MICROBIAL PRODUCTION OF AMINO ACIDS

I. DL-alanine production by an *Arthrobacter* species

BY T. M. RUDRA SETTY AND J. V. BHAT

(Microbiology & Pharmacology Laboratory, Indian Institute of Science, Bangalore-12, India)

(Received: April 3, 1969)

ABSTRACT

One hundred and fifteen Arthrobacter *strains isolated from soils, sewage and activated sludge, were screened for their production of amino acids when grown in a simple basal salts medium with glucose and urea. Thirtytwo isolates produced significant amounts of alanine and glutamic acid along with traces of other amino acids. Seven of these isolates produced large amounts of alanine into the medium, of which isolate* C_{19} d *was selected for detailed study. Under optimal cultural conditions on the laboratory scale, a maximal production of* 13 g *of alanine per litre of the medium was obtained. Alanine was recovered in a crystalline form from the broth quantitatively by column chromatographic method.*

Though alanine occurs in small amounts in broths of various types of microorganisms, bacteria in particular have been observed to excrete it in appreciable quantities. Of the bacteria so far isolated those belonging to the genera *Brevibacterium* and *Corynebacterium* have been shown to be the most efficient. *Brevibacterium pentaso-aminoacidicum* and *Brevibacterium-pentaso-alanicum*, for example, have been reported to yield respectively, 40 per cent alanine from glucose and 25 per cent alanine plus 15 per cent glutamic acid from xylose[1,2]. Some other isolates in the genera isolated from cheese, soil and sewage have also been reported to yield even 40—50 per cent alanine from glucose[4]. *Corynebacterium gelatinosum*, isolated and studied by Samejima *et al*[5], accumulated 17—18 per cent alanine from glucose.

As many as one hundred and fifteen *Arthrobacter* strains isolated in this laboratory[3], from soils sewage and activated sludge, were screened for their ability to excrete amino acids. Of these, seven isolates were found to accumulate large amounts of alanine from glucose. One among them, *viz.*, strain $C_{19}d$ was found particularly efficient for alanine production and therefore was selected for detailed study and the cultural conditions necessary for maximal production of alanine by this strain were determined.

EXPERIMENTAL

Culture.—The cultural conditions are shown in Table 1.

Estimation of growth.—Bacterial growth was measured turbidimetrically with a Bausch and Lomb colorimeter. The culture broth was diluted twentyfold and its optical density was measured at 540 $m\mu$.

Estimation of alanine. Amino acid was estimated by paper chromatography according to the method described by Giri *et al*[1]. Whatman No. 1. filter paper was used throughout.

Estimation of glucose. Glucose was estimated by Somogyi's method[6].

TABLE 1

Cultural conditons

Components of the medium	Seed medium %	Fermentation medium %
Glucose	7.5	10.0
Urea	0.5	1.0
K_2HPO_4	0.05	0.05
KH_2PO_4	0.05	0.10
$MgSO_4.7\ H_2O$	0.025	0.025
$MnSO_4.H_2O$	0.001	0.001
$FeSO_4.7\ H_2O$	0.001	0.001
Yeast extract	0.01	0 02
Temperature	28°C	28°C
Volume/500 ml flask	100 ml	100 ml
r.p.m.	250	250
Inoculum size	1 loopful	1%
Culture time	24 hrs	96 hrs
pH	7.0	7~8.2

Recovery of alanine from the culture broth. The broth after centrifugation was adjusted to *pH* 2.0 with 1N H_2SO_4 and filtered. The filtrate was passed through a column of amberlite IR−120 (H^+), which adsorbed all the amino acids. After washing with distilled water, the column was eluted with 0.1 N NH_4OH. Fractions containing amino acids were combined and evaporated in vacuum to a convenient volume. The neutral concentrate was then passed through a column of aluminium oxide (chromatographic grade) to remove

glutamic acid from the solution. The column was washed several times with distilled water and the eluate and the washings were collected together and evaporated in vacuum to a convenient volume. Additions of ethanol to this concentrated eluate induced crystallisation of nalaine. One or two recrystalisation gave a pure sample of alanine. Its identity was established by chromatographic methods and through its infrared spectrum.

Effect of urea concentration on alanine production. From the point of product formation, urea nitrogen was found to be more suitable than other nitrogen sources like ammonium sulphate and ammonium nitrate. The concentration of urea itself in the medium had a profound effect on alanine production. With the basal medium containing 10 per cent glucose and 0.5 per cent urea, the nitrogen content became limiting for alanine production after 72 hr though the growth continued even after 72 hr presumably utilising the amino acid nitrogen (Fig, 1). When the urea concentration was raised to 0.75 per cent, production of alanine continued at a uniform rate until the end of 96 hr and thereafter there was a noticeable fall in its production (Fig. 2), With 1.0 per cent urea in the medium, alanine production increased at a rapid

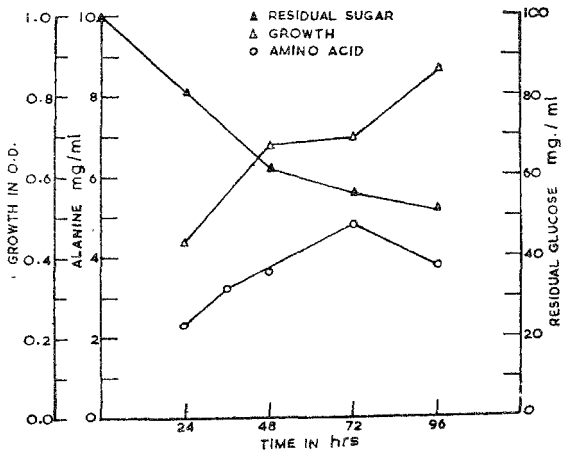

FIG. 1

Effect of urea deficiency on alanine production by an Arthrobacter sp. No. $C_{19}d$ 500 ml shake flasks with 100 ml medium: Mineral medium with 10% glucose, 0.5% urea temp. 28°C

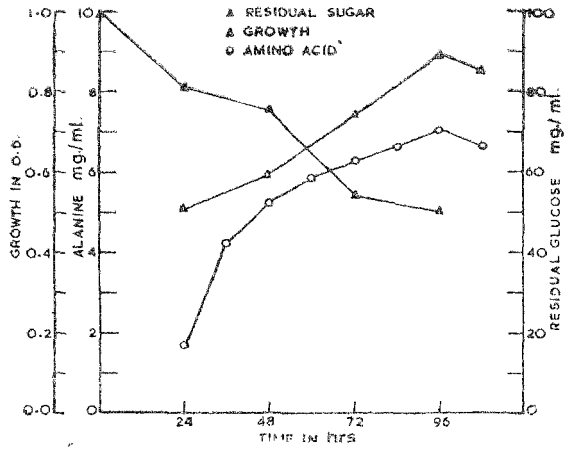

FIG. 2

Alanine production by an Arthrobacter sp. No. $C_{19}d$ with 0.75%, urea in the medium 500 ml flasks with 100 ml medium: mineral medium with glucose 10%, urea 0.75%, temp. 28°

rate and reached the peak at the end of 96 hr; however, a significant fall in bacterial growth was evidenced (Fig. 3). Further increase of urea in the medium to 1.25 per cent proved inhibitory to the growth of the organism as as well as the production of alanine (Fig. 4). In effect, though 0.75 per cent urea appears to be adequate, 1.0 per cent seems to be preferable since it prevents excessive growth of the bacteria and contributes to the product formation.

Effect of pH on alanine production. During fermentation the *pH* of the medium was controlled by using suitable proportions of KH_2PO_4 and K_2HPO_4 as buffers in the medium. Fig. 5 shows that the *pH* of the medium, containing 10 per cent glucose and 1.0 per cent urea, rose from the 7.3 to 8.5 and that the alanine production occurred mainly between a *pH* range of 7.5 to 8.2. It is also clear from the figure that the rate of alanine production increased as the *pH* approached 8.2 but slowed down when the *pH* reached 8.5 and again shot up when the *pH* came down to 8.2, indicating thereby the *pH* optima at 8.2 level.

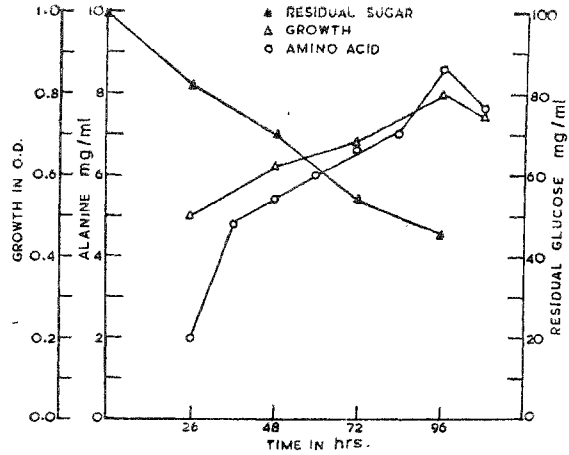

FIG. 3

Alanine production by an Arthrobacter sp. No. $C_{18}d$ at optimal urea concentration 500 ml shake flasks with 100 ml medium: Mineral medium, glucose 10%, urea 1.0%, temp. 28°C

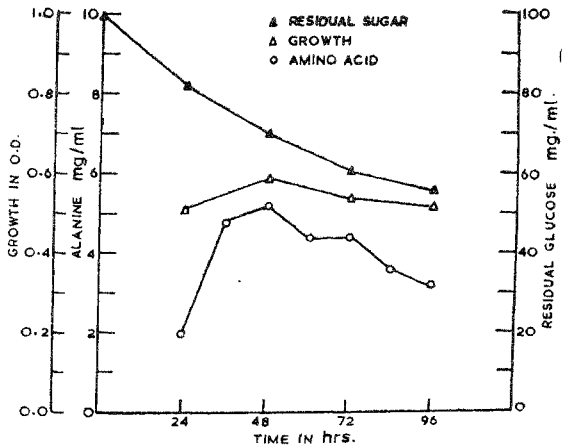

FIG. 4

Effect of excess urea on alanine production by Arthrobacter sp. No. $C_{18}d$. 500 ml shake flasks with 100 ml medium: Mineral medium, glucose 10%, urea 1.25%, temp. 28°C

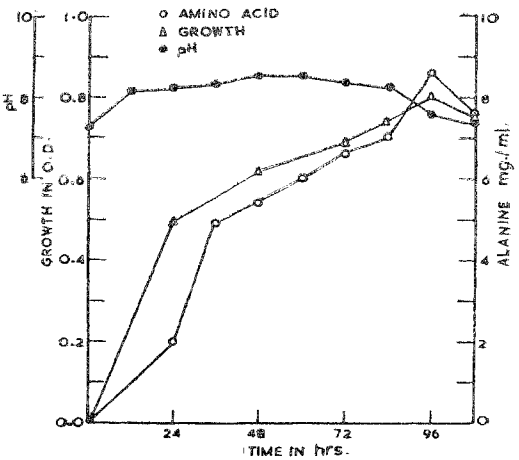

FIG. 5

pH changes during alanine production by Arthrobacter sp. No. $C_{19}d$ 500 ml shake flasks with 100 ml medium. Mineral medium with glucose 10%, urea 1.0% temp. 28°C

The result of the experiment in which the *pH* of the medium was maintained at 8.2, depicted in Fig. 6, shows that the rate of product formation was steadily rising till the end of the fermentation. Alanine yield was good at this *pH*, as other amino acids, i.e., glutamic acid and valine were formed only in traces. On the other hand, when fermentation was carried out at *pH* 8.5 relatively small amounts of alanine were formed (Fig. 7).

Effect of temperature on alanine production. Alanine production at 28°C and 32°C was then compared. The yield in identical medium at the end of four days were respectively 10.8 and 13.0 mg/ml of the medium suggesting thereby 32°C as the more suitable temperature for its synthesis from glucose and urea.

CONCLUSION

Under optimum cultural conditions, the *Arthrobacter* culture $C_{19}d$ could accumulate a maximum of 13 mg/ml alanine in the medium on a laboratory scale. This works at 26 per cent level on the basis of glucose utilized.

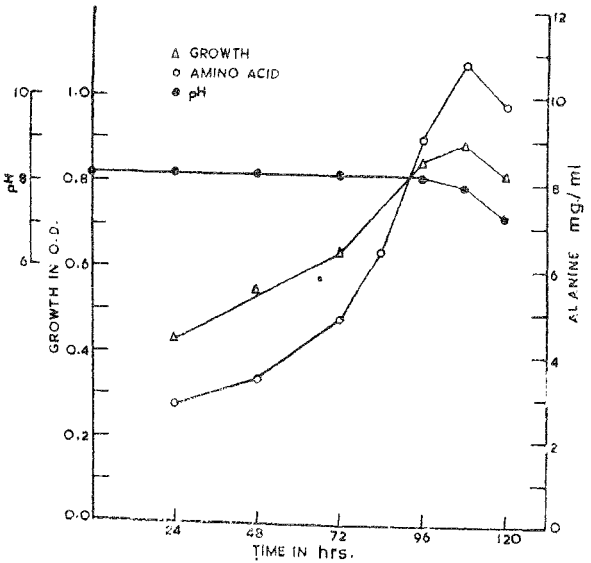

FIG. 6

Alanine production by Arthrobacter sp. No. $C_{19}d$ at optimum *pH* 8.2. 500 ml shake flasks with 100 ml medium: Mineral medium with glucose 10%, urea 1.0%, temp. 28°C

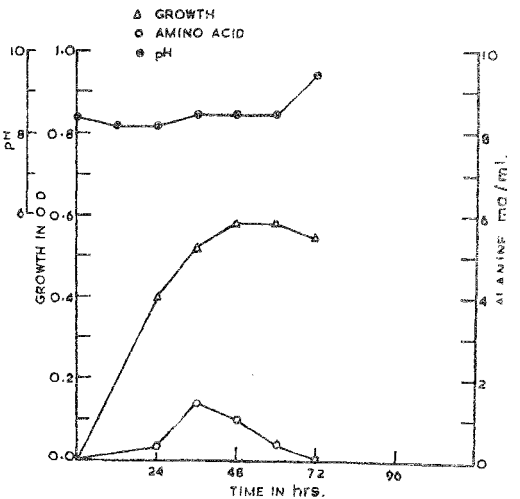

FIG. 7

Alanine production by Arthrobacter sp. No. $C_{19}d$ at *pH* 8.5. 500 ml shake flasks with 100 ml medium: Mineral medium with glucose 10%, urea 1.0%, temp. 28°C

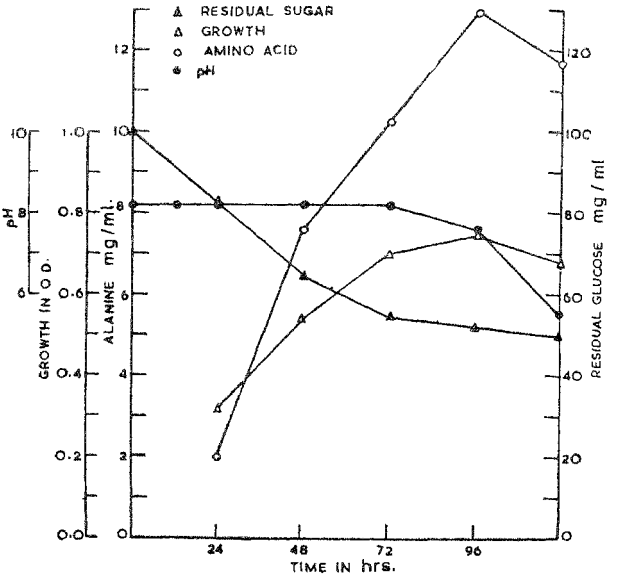

FIG. 8

Alanine production by Arthrobacter sp. No. $C_{19}d$ at optimum *pH* and temperature. 500 ml shake flasks with 100 ml medium: Mineral medium with glucose 10%, urea 1.0% temp. 32°C, *pH* 8.2

REFERENCES

1. Giri, K. V., Radhakrishnan, A. N. and Vaidyanathan. C. S. . . *J. Indian Inst. Sci.*, 1953, 35, 145.
2. Hirose, Y. and Yamada, K. . . Amino Acids, 3, Paper No. 2 1961. (c.f. Recent Progress in Microbiology VIII, p. 320, 1962, edited by N. E. Gibbons, University of Toronto Press).
3. Mullakhanbhai, M. F. and Bhat, J. V. . . *J. Indian Inst. Sci.*, 1966, 48, 25.
4. Okumura, S., Okada, H., Ozaki A., Kono, K and Sakaguchi, K. Amino Acids 2, Paper No. 4, 1960. (c. f. Recent Progress in Microbiology VIII, 1962, 320)
5. Samejima, H., Nara. T , Fujita, C. and Kinoshita, S. . . Nippon Nôgeikagaku Kaishi, 34, 832-838, 838—844, 1960. (c. f. Recent Progress in Microbiology VIII, 1962, 320.)
6. Somogyi, M. . . . . *J. Biol. Chem.*, 1926, 70, 607.
7. Yamada, K. and Hirose, Y. . . Amino Acids, 2, Paper No. 2, 1960. (c. f. Recent Progress in Microbiology VIII, 1962, 320).

SUPPLEMENT TO GLOSSARY OF INDIAN MEDICINAL PLANTS

By

B. N. CHOPRA, I. C. CHOPRA AND B. S. VARMA

In the year 1956, the Council of Scientific & Industrial Research, New Delhi published a Glossary of Indian Medicinal Plants with a view to presenting concise information regarding their properties, uses and important constituents. Over 2,600 species, belonging to about 1,350 plant genera have been dealt with The information is given under the botanical names of the plants, which are arranged in their alphabetical sequence; trade and vernacular names are also mentioned. The Glossary gives distribution of the plants, diseases for which the particular plant is used, and the active principles. Adequate literature references to the sources of information are also provided. The book ends with two comprehensive indexes one pertaining to the vernacular and trade names, and the other to the chemical constituents.

In order to bring the Glossary up to date, this Supplement has been brought out. It follows the style of the Glossary and covers all relevant information published during the period 1955-64. The supplement provides additional information on over 700 species already mentioned in the Glossary, and includes about 380 new species. Indexes covering additional vernacular and trade names and chemical constituents have been provided. The supplement like the original Glossary, will be useful not only to the practitioners of indigenous system of medicine, but also to all others who are interested in drugs of vegetable origin and common bazaar medicines.

Pages xii + 119 Royal 8 vo

Price Rs. 14.00 Sh. 28, or $ 4.50

Copies available from:

SALES AND DISTRIBUTION SECTION

PUBLICATIONS & INFORMATION DIRECTORATE

HILLSIDE ROAD, NEW DELHI-12