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OPTIMIZATION OF PHYSICAL CONDITIONS FOR L-THREONINE PRODUCTION BY AN A,€-DIAMINOPIMILIC ACID AND THIAMINE-HCL DUAL AUXOTROPHIC MUTANT *CORYNEBACTERIUM GLUTAMICUM* X1870

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ABSTRACT
The production of L-threonine by α,€-diaminopimilic acid and thiamine-HCl dual auxotrophic mutant *Corynebacterium glutamicum* X1870 was studied by optimizing different physical conditions. Production was increased significantly from 10.7mg/ml to 15.1 mg/ml with pH, 6.0; temperature, 29^0C; fermentation time, 96h; volume of medium, 30 ml and inoculums size, 4%.

[KEYWORDS: L-threonine, α,€-diaminopimilic acid, thiamine-HCl, dual auxotrophic, *Corynebacterium glutamicum*].

INTRODUCTION
L-threonine is an essential amino acid, used as a food supplement to improve their nutritive quality.[1] Due to its huge market demand, commercial production using chemical synthesis has been started several decades ago, however production of stereo-specific L-threonine using microorganisms has gained superiority over its chemical synthesis.[2] Several microbial strains have been examined.[2-4]

A new auxotrophic mutant of *Corynebacterium glutamicum* has been developed in our previous experiment.[5] Different physical conditions also affect the microbial growth and production of amino acids.[6,7] As each bacterium has a definite range of culture conditions for their maximum growth and optimum productivity, it is essential to optimize culture conditions to maximize the productivity. The present study was aimed to improve the productivity by optimizing the physical conditions.

MATERIALS AND METHODS
Microorganism and mutagenesis: A high L-threonine yielding strin of *Corynebacterium glutamicum* was developed by induced mutation using UV irradiation followed by penicillin selection. A high L-threonine yielding α,€ diaminopimilic acid and thiamine-HCl dual auxotrophic mutant strain *Corynebacterium glutamicum* X1870 was developed which could accumulate 10.7 mg/ml L-threonine before optimization.[8]

Composition of growth medium and preparation of inoculum: The growth medium is composed of glucose, 1%; urea, 0.8%; beef extract, 0.3%; yeast extract, 0.2%; $MgSO_4.7H_2O$, 0.002%; water, 1L and the pH was adjusted to 7.0.[8]

Medium for L-threonine production: The composition of the production medium was: glucose, 10%; urea, 1%; KH_2PO_4, 0.1%; K_2HPO_4, 0.1%; $MgSO_4.7H_2O$, 0.02%; thiamine-HCl, 60µg/L; DAP, 60mg/L; water, 1L and pH was adjusted to 7.0.[8]

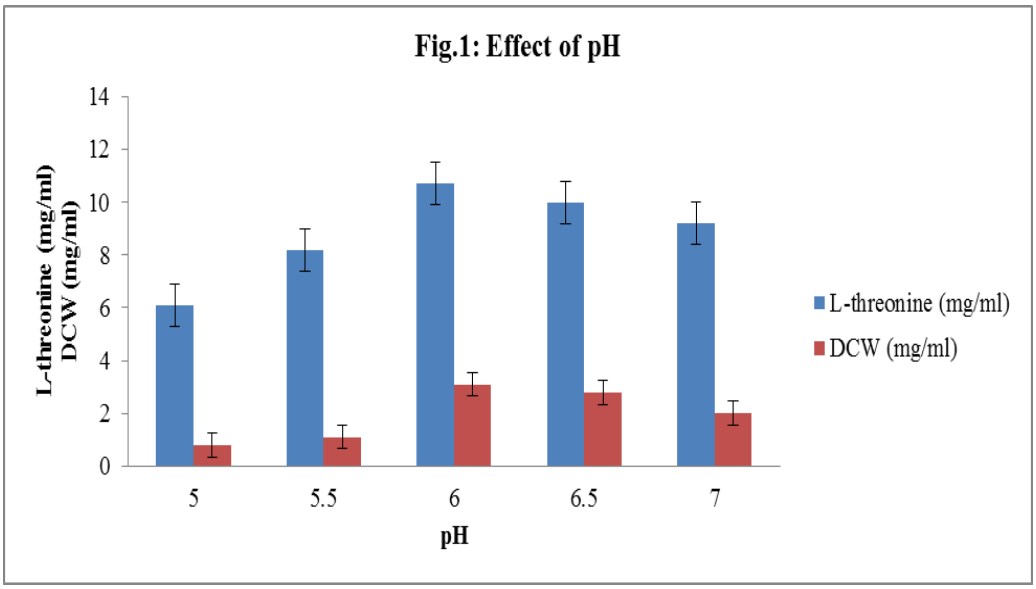
Analysis for L-threonine: Descending paper chromatography was employed for the detection of L-threonine followed by quantification.[8-10]

Estimation of dry cell weight: After centrifugation, the bacterial cell precipitate washed twice with double distilled deionized water and dried at 100^0C to attain constant dry cell weight.[11]

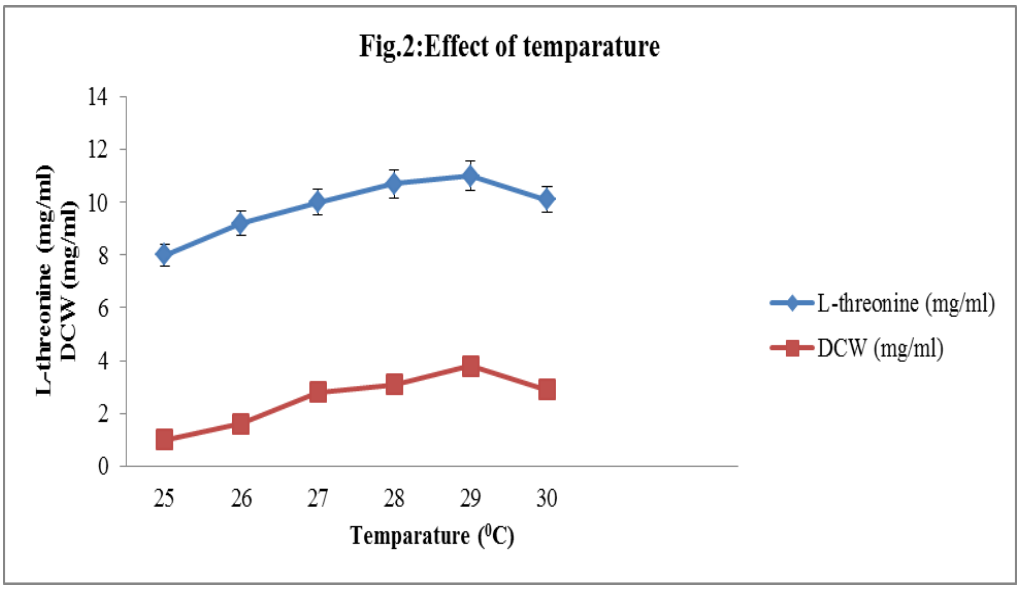
Statistical analysis: All data were analyzed using software 'Prism 4.0' (graph pad Inc., USA). One way ANOVA Dunett's post hoc multiple comparison test were done. Values were expressed as mean±SEM, where n=6.

RESULTS AND DISCUSSION
Effect of initial pH: Microbial growth is sensitive to pH and on the other hand, growth changes the pH of thr production medium due to accumulation of secondary metabolites.[12-15] It also influences the transport of the L-

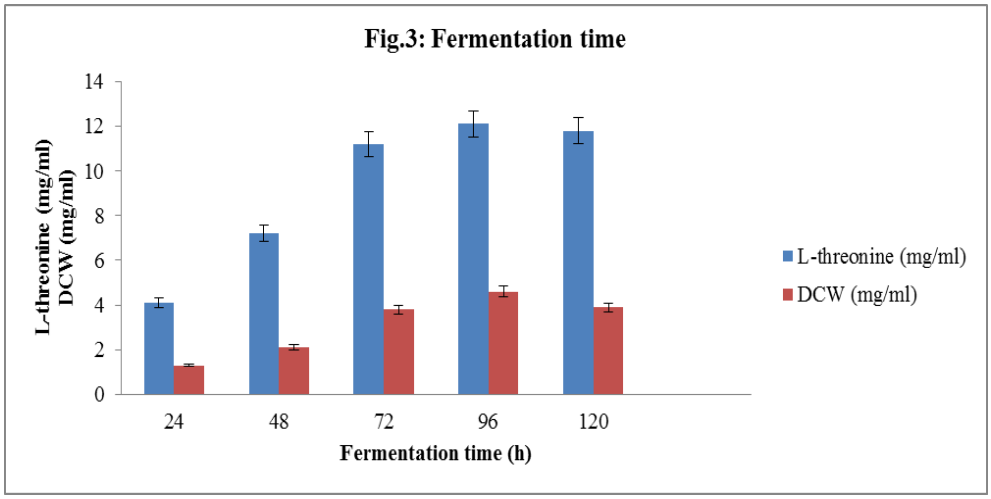
amino acids across the microbial cell membrane.[16] The pH of the production medium was adjusted using 0.1(N) HCl and 0.1(N) NaOH. The optimum pH for L-threonine production was determined among five different pH (5.5, 6,6.5,7 and 7.5) and maximum production was obtained with maximum cell growth at pH 6.0 (Fig.1). Ganguly and Banik (2010) obtained maximum L-glutamic acid using a mutant *Micrococcus glutamicus* AB100 at pH 6.5.[17] *Micrococcus glutamicus* SG300 produced maximum L-lysine in the fermentation broth at pH 7.5.[18]



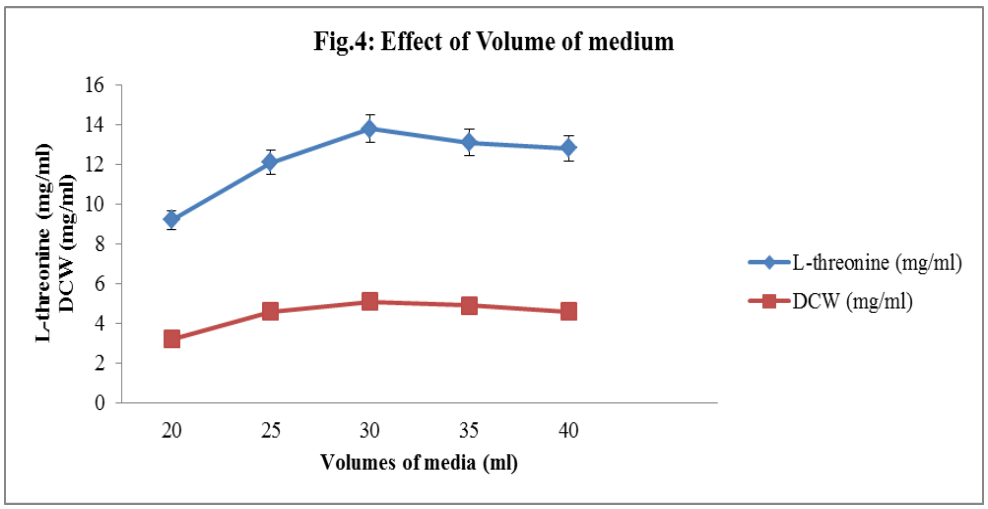
Effect of temperature: Growth of a microorganism is a function of temperature as it controls metabolism.[19] Change in temperature alters the utilization of metabolites and thus growth pattern.[20] In this present study, maximum productivity was obtained with 29^{0}C (Fig.2).



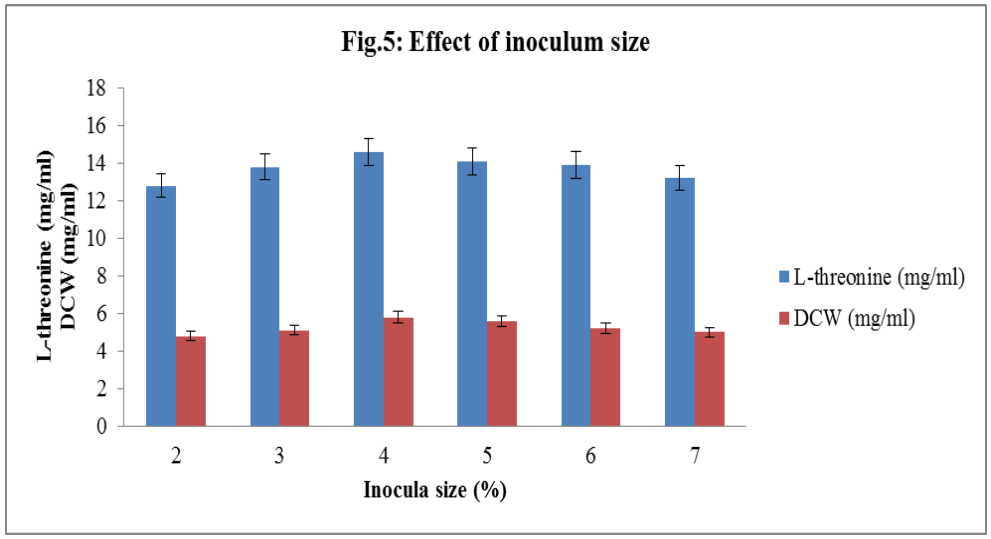
Effect of fermentation time: To determine the optimum fermentation time, the bacterial culture was incubated for 24-120h. Optimum growth and productivity was obtained with 96h of incubation (Fig.3).



Effect of volume of medium: Too much volume of medium may dilute the product, whereas too low medium may give insufficient nutrient supply.[21] In this present study, maximum production was obtaine with 30ml of volume of meium (Fig.4).



Effect of inoculum size: Inoculum size has a significant influence on L-amino acid fermentation.[22] Smaller inoculums size increases the growth period as it gives insufficient biomass.[23] In this present study, maximum productivity was obtained with 4% inoculums size with $4X10^8$ cells (Fig.5).



CONCLUSION

The present study revealed that, L-threonine production by the mutant *Corynebacterium glutamicum* X1870 was greatly influenced by different physical conditions. Production was significantly ($p<0.01$) increased from 10.7mg/ml to 15.1 mg/ml after optimization.

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