Strain from buffalo milk

After 72 h of fermentation, maximum amount of amino acids were produced in L-6 medium. The maximum lysine production was 1.8 g L^{-1} by MB-6. MB-5 was the only strain that produced glutamic acid with an amount of 4.5 g L^{-1} in L-6 medium. MB-1, MB-4, MB-5, MB-6, MB-7 and MB-8 produced alanine with an amount of 1.5, 1.7, 5.1, 1.2, 1.2 and 2.6 g L^{-1} respectively. In L-6 medium MB-5 was the best strain because it produced significant amount of amino acids i.e., lysine (1.3 g L^{-1}), glutamic acid (4.5 g L^{-1}) and alanine (5.1 g L^{-1}) after 72 hours of fermentation (Table 3).

Amino acid production by isolates from cow milk

Production of amino acids in medium M-I

Three strains were selected as best amino acid producers from cow milk. These strains were MC-1, MC7 and MC-13. Maximum amino acids production was given by MC-1 strain that produced both aspartic acid and alanine. Maximum aspartic acid production was given after 24 h, which was 1.8 g L^{-1} and maximum alanine production was given after 72 h, which was 1.1 g L^{-1}. MC-2 and MC-7 also produced alanine in an amount of 1.1 g L^{-1} after 72 h. MC-13 produced glutamic acid and isoleucine only after 48 h (Table 4).

Production of amino acids in medium M-II

In medium M-II, MC-1 and MC-7 produced aspartic acid in significant quantity, whereas MC-13 produced glutamic acid as well as alanine at different fermentation periods (Table 4). MC-14 produced aspartic acid (1.4 g L^{-1}), glutamic acid (0.9 g L^{-1}) and alanine (1.3 g L^{-1}) after 72 h of fermentation.

Production of amino acids in medium L-6

In medium L-6, lysine was produced by all the strains except MC-10 strain. Maximum lysine production (1.8 g L^{-1}) was obtained by MC-13 after 72 h. The other major amino acids which were produced by L-6 medium were isoleucine and alanine (Table 4).

Discussion

The selection of raw material is very important in microbial fermentation. Bashir (2000) reported that 7-10% molasses concentration was suitable for amino acid production. During the recent study 10% molasses concentration was found suitable. When molasses media were used, the major amino acids produced were aspartic acid, alanine, valine and glutamic acid. The results are in line with the findings of Ahmad and Nadeem (1993) who reported that wild type bacteria produced valine, alanine and glutamic acid; as in both studies, the raw material is same for amino acid production. Molasses has variety of nutrients, which are beneficial for both bacterial growth as well as the amino acid production. The amino acids were produced in molasses media

(M-I and M-II) because of low fats, high carbohydrates and high concentrations of magnesium, potassium, iron, sodium and chloride of molasses.

The second carbon source that was used in the study was glucose. Bashir (2000) reported that 10% glucose concentration enhanced the amino acid production during the experiment, which was in line of our work. When glucose medium was used, the major amino acids produced were lysine, isoleucine and alanine. It is because of the presence of glucose as carbon source in L-6 medium for amino acids fermentation. The results were in line with Rutkov (1984), who produced lysine and isoleucine by batch cultivation of C. Glutamicum in glucose-NH_4Cl medium and analyzed that maximum lysine and isoleucine were produced after 30 h of fermentation in a medium containing 6% glucose and 1.2% NH_4Cl.

Lysine was produced in L-6 medium (glucose medium) by almost all bacterial strains from both the milk sources. Recent study verifies the findings of Sen and Chatterhee (1983) who claimed that glucose as carbon source and $(NH_4)_2SO_4$ as nitrogen source were suitable for lysine production.

Very low production of amino acids was observed during initial 24 h of fermentation. The maximum production of amino acids was observed within 48-72 h. The results are in line of Sen and Chatterhee (1983) who reported the maximum production of amino acids after 72 h of continuous shaking fermentation (150 rpm).

Bacteria, which were isolated from cow milk, produced very nominal quantity of amino acids in M-I and M-II as compared to those produced by the bacteria isolated from buffalo milk. From this study it was observed that the isolates from buffalo milk were more productive as compared to those obtained from cow milk.

It was found that medium M-II was better for amino acid production as compared to M-I. The only difference between two was the presence of NH_4NO_3 in M-II. Thus NH_4NO_3 was selected as the better nitrogen sources for amino acid production.

In conclusion, aspartic acid, alanine, glutamic acid, lysine and isoleucine were the major amino acids produced from the bacterial strains of both the milk sources. On the basis of performance of all the bacterial strains, four strains (MB-1, MB-5, MB-13 and MV-14) isolated from buffalo milk and three strains (MC-1, MC-7 and MC-13) from cow milk were selected as the best amino acid producer strains and thus selected for further study.

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