Improved Method for Rapid Microbioassay of Amino Acids

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A rapid method for microbioassay of amino acid was previously reported. In the rapid method, however, the varied compositions of synthetic media for intermediate culture were required for the assay of respective amino acid. The method becomes more advantageous if a single medium can be uniformly used for the intermediate culture in the rapid assay of various amino acids.

In the improved method of present study, the growing cells were centrifuged after intermediate culture to prevent the carrying over of the amino acid to be determined into assay culture. Thus, it became possible to use a complete medium for the intermediate culture. Protein hydrolysate could also be used for the complete medium which required no complicated amino acid mixture.

Selection of medium for intermediate culture

In order to reduce the period of time required for the intermediate culture, effect of composition of the medium on the growth rate of Leuconostoc mesenteroides P-60 was investigated at 37°C.

Addition of yeast extract to the complete medium was found to markedly accelerate the growth at initial stage when large amounts of cells of stationary phase were used as inoculum. The growth attained to the same level within 2 hr as that obtained by 2.5 hr-incubation in the medium without yeast extract. But no further acceleration was observed by increasing yeast extract above 0.5%.

As to nitrogen sources, no difference of the growth was found among the amino acid mixture proposed by Henderson and Snell, Casamino acids, Proteose peptone, and Bactotrypton. Effect of carbohydrates on the growth was also examined. Glucose, fructose and xylose were utilized for the rapid growth of microorganism in this order. When sucrose, maltose, lactose and sorbose were used as carbon sources, growth did not occur during 5 hr-incubation period.

Effect of growth phase of inoculum on assay culture

The influence of growth phase of inoculum on the growth rate was previously studied in detail. As the results, the cells of logarithm phase were adopted as inoculum for the assay culture of previous rapid microbioassay. In the improved method, the cells of logarithm phase were also found to be suitable for inoculum for the assay culture. Furthermore, the time required for attaining to the appropriate growth phase was reduced from 2.5 hr to 2 hr by addition of yeast extract.

Procedure for improved rapid microbioassay

The improved rapid microbioassay, in which a simplified semi-synthetic medium was used for intermediate culture, was accomplished by the following procedure. Inoculum culture was carried out in 10 ml of the complete medium according to the previous directions.

The whole culture was transferred to 140 ml of the semi-synthetic medium (Table I) and incubated at 37°C for 2 hr (Intermediate culture). The cells of logarithm phase were centrifuged and resuspended in 150 ml of double strength basal medium, the composition of which was the same as described in the previous paper. One milliliter of this suspension was added to each assay tubes (14 × 120 mm) containing 1 ml of samples or standard solutions. Assay culture was carried out at 37°C for 2.5 or 3.5 hr and turbidimetric estimation of growth was made directly in the assay tubes using the electric photometer (Hitachi, model EPO-B).

The typical growth response curve to L-isoleucine on 2.5 hr- and 3.5 hr-assay cultures is shown in Fig. 1.
Growth after 2.5 hr-incubation did not increase at higher concentrations of L-isoleucine and the amino acid could be assayed only up to a concentration of 20 μg/ml. However, good growth curve was obtained by 3.5 hr-incubation, resulting in expansion of the assay range up to a concentration of 30 μg/ml. Other amino acids also could be determined by using the same inoculum as that for L-isoleucine assay. Figure 1 also represents typical standard curves of several amino acids. Application of the improved method for the assay of L-isoleucine in the broth obtained by the culture of Serratia marcescens gave the same assay values with those obtained by the conventional method and no difference in reproducibility was found between both the methods.

The development of a simplified complete medium made the previous method more convenient and the growth promoting effect of yeast extract led to reduction of incubation period for the intermediate culture. An automated procedure of the improved rapid method is now being studied, and the results will be published elsewhere.

REFERENCES