L-ASPARAGINASE AND GLUTAMINASE ACTIVITIES IN THE CULTURE FILTRATES OF *ASPERGILLUS NIDULANS*

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A LARGE number of fungal strains of ascomycetes and *fungi imperfecti* produce L-asparaginase and glutaminase in their culture filtrates or within the mycelium. These enzymes, extracted from the mycelia of *Aspergillus terreus*[1] and *Fusarium trincitum*[2], have been found to have antitumour activities. Recently, the properties of L-asparaginase have been studied in the mycelial extracts of *Aspergillus nidulans*[3,4]. *The present investigation reports the conditions for* the optimal expressions of L-asparaginase and glutaminase in the culture filtrates of *Aspergillus nidulans.*

A riboflavin and biotin requiring green conidial (*ribo A*1, *bi A*1) strain from the Departmental stock (FGSC No. 158) was used during the course of the present investigation. Composition of complete (CM) and minimal media (MM) and culture conditions have been described earlier[5,6].

Cultures of the desired strain were raised by inoculating approximately 5×10^7 conidia/50 ml of the complete or minimal media or in media containing L-asparagine or glutamine at a final concentration of 0·2 to 1·0%. Flasks were incubated at 37° C and 150 *rpm in a New Brunswick Gyrotory G25* incubator shaker. Test samples were withdrawn at 24 hr intervals upto 96 hr. Culture filtrates were centrifuged at 12,000 × g for 30 min, before estimating the enzyme activities. Imada's procedure[7] was followed for estimating the activities of these enzymes.

A unit of the enzyme catalyzes the formation of 1 μ mole of ammonia/min, under the conditions of the assay. Standards were prepared by using ammonium sulphate.

L-Asparaginase and glutaminase activities of the *ribo A*1, *bi A*1 strain were estimated in the culture filtrates *in* phosphate, citrate and Tris buffers at 5·5 through 8·0 pH values. Maximum activities were obtained in phosphate buffer at pH 7·0.

Enzyme activities were determined in the culture filtrates at a regular interval of 24 hr up to 96 hr in *cultures grown in complete media* (CM), minimal media (MM) and media containing different percentages of L-asparagine (*Fig. 1*) or glutamine (*Fig. 2*) as the sole source of nitrogen. Results showed that in general, both the enzymes follow a similar pattern. However, glutaminase activity was higher at any particular stage of the submerged growth as compared to the L-asparaginase activity.

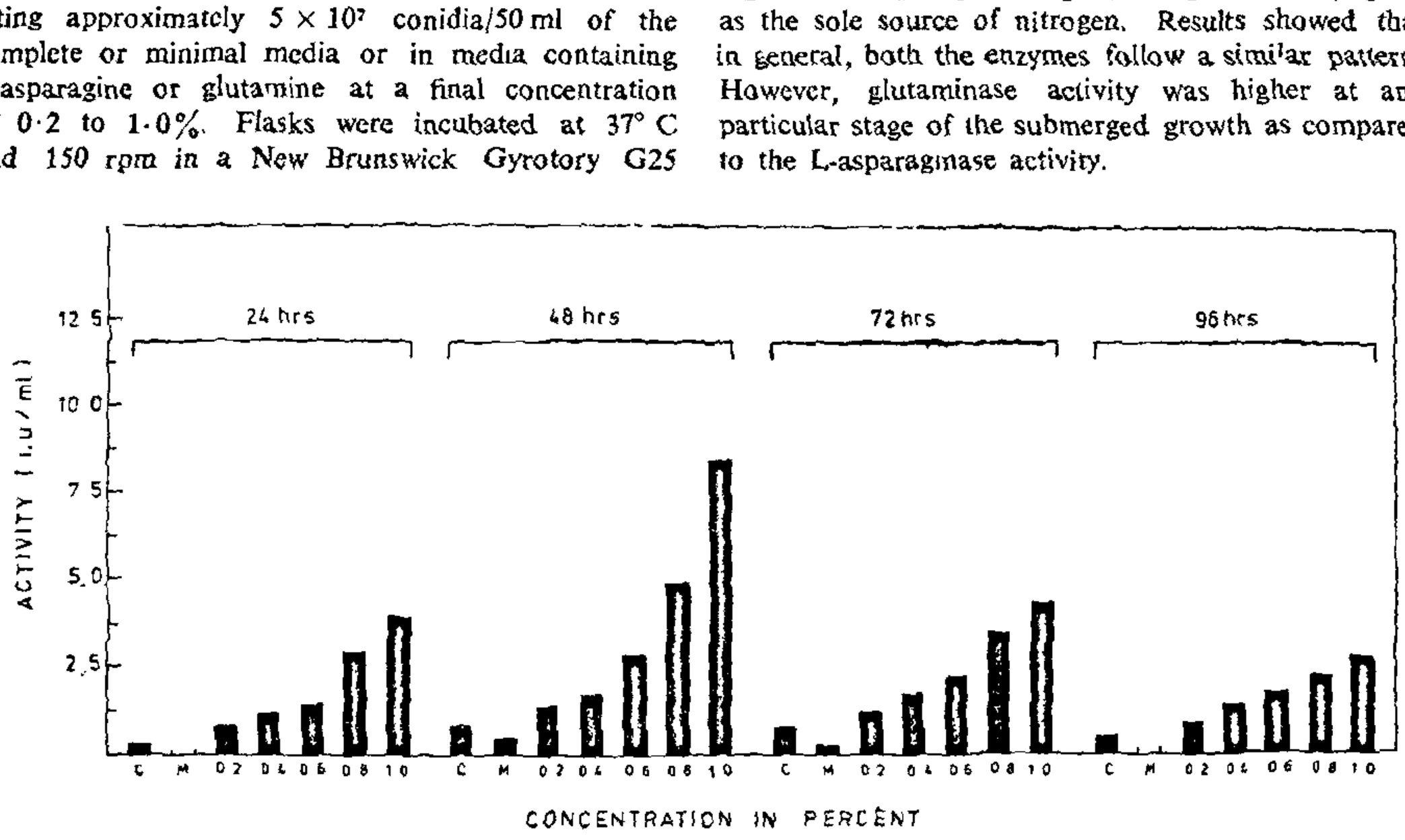

FIG. 1. L-Asparaginase activity (in international units) of the *ribo A*1, *bi A*1 strain of *A. nidulans* in media containing different concentrations of L-asparagine (used as the sole source of nitrogen) as a function *of time. C: complete media, M: minimal media.*

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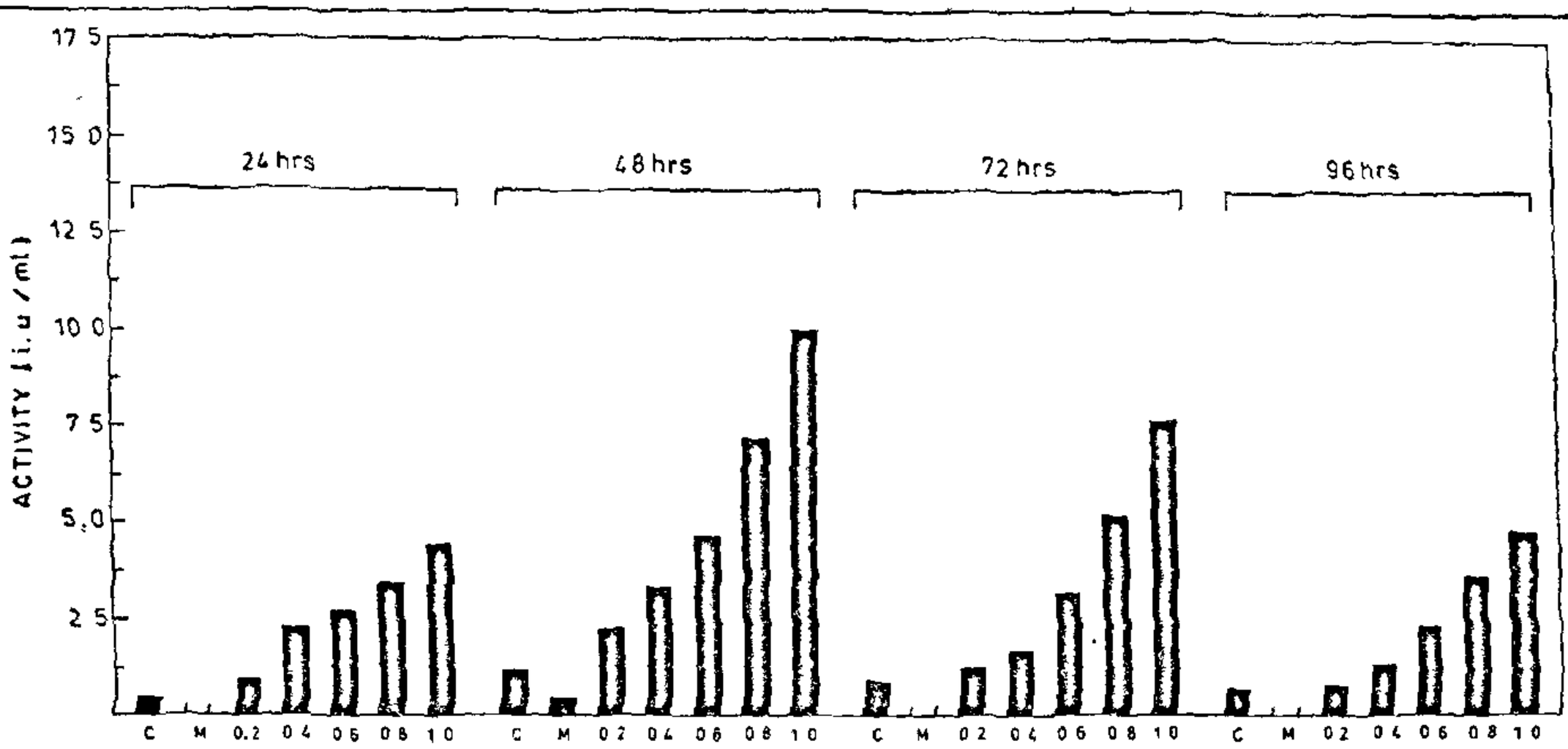

FIG. 2. Glutaminase activity (in international units) of the *ribo A*1, *bi A*1 strain of *A. nidulans* in media containing different concentrations of glutamine (used as the sole source of nitrogen) as a function of time. (C : complete media, M : minimal media.)

Effects of enzyme and substrate concentrations on the enzyme activities were studied by raising cultures in the medium containing 0·6% of L-asparagine or glutamine as the sole nitrogen source for 48 hr. There was a linear increase in the activities of both the enzymes with increasing amounts of the enzyme extract.

Both the enzyme activities were low in minimal media as compared to complete media. In the latter, highest activities were seen after 48 hr of incubation, which decreased on further incubation. When L-asparagine was used as a nitrogen source in a minimal medium, a linear increase in the L-asparaginase activity was recorded in relation to L-asparagine concentration in the growth medium. As in the complete medium, maximum activity of the enzyme was after 48 hr of growth in the minimal medium.

Glutaminase activity of the *ribo A*1, *bi A*1 strain, in general, followed a pattern similar to that of L-asparaginase. However, at any particular stage of growth of the culture, the activity of glutaminase was more as compared to the activity of L-asparaginase. A direct correlation between the activities of these enzymes and submerged conidiation could not be established. Still, it was evident that maximal levels of these enzymes were present during conidiation that is, after 48 hr of incubation.

Investigations carried out with a variety of micro-organisms[8,9] have shown the importance of concentration and type of nitrogen and carbon source used for growth. A 10-fold increase in L-asparaginase and glutaminase activities of *A. nidulans* was recorded when L-asparagine or glutamine was used as the nitrogen source. This is also supported from the findings that asparagine and glutamine are not substrates for the formamidase or acetamidase. However, there may be a specific asparaginase and a specific glutaminase for the use of these amides[10]. From the present investigation it is evident that the rise and fall in the L-asparaginase and glutaminase activities are parallel during submerged differentiation. Therefore, it is presumed that these two amidase activities are expressed by a single enzyme, rather than two different ones. Alternatively, they are coordinately controlled.

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