SOIL AND WATER-BORNE BACTERIAL ISOLATES FROM PULIYANKULAMA FOR L-ISOLEUCINE AND L-LYSINE PRODUCTION ON DIFFERENT SUBSTRATES

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Amino acids (AAs) are the components of proteins. Key applications of AAs include food, chemical, medicine & cosmetic industries and feed additives. L-isoleucine (L-Ile) is a branched-chain AA found in many proteins. It is important in hemoglobin synthesis and regulation of blood sugar & energy levels. L-lysine (L-Lys) becomes important for calcium absorption, maintaining nitrogen balance in the body and for the production of antibodies, hormones & enzymes. The adequate intakes of L-Ile and L-Lys for infants aged 0-6 months are 529 and 640 mg/day respectively[1]. L-Ile and L-Lys are essential AAs that they cannot be synthesized by the body and thus should be available in foods and feeds. Fermentation yields optically active and biologically required L-forms of AAs directly over the other methods[2]. However, bacterial production of AAs has little been exploited in Sri Lanka. Therefore, a study was done to isolate local bacteria to produce L-Ile and L-Lys.

Twelve soil-borne bacterial isolates (I_1 - I_{12}) and 4 water-borne isolates (I_{13} - I_{16}) were obtained from the farm, Faculty of Agriculture, RUSL. Soil samples were collected from paddy field and cattle, poultry & goat sheds whereas water samples were from an irrigation canal and a duck-fish integration pond. 2 g of soil was mixed with 10 ml sterile water to isolate bacteria. It was followed by a number of serial dilutions, which were passed through Millipore filters (0.45 µm). The filters were then placed on nutrient agar (NA) plates and incubated overnight at 37 °C in a shaking incubator. Next day, the colonies appearing were picked up and streaked on Eosin Methylene Blue (EMB) agar for identification. For isolation of bacteria from water, each sample (2 ml) was diluted to 5 ml and pre-filtered to remove dust. Then, 2 ml was passed through Millipore filter. The filters were placed on NA and incubated overnight at 37 °C. The rest of the procedure was the same as for bacterial isolation from soil. For isolation of *E. coli*, filters were placed on McConkey agar and incubated overnight at 37 °C. The following day, pink colonies (supposed to be *E. coli*) were picked up, streaked upon EMB agar and incubated overnight at 37 °C. Following day metallic green isolates were picked and slant cultured. It was further confirmed by Gram's staining and microscopic observations. 0.5 ml of bacterial suspension at an optical density of 0.7 from each slant was inoculated in 50 ml of 3 fermentation media separately (Table 1) in triplicate at pH of 7 and incubated at 28±1 °C with 100 rpm shaking. Fermentation broth was collected at 24, 48 and 72 hrs intervals and subjected to paper chromatography. Paper chromatography using Whatman® cellulose 1 Chr papers was performed with standard L-Ile and L-Lys to find their *Rf* values and to plot standard curves. Coloured spots corresponding to the *Rf* value of L-Ile (0.550) and L-Lys (0.086) were eluted in methanol separately and the absorbance was measured at 550 nm on spectrophotometer, model: Spectro US-VIS Double Beam PC. Quantification was done using standard curves. The data were analyzed using ANOVA procedure and mean separation by DMRT at 5% P-value.

Table 1. Composition of fermentation media

Ingredients (%)	FM-1 medium	L-6 medium	M-1 medium
Glucose	-	10	-
Trypticase	-	0.75	-
Molasses	10	-	10
Peptone	1	-	-
Meat extract	0.5	-	-
NaCl	0.25	-	-
$CaCO_3$	-	2	2
KH_2PO_4	-	0.07	0.05
K_2HPO_4	-	0.04	0.05
$MgSO_4.7H_2O$	-	0.03	0.025
$(NH_4)_2SO_4$	-	3	2

The results revealed that out of 16 isolates only two isolates (I_{15} and I_{16}), which were recovered from a duck-fish integration pond, produced L-Ile in FM1 medium. A 3-way interaction between the isolates, media type and incubation time was found to be significant at 5% probability level. I_{14} and I_{15} (Table 2).

Again an ANOVA was performed after fixing the strain at I_{16} on FM1. The highest L-Ile production (0.73 g/L) was observed after 48 hrs of fermentation. However, it was not significantly different from the amount (0.65 g/L) obtained after 24 hrs. Eight isolates demonstrated to produce L-Lys on different media. Of which, only 6 isolates produced L-Lys in FM1 medium. The results of the statistical analysis revealed that there was a significant interaction between the strain, media type and the duration of fermentation. However, to select the potential strain to produce L-Lys, an ANOVA was performed after fixing the time at 48 hrs on FM1 medium. The model was found to be significant at 5% P-value. The DMR test revealed that the highest L-Lys production by I_8 was not significantly different from that of I_2 although different from I_{16}, I_3, I_{14} and I_{15} (Table 2).

Table 2. L-Ile and L-Lys production on FM1 after 48 hrs of fermentation

Strain	Quantity of L-Ile (g/L)	Quantity of L-Lys (g/L)
I_{16}	0.73^a	0.51^b
I_{15}	0.51^b	0.43^d
I_8	-	0.56^a
I_2	-	0.54^a
I_3	-	0.48^c
I_{14}	-	0.48^c

A simple ANOVA was carried out fixing the strain at I_8 on FM1 to compare the fermentation durations. The results revealed that although the highest L-Lys production (0.60 g/L) was obtained after 72 hrs of fermentation, it was not significantly different from the production (0.56 g/L) after 48 hrs. All the isolates were Gram negative, rod shaped cells.

Fermentation process is non-linear and time dependant. The L-Ile production was found to be decreasing with time after 48 hrs of fermentation suggesting negative regulation of AA production by the accumulation of the products[3]. It can be concluded that the isolate I_{16} can be used as a potential strain for L-Ile production on FM1 whereas I_8 and I_2 can be used for maximizing L-Lys production on Fm1.

REFERENCES

1. Biochem Compounds. 2012. Isoleucine compound summary: Author. Retrieved on 10.11.2012 from http://pubmed.ncbi.nlm.nih.gov/summary/summary.cgi?cid=6306.
2. Yamada, K., S. Kinoshita, T. Tsunoda and K. Aida. 1972. *The microbial production of amino acids*. John Willey and Sons, New York. pp: 68-71.
3. Zhang, X., T. Sun, Z. Y. Sun, X. Lin and D. X. Gu. 1998. Time dependant kinetic models for glutamic acid fermentation. Enzyme and Microbial Technology, 22: 205-209.