Original Article

To Assess the Biogenesis of Lysine by Penicillium Expansum Using Agricultural Waste as Energy Source

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ABSTRACT

Objective: To Assess the Biogenesis of lysine by penicillium expansum using agricultural waste as energy sources.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the CMH Institute of Medical Sciences Bahawalpur from May 2018 to September 2018.
Materials and Methods: The waste of Millet husk, sorghum husk and Banana stem were used throughout the study. Microorganism: Penicilliumexpansum was obtained from the Department of Botany, University of Glasgow, U.K. and was used in this study. The stock culture was maintained on agar slant, containing (G/L) dextrose 20: peptone 10: agar 20 and distilled water. The sterilized slants were inoculated with penicillium expansum and incubated at 27oC to obtained growth.
Results: In present study paper chromatographic method was carried out for separation and identification of amino acids synthesized by penicillium expansum in the culture broth using different solvent systems, who indicates that the selection of filter paper, solvent mixture and other conditions for paper chromatography are very important, because these determine Rf values and degree of separation.
Conclusion: This study concludes that biogenesis of lysine by penicillium expansum using agricultural waste as energy sources.
Key Words: Lysine, penicillium expansum, agricultural waste

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INTRODUCTION

Certain fungi are capable to synthesize amino acids[1]. Amino acid synthesis capability of microorganism depends on the media composition, physical parameters and organism employed. The microbial synthesis of amino acids on an industrial scale has developed rapidly in the past decade. The impetus for these advances originated chiefly from the interest in the nutritional applications of lysine and other essential amino acids. It is judge that more than 600,000 metric tons of Lysine are fabricate annually and, unsettled to the utilization of new uses in pharmaceuticals, cosmetics and polymer materials, the market shows a growth potential of 7–10% per year.[2]

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Lysine application as a diet supplement in human being and domestic animals sought particularly in the under developed and over-populated area of the world where the chief staples are deficient in this amino acids, recently the production of lysine has received great interest as a consequence of the encouraging results obtained in feeding trails with cattle, dogs and poultry that are supplied a normal diet supplemented with lysine[4]. Lysine may be corresponding with indemnity against osteoporosis.[5]

L-Lysine has a known anxiolytic action through its effects on serotonin receptors in the intestinal tract. One study showed that overcharge of the 5-HT4 receptors in the gut is integrated with anxiety-induced intestinal pathology.[6] A study conducted in 2010 that addition with sufficient doses of Lysine could avert the development of Alzheimer Disease.[7]

Several European countries and South American counties are feeding the under nourished children with bread prepared with lysine as a nutritional supplement. Several commercial enterprises are employing lysine to fortify different cereal brands. Japan leads the world in microbial production of lysine. Only kyowa fermentation industry produces over 1000 tons of lysine per year.[8]

Selection of microorganism and cheapest nutritional media for the growth of microorganism and synthesis of desired amino acids is an essential parameter. Cultures

which are known for producing some quantity of essential amino acid are suitable sources and various methods can be used to enhance the production of amino acids[11]. The present study was directed primarily towards the production of extracellular lysine by penicilliumexpansum using agricultural waste as a carbon source.

MATERIALS AND METHODS

In present study the waste of Millet husk, sorghum husk and Banana stem were used throughout the study.

Microorganism: Penicilliumexpansum was obtained from the Department of Botany, University of Glasgow, U.K. and was used in this study. The stock culture was maintained on agar slant, containing (G/L) dextrose 20: peptone 10: agar 20 and distilled water. The sterilized slants were inoculated with penicilliumexpansum and incubated at 27oC to obtained growth.

Degradation of millet, sorghum husk and Banana stem with 0.6N H2SO4: 10 g of millet, sorghum husk and Banana stem was separately mixed with 1000 ml of 0.6N sulfuric acid. Thesemixtures were frequently agitated on flame for one hour, maintaining the level of solution constant. After cooling at room temperature, the slurry was autoclaved at 1.5 kg/cm2 for 30 minutes. The autoclaved slurry was cooled at room temperature and unsolubilized material was removed by filtration through suction pump. The filtrate of solubilized millet, sorghum husk and banana stem was incorporated into the culture medium as a carbon sources.

Culture medium: culture medium was used for the growth of penicilliumexpansum. Without altering chemical composition. Cultivation condition: 100 ml of culture media supplemented with millet, sorghum husk and banana stem soluble filtrate was taken in 500 ml conical flasks plugged with cotton wool and autoclaved at 1.5 kg/cm2 for 20 minutes. The sterilized media cooled at room temperature were inoculated with 2.0 ml of penicilliumexpansum spores 50x per ml. these flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 26+2oC adjust at 200 rev/min. The culture broth was separated from mycelium after an interval of 48 hours incubation period by filtration through whatman No. 1 filter paper.

Determination of mycelial biomass: The quantity of the mycelium was noted after washing with distilled water and drying at 105-110oC in a hot oven a constant weight was obtained.

Determination of final pH values: The final pH value of the culture broth was determined using WPA pH meter.

Amino acid analysis by paper chromatography: The amino acid were identified from culture broth by one and two dimensional paper chromatography. A 50 ul of culture broth and authentic samples as a marker were applied on whatman No. 1 filter paper. The paper strips were developed in the following solvent systems:

A- Butanol-acetic acid –water (5:2:2) v/v/v).
B- Ethanol- Ammonium hydroxide (2:2:1v/v/v
C- Chloroform –Methanol –Ammoniam hydroxide ,
D- Phenol-water 80:20 v/v),
E- Propanol: water 7:3v/v)

In one dimensional procedure where as in the two dimensional, Butanol-acetic acid-water (5:2:2 v/v/v) and phenol –water (4:1 v/v) were used. After drying the chromatograms were sprayed with ninhydrin solution and dried in oven for five minutes at 80oC. Each spot was then identified by comparing with that of authentic amino acids.

Spectrophotometric determination of Lysine: Determination of lysine from culture broth: lysine was determined from culture broth of penicilliumexpansum according to the procedure of Kibrick.

RESULTS

Paper chromatographic method was carried out for separation and identification of amino acids synthesized by penicilliumexpansum in the culture broth using different solvent systems. Different Rf values were recorded with different solvent systems using one and two dimensional paper chromatography as shown in Table 1 &2. Time course of lysine production by penicillium expansum using 0.6NH2SO4 pretreated millet husk and sorghum husk as a carbon source are presented in Table 3 and 4. However the production pattern of lysine by penicillium expansum on 0.6N H2SO4 pretreated banana stem waste mineral medium was found totally different than pretreated millet and sorghum husk mineral medium as presented in Table-5.

Table No. 1: Rf values (x100) standard acids and pencillium expanses culture amino acids by one dimensional paper chromatographic analysis using solvents from A to E.

Sr. No.	Amino acids	Rf in sovent A		Rf in sovent B		Rf in sovent C		Rf in sovent D		Rf in sovent E		
		Stand sample		Stand sample		Stand sample		Stand sample		Stand sample		
1.	Methionine	68	66	73	75$	87	-	80	`81	72	72	--
2.	Lysine	22	--	21	62	-	83	81	38	-	12	--
3.	Alanine	47	--	--	59	56	86	-	61	62	47	--
4.	Glutamine	32	31$	50	-	75	74$	59	-	40	-	--
5.	Leucine	84	--	--	79	-	92	-	81	-	73	--
6.	Isoleucine	79	--	83	86$	87	-	82	81	80	78	--
7.	Phenylalanine	80	--	82	86$	94	-	81	--	87	-	--
8.	Arginine-	32	31$	50	-	72	70$	76	--	11	-	--

9.	Tyrosine-	60	-	68	-	83	-	65	--	57	57$	--
10.	Tryptophan	70	-	65	-	85	-	77	-	68	64$	-
11.	Threonine	40	93$	51	-	91	-	65	-	42	-	-
12.	Histidine	29	-	36	-	85	-	72	73	30	-	-
13.	Glycine	32	-	31$	59	-	85	-	45	44	48	-
14.	Asparagine	25	-	44	-	75	74$	20	20$	23	-	-
15.	Serine	30	-	31$	38	40	85	-	39	-	30	-
16.	Valine	70	-	-	77	75$	91	-	80	-	70	-
17.	Aspartic acid	28	-	30	-	73	-	20	20$	32	-	-
18.	Glutanio acid	40	39$	45	-	84	-	37	36	29	-	-
19.	Cysteine	48	-	-	52	-	86	-	60	-	44	-
20.	Cystine	16	-	14	32	-	70	-	24	25	16	-
21.	Proline	59	-	54	75	75$	89	-	87	-	68	64$

Table No.2: Rf values amino acids

Sr. No.	Amino acids	Rf value of standard	Rf values of broth amino acid phenol-water
1.	Methionine	77	71
2.	Lysine	30	28$
3.	Alanine	62	60$
4.	Glutamine	62	60$
5.	Leucine	84	84$
6.	Phenylalanine	84	84$
7.	Asparagine	38	-
8.	Serine	29	28$
9.	Valine	90	93
10.	Arjinine	42	46
11.	Tyrosine	54	54
12.	Glutanic	30	28$
13.	Cysteine	21	-
14.	Threonine	15	14
15.	Isoleucine	77	-
16.	Histidine	67	65$
17.	Aspartic acid	14	14$
18.	Tryptophan	80	84$
19.	Glycine 29	46	46$

Table No.3: Effect of pretreated Millet husk as a carbon source on the biosynthesis of lysine by penicillum expansum culture was grown at 28+ 2^{0}C in a cooled orbital shaking incubator.

Time Period Hours	Initial pH	final pH	Mycella weight g/100 ml broth	total protein mg/ml	reducing sugar mg/ml	lysine
24	6.0	5.67	0.585	2.84	0.023	2.00
48	6.0	5.94	0.65	2.6	0.024	2.00
72	6.0	7.34	0.585	2.08	0.019	2.90
96	6.0	7.85	0.405	0.36	0.018	2.80
120	6.0	7.8	0.4	0.155	0.016	2.80
144	6.0	7.7	0.4	0.11	0.014	2.80
168	6.0	7.77	0.4	0.1	0.014	2.80
192	6.0	7.75	0.4	0.1	0.009	1.20
216	6.0	7.72	0.4	0.095	0.008	1.20
240	6.0	7.7	0.4	0.095	0.007	1.20
264	6.0	0.07	0.4	0.085	0.006	1.20

Table No.4: Effect of pretreated sorghum husk as a carbon source on the biosynthesis of lysine by penicillum expansum. Culture was grown at 28+2^{o}C in a cooled orbital shaking incubator.

Time Period Hours	Initial pH	final pH	Mycella weight g/100 ml broth	total protein mg/ml	reducing sugar mg/ml	lysine
24	6.0	0.126	0.55	2.84	0.026	2.00
48	6.0	0.146	0.62	2.4	0.023	2.40
72	6.0	0.177	0.55	2.6	0.018	1.44
96	6.0	0.199	0.405	0.86	0.018	1.40
120	6.0	0.113	0.345	0.8	0.018	1.40
144	6.0	0.106	0.33	0.75	0.015	1.40
168	6.0	0.103	0.325	0.09	0.015	1.40
192	6.0	0.102	0.325	0.075	0.014	1.40
216	6.0	0.098	0.295	0.075	0.013	1.40
240	6.0	0.095	0.285	0.065	0.012	1.20
264	6.0	0.09	0.215	0.065	0.012	1.20

Table No.5: Effect of pretreated banana stem waste as a carbon source on the biosynthesis of lysine by penicillum by penicillumexpansum culture was grown at 28±2^{0}C in a cooled orbital shaking incubator.

Time Period Hours	Initial pH	final pH	Mycella weight g/100 ml broth	total protein mg/ml	reducing sugar mg/ml	lysine
24	6.0	5.55	0.072	0.165	1.64	0.9
48	6.0	6.86	0.134	0.165	0.55	1.20
72	6.0	7.18	0.064	0.225	0.34	1.50
96	6.0	8.6	0.061	0.26	0.14	1.40
120	6.0	8.53	0.06	0.285	0.14	1.20
144	6.0	8.56	0.06	0.215	0.11	0.95
168	6.0	8.65	0.047	0.205	0.1	0.80
192	6.0	8.75	0.047	0.2	0.1	0.80
216	6.0	8.73	0.046	0.195	0.1	0.75
240	6.0	8.69	0.045	0.19	0.1	0.70

DISCUSSION

Different essential amino acids were found by the use of different solvents. These results are in agreement with the finding of stepka[12], who indicates that the selection of filter paper, solvent mixture and other conditions for paper chromatography are very important, because these determine Rf values and degree of separation.

It is observed that the amount of lysine production was increased upto 48 hours and then declined abruptly. It was noted that rise of pH was continuously in increasing order with increase of incubation period but the concentration of sugar and protein were found in decreasing order. These results are accordance with the finding of other workers in case of amino acid synthesis by bacteria, yeast and fungi on synthetic and natural media.

CONCLUSION

This study concludes that biogenesis of lysine by penicillium expansum using agricultural waste as energy sources.

Author's Contribution:

Concept & Design of Study:	Jawad Mumtaz Sodhar
Drafting:	Syed Asif Jahanzeb Kazmi, Alina Saqib
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Final Approval of version:	Jawad Mumtaz Sodhar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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