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BIOCONVERSION OF CANE MOLASSES INTO AMINO ACIDS

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Soil and water were explored to obtain bacterial isolates for the production of amino acids in fermentation medium containing molasses, supplemented with some salts in order to optimize the growth conditions. Nearly all the bacterial isolates produced alanine in the fermentation broth, while some of them also produced aspartic acid, valine and glutamic acid in traces. Nearly all the isolated bacteria produced alanine in the fermentation broth, while some of them also produced aspartic acid . Maximum alanine (4.90 g/l) was produced by an isolate NIAB B-2784 after 72hr followed by 4.83 g/l (72 hr), 4.61 g/l (96 hr) by NIAB N-130 and 4.30 g/l (96 hr) by NIAB S-15. Few bacterial isolates also produced lysine in the fermentation broth headed by NIAB SK-179 producing about one gram of lysine. All the isolates showed varying behavior regarding time scale production and pH. The study showed that there was a wide scope for bioconversion of sugar industrial waste, cane molasses, into value added products, like amino acids, through bacterial fermentation.

INTRODUCTION

Blackstrap molasses is thick, dark residual liquid food (syrup) that remains after the last extraction of sugar from sugar cane (Anon.2006). Molasses not only provide a high concentration of C6 sugars to support the fermentation process and some other fermentable carbohydrates as well. It also contains a high concentration of "B" vitamins, especially biotin, which enhances fermentation rates (Paturau, 1982). Amino acids are known to be the building blocks of various proteins (Meister, 1965). Commercially the amino acids are being applied in various sectors such as food industry (about 60 %), feed additives (31 %), medicine and cosmetics (4%) and starting material in the chemical industry (5%) (Crueger and Crueger, 1990). The amino acids are important additives to the feed of dairy cattle to ensure proper protein synthesis and subsequent milk production in the animals (Dinn *et al.*, 1996). They also play a variety of roles in metabolism as components of proteins, and some even serve as neurotransmitters (Kazmierczak, 1993) as well as minimize the deficiencies of essential amino acids (Malumbers *et al.*, 1995). It has been observed that deficiency of even a single amino acid from the diet can expose the individual to certain serious diseases (Meister, 1965).

Microorganisms have been used commercially for over 40 years to produce amino acids (Eggeling and Sahm, 1999). Corynebacteria, along with other genera such as *Escherichia*, *Serratia* and *Bacillus,* are the main group of microorganisms used in the production of amino acids (Niederberger, 1989). Fermentative processes have the advantage of yielding optically active and biologically required L-form of amino acids, directly. These processes also help to utilize the agro-industrial wastes such as molasses (cane and beet), starch waste (maize and potato), whey, gas oil, domestic sewage, cellulose waste, sulfite waste liquor and corn steep liquor in order to up-grade them decreasing the environmental pollution. In addition, amino acids are found cheaper to be produced by fermentation process as compared to chemical synthesis (Soda *et al.*, 1983; Nadeem and Ahmad, 1999).

In Pakistan, bacterial production of amino acids has not been well exploited yet. So, in this study, efforts were made to produce amino acids using cane-molasses, an industrial waste for the purpose to exploit our local natural resources, and also a step to reduce the environmental pollution.

MATERIALS AND METHODS

More than two hundred bacterial isolates obtained from soil and water was screened for amino acid production in molasses medium. Nutrient, McConkey and EMB agars were used for isolation and propagation of bacteria particularly *E. coli* and *Corynebacterium glutamicum*, later checked for amino acid production in molasses medium.

Fermentation study

The fresh cultures of isolates were inoculated into fermentation medium in flasks (Erlenmayer, 250 ml) contained 50 ml of molasses medium. Flasks were incubated up to 96 hours at 30 ± 1°C and 150 rpm in a temperature controlled gyratory shaker (Sanyo orbital incubator D020206). Three ml of the fermented broth was taken after every 24 hours of incubation, centrifuged to separate the cell mass and then cell free supernatant was monitored for amino acid production to make qualitative as well as quantitative estimation and to study the time scale production of various amino acids.

Table 1. Fermentation medium used for the production of amino acids

Ingredients	Percentage
Cane Molasses	10.0
$CaCO_3$	2.0
KH_2PO_4	0.05
K_2HPO_4	0.05
$MgSO_4.7H_2O$	0.025
$(NH_4)_2SO_4$	2.0
pH *	7.2

* Adjusted with 1 M KOH

Amino acid analysis

Qualitative analysis of amino acids was done by paper chromatography (Lederer and Lederer, 1957) applying 10µl sample on Whatman1 filter paper. The paper was irrigated in n-butanol: acetic acid: water (4:1:5) solvent system for 18 hours. It was then heat dried and sprayed with 0.1 % ninhydrin alcoholic solution. Colored spots of amino acids appeared upon drying at 70 °C for 15 minutes and were identified by computing R_f values. Any confusion caused by the overlapping of some amino acids was removed by paper electrophoresis. The proximate quantitative estimation of amino acids was done through Spectrophotometry (Stenesh, 1984) by cutting the spots and eluting their entire color in 3ml of methanol. These elutes were then read at 550 nm using methanol as blank. Each amino acid was quantified with the help of standard curve of respective amino acid.

RESULTS AND DISCUSSION

Out of two hundred bacterial isolates, 52 percent of bacterial isolates produced amino acids in molasses medium. Amino acid production by some prominent isolates is given in (Table 2). Nearly all the bacteria produced alanine in the fermentation broth, while some of them produced aspartic acid in good amount. Maximum alanine (4.90 g/l) was produced by an isolate NIAB B-2784 after 72hr followed by 4.83 g/l (72 hr), 4.61 g/l (96 hr) by NIAB N-130 and 4.30 g/l (96 hr) by NIAB S-15. Seven isolates produced lysine, while glutamic acid and valine were also produced by twenty one and eleven isolates respectively.

Bacteria are the smallest microorganisms and exist in several shapes. Some of them exist in spheres (cocci) while others as cylinders (bacilli) and spirals (spirilla) (Brock *et al.*, 1986). They are present in extreme environments (from 0- 100°C) and used for the production of a variety of compounds like alcohols, antibiotics and amino acids. In fact, different kinds of bacteria are well adapted to their respective environment and produce metabolites utilizing natural resources (Sarles *et al.*, 1956). The present study was therefore aimed at the up-gradation of industrial waste (cane molasses) through bacterial fermentation to save the cost of amino acid import and decrease the environmental pollution generated by disposal off of cane molasses.

The selection of raw material is very important in microbial fermentation and involves carbon sources that produce carbon skeleton for amino acids, and energy source for fermentable microorganisms. A number of raw materials, such as molasses (cane, beet, citrus), starches from corn, cassava, sweet potato and banana along with cellulose, acetic acid, ethanol and methanol are used as carbon sources for amino acid production (Minoda, 1986). Among these raw materials, cane molasses is utilized for amino acid fermentation most of the time, due to easy availability and less price. Bashir (2000) reported 7-10 percent molasses concentration suitable for amino acid production. During the recent study, 10 percent molasses concentration was found suitable and resulted in accumulation of alanine and aspartic acid in the fermentation broth. The results were in line with the finding of Ahmad and Nadeem (1993) who reported that wild type bacteria produced valine, alanine, aspartic acid and glutamic acid when molasses was used as carbon source. 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 medium because of low fats, high carbohydrates (sucrose, fructose, glucose and dextrose) and high concentrations of magnesium, potassium, sodium, calcium and chloride . In the present study, molasses was found to have capability of up-gradation into amino acids (alanine, aspartic acid, valine and glutamic acid) by bacterial fermentation.

In molasses media, addition of ammonium salts has been reported as nitrogen supplementation for amino acid production. Ghosh and Sen, (1996) reported amino acid synthesis in decreasing order by the addition of ammonium sulfate, ammonium chloride and ammonium nitrate. Ammonium sulfate was used as nitrogen source in the present study. Bacteria showed varying behavior in amino acid production in molasses medium containing ammonium sulfate.

The pH of medium plays an important role in biosynthesis of amino acids. The pH of medium, adjusted to 7.2 for amino acid production gave acceptable results. Nakayama (1987) reported a range of pH (7.0-8.0) for glutamic acid production whereas Litchfield (1985) claimed that the pH range for amino acid production process is 6.0-7.2 by bacterial fermentation.

Table 2. Production of amino acid in molasses medium by bacterial isolates obtained from different sources

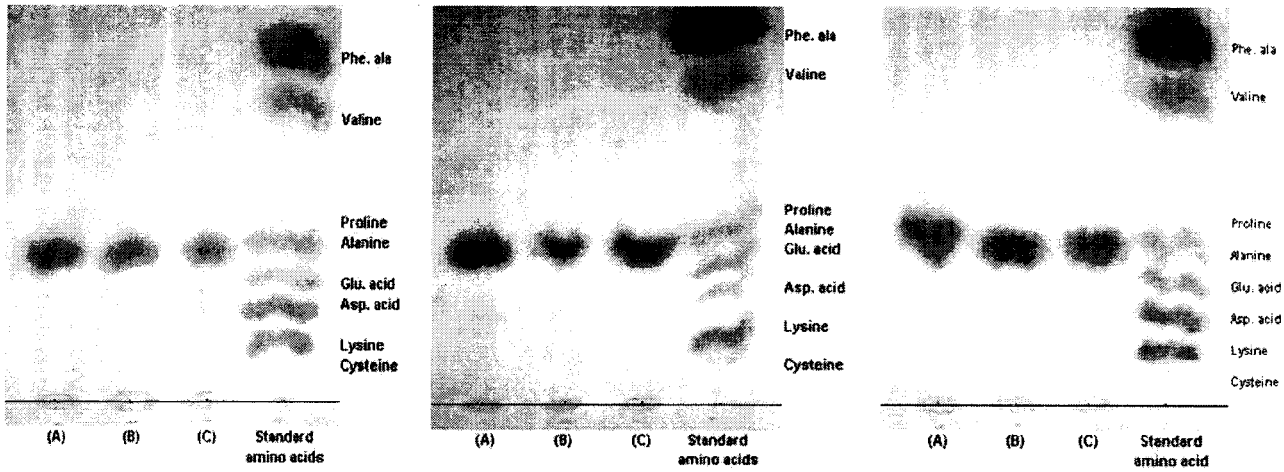
Isolates	pH	Amino acid Produced (hr)	Quantity (g/L)	Remark*
NIAB S-39	7.84	Ala (48)	1.93	-
		Val (48)	0.11	
NIAB W-32	7.22	Ala (72)	3.34	Asp, Val
NIAB S-14	7.1	Ala (48)	0.13	Lys, Asp, Glu
	7.5	Ala (72)	2.08	
NIAB3527-5	7.20	Ala (96)	2.69	-
NIAB W13-6	8.03	Ala (72)	1.72	
	7.33	Ala (96)	3.41	
		Val (96)	0.38	
NIAB SK-179	7.02	Lys (24)	0.20	-
	7.23	Cys (48)	0.41	
		Lys (48)	0.99	
		Ala (48)	1.90	
	8.11	Cys (72)	0.66	
		Lys (72)	0.98	
		Ala (72)	2.19	
	8.20	Cys (96)	0.65	
		Lys (96)	0.79	
		Ala (96)	2.88	
NIABB-2784	8.20	Ala (48)	2.80	-
		Val (48)	0.68	
	8.29	Asp (72)	1.35	
		Ala (72)	4.90	
	9.02	Ala (96)	4.25	
		Val (96)	1.52	
		Phe (96)	0.59	
NIAB N-130	8.34	Ala (48)	2.78	Phe
	8.22	Asp (72)	1.43	
		Ala (72)	4.83	
		Val (72)	0.56	
	8.50	Asp (96)	1.67	
		Ala (96)	4.61	
		Val (96)	0.67	
NIAB S-15	7.03	Ala (24)	0.52	Asp
	6.74	Ala (48)	1.94	
	7.41	Ala (72)	2.84	
	7.80	Ala (96)	4.30	

* Amino acids produced in negligible quantity

Temperature is also a key factor during the fermentation processes both for bacterial growth and amino acids biosynthesis. Generally, majority of bacteria able to grow in a range of 25-40°C resulted in production of various amino acids (Tortora *et al.*, 1998). The optimal temperature for amino acid fermentation is usually 30-35°C. The fermentation carried out at 30 ± 1°C produced acceptable results.

It was concluded that molasses has the capability to be up-graded into alanine, aspartic acid, glutamic acid, valine and lysine by bacterial fermentation. Addition of different salts and nitrogen sources in the medium resulted in the production of different amino acids by bacterial fermentation of cane molasses. These finding would help in proper utilization of industrial wastes (cane molasses) to produce a variety of amino acids.

Fig. 1. Production of amino acids in molasses medium M-I by bacterial isolate (W-32) after different time intervals



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