Journal of General Microbiology (1973), **76**, 85–99
Printed in Great Britain

Asparaginase and Glutaminase Activities of Micro-organisms

By A. IMADA, S. IGARASI, K. NAKAHAMA AND M. ISONO
Microbiological Research Laboratories, Central Research Division, Takeda Chemical Industries, Jûsô, Osaka, Japan

(*Received* 14 *September* 1972; *revised* 28 *November* 1972)

SUMMARY

L-Asparaginase and L-glutaminase activities were detected in many micro-organisms and the distribution of these activities was found to be related to the classification of micro-organisms.

Among 464 bacteria, the activities occurred in many Gram-negative bacteria and in a few Gram-positive bacteria. Most members of the family Enterobacteriaceae possessed L-asparaginase. L-Asparaginase and L-glutaminase occurred together in a large proportion of pseudomonads. Among Gram-positive bacteria many strains of *Bacillus pumilus* showed strong L-asparaginase activity. Amidase activities were also observed in several strains in other families.

L-Asparaginase activity was not detected in culture filtrates of 261 strains of species of the genera *Streptomyces* and *Nocardia*, but L-asparaginase and L-glutaminase were detected when these organisms were sonicated.

The amidase activities in culture filtrates of 4158 fungal strains were tested. All the strains of Fusarium species formed L-asparaginase. Organisms of the genera *Hypomyces* and *Nectria*, which are regarded as the perfect stage of the genus *Fusarium*, also formed L-asparaginase. Several Penicillium species formed L-asparaginase. Two organisms of the family Moniliaceae formed L-glutaminase together with L-asparaginase, and a few ascomycetous fungi formed L-asparaginase or L-glutaminase.

Among 1326 yeasts, L-asparaginase or L-glutaminase occurred frequently in certain serological groups of yeasts: VI (Hansenula) group, Cryptococcus group and Rhodotorula group. Many strains of Sporobolomyces species also showed L-asparaginase activity. Several strains of Cryptococcus and Rhodotorula group possessed L-glutaminase and L-asparaginase. L-Glutaminase alone was formed in many strains of *Candida scottii* and *Cryptococcus albidus*, both of which are related to Basidiomycetes.

INTRODUCTION

Kidd (1953) observed that certain transplanted murine leukaemias were suppressed by treatment with guinea-pig serum. Broome (1961) proposed that the antileukaemia activity was attributable to the action of L-asparaginase (EC. 3.5.1.1). Several investigations have confirmed this proposal (Broome, 1963*a*, *b*; Old, Boyse, Campbell & Daria, 1963; Schwartz, Reeves & Broome, 1966; Yellin & Wriston, 1966*a*, *b*).

It is well known that certain micro-organisms possess L-asparaginase and some of them show antileukaemia activity. Mashburn & Wriston (1964) proved that partially purified L-asparaginase of *Escherichia coli* suppressed 6C3HED lymphosarcoma of mice. L-Asparaginase of *Escherichia coli* was purified and is now being used experimentally as an antileukaemia agent for human leukaemia patients, and has been found to be very effective for acute lymphatic leukaemia of children (Hill *et al.* 1967; Oettgen *et al.* 1967). Besides L-asparaginase of *Escherichia coli*, the enzymes of organisms of Enterobacteriaceae have been found to suppress the growth of 6C3HED lymphosarcoma (Rowley & Wriston, 1967;

Eremenco, Evseev & Nikolaev, 1968; Wade *et al.* 1968; Peterson & Ciegler, 1969*a*; Yamada, 1970; Boyd & Phillips, 1971; Tosa *et al.* 1971). Reddy, Jayaram, Sirsi & Ramakrishnan (1969) found that L-asparaginase from *Mycobacterium tuberculosis* suppressed Yoshida ascites sarcoma in rats. De-Angeli *et al.* (1970) found that L-asparaginase of *Aspergillus terreus* suppressed Walker 256 ascites carcinoma in rats.

Anticancer activity of L-glutaminase (EC 3.5.1.2) has also been proposed. Greenberg, Blumenthal & Ramadan (1964) and El-Asmar & Greenberg (1966) observed that L-glutaminase of a pseudomonad inhibited the initial growth of several murine carcinomas but had little effect on the survival time of animals. Recently, Roberts, Holcenberg & Dolowy (1970) found that L-glutaminase of a Gram-negative rod-shaped bacterium suppressed Ehrlich ascites carcinoma.

L-Asparaginase from *Saccharomyces cerevisiae* (Greenberg *et al.* 1964), *Bacillus coagulans* (Mashburn & Wriston, 1964; Law & Wriston, 1971), *Alcaligenes faecalis* (Yamada, 1970; Tosa *et al.* 1971), *Candida utilis* (Sakamoto *et al.* 1970) and *Fusarium tricinctum* (Scheetz, Whelan & Wriston, 1971) had no antilymphoma activities.

L-Asparaginases from other sources, such as *Penicillium camemberti* (Dox, 1909), *Aspergillus niger* (Bach, 1928; Schmalfuss & Mothes, 1930), *Brucella abortus* (Altenbern & Housewright, 1954), pseudomonads (de Groot & Lichtenstein, 1960; Ramadan, El-Asmar & Greenberg, 1964), *Staphylococcus aureus* (Tsuji, 1957), *Bacillus stearothermophilus* (Manning & Campbell, 1957), *Mycobacterium avium* (Tsuji, 1957), and *Rhodopseudomonas capsulatum* (Tchan, Asano & Kobayashi, 1971), have not been tested for their antilymphoma activities. Recently, Peterson and Ciegler (1969*b*) and Wade, Robinson & Phillips (1971) surveyed the L-asparaginase and L-glutaminase activities of a number of bacteria and found a wide distribution of both activities. Arima, Sakamoto, Araki & Tamura (1972) noticed that many bacteria, fungi and yeasts produced extracellular L-asparaginase.

L-Asparaginase activity occurs in a number of micro-organisms. However, the anti-6C3HED lymphosarcoma activity was shown only by the enzymes from Enterobacteriaceae, and these enzymes are now used for clinical experiments. It may be anticipated that the injection of enzymes for a prolonged period induces the formation of antibody which neutralizes the enzyme activity. Anaphylaxy shock is also supposed to occur. With respect to L-asparaginase of *Escherichia coli*, several unfavourable side actions, including nausea, anorexia and liver dysfunction, have been pointed out (Haskel *et al.* 1969).

The present report shows the distribution of L-asparaginase and L-glutaminase activities among micro-organisms. The relationship between this distribution and the classification of micro-organisms is discussed.

METHODS

Chemicals. Nutrient broth, yeast extract and malt extract were obtained from Difco Laboratories, Detroit, Michigan, U.S.A.; Polypepton, from Daigo Nutritive Chemicals, Osaka, Japan; corn-steep liquor (CSL), from Corn Product Co., New York, U.S.A.; and Pharmamedia, from Traders Mill Co., Fort Worth, Texas, U.S.A. Other chemicals were purchased from Wako Pure Chemicals Co., Osaka, Japan.

Organisms. Most microbial strains were obtained from the Institute for Fermentation, Osaka. Several strains of *Pseudomonas aeruginosa* isolated from clinical sources were obtained from Medical School, Osaka University.

Culture methods. CSL-medium of Roberts, Burson & Hill (1968) was used for bacteria. ST-2 medium (pH 7·0) which was used for Streptomyces and Nocardia contained (g/l distilled water): soluble starch, 50; Polypepton, 10; beef extract, 5; and NaCl, 5. M-medium

(pH 6·0) used for yeasts and fungi contained (g/l distilled water): sucrose, 30; Polypepton, 5; beef extract, 5; yeast extract, 2; malt extract, 2; KH_2PO_4, 5; K_2HPO_4, 1·5; $MgSO_4.7H_2O$, 0·5. DP-medium (pH 6·0) used for fungi contained (g/l distilled water): dextrin, 30; Pharmamedia, 40; KH_2PO_4, 5; K_2HPO_4, 1·5; $MgSO_4.7H_2O$, 0·5.

Bacteria, actinomycetes and yeasts were cultivated in 4 ml of media in 14 mm diam. test tubes with shaking. Fungi were cultivated in 8 ml of media in 20 mm diam. test tubes with shaking. Cultivation temperature was 28 °C.

Enzyme preparation. The enzyme activities of bacteria were examined by using whole culture broth as the enzyme preparation. In the experiment shown in Table 2, bacterial suspension (bacteria harvested from 1 ml of culture were suspended in 1 ml of distilled water) and culture filtrate were used as the enzyme preparation. The activities of actinomycetes were examined by using culture filtrate or cell-sonicate (actinomycetes harvested from 1 ml of culture were suspended in 1 ml of distilled water, sonically disrupted for 3 min, and cell debris was centrifuged off at 12000 *g* for 30 min). The activities of fungi were examined by using culture filtrate, and those of yeasts by using whole culture broth as the enzyme preparations.

Assay of L-*asparaginase and* L-*glutaminase.* L-Asparaginase was assayed as follows. A reaction mixture containing 0·5 ml of 0·04 M-L-asparagine, 0·5 ml of 0·5 M-buffer, 0·5 ml of an enzyme preparation, and distilled water to a total volume of 2·0 ml was incubated at 37 °C for 30 min. The reaction was stopped by adding 0·5 ml of 1·5 M-trichloroacetic acid. Blank tubes were run by adding the enzyme preparation after the addition of trichloroacetic acid. To 3·7 ml of distilled water, 0·1 ml of the above mixtures and 0·2 ml of Nessler's reagent were added. After keeping the mixture at 15 to 20 °C for 20 min, extinction at 450 nm was measured with a Spectronic 20 colorimeter (Schimadzu Bausch & Lomb) using $\frac{1}{2}$ inch cells, and the amount of released ammonia was determined. One international unit (i.u.) of L-asparaginase is the amount of enzyme which liberates 1 μmol of ammonia in 1 min.

L-Glutaminase was assayed by using L-glutamine in place of L-asparagine.

For the examination of these amidase activities, acetate buffer, pH 5·6, and tris(hydroxymethyl)aminomethane.HCl buffer, pH 8·4, were used for bacterial enzymes and tris(hydroxymethyl)aminomethane.HCl buffer, pH 7·2, for the enzymes of actinomycetes, yeasts and other fungi.

Measurement of growth. Growth of bacteria was expressed in extinction at 600 nm of the cultures, which was measured by a Spectronic 20 colorimeter.

RESULTS AND DISCUSSION

L-*Asparaginase and* L-*glutaminase activities of bacteria*

For the examination of bacterial L-asparaginase and L-glutaminase activities, CSL-medium of Roberts *et al.* (1968) was chosen, since it generally gave good growth of a variety of bacteria with comparatively high amidase activities. The cultivation period was set at 40 h, since deviation of the amount of growth of bacteria was less at 40 h cultivation than at 16 h without significant difference in the specific activities, which is the activity per growth (see Table 2). Whole culture broth was employed as the enzyme preparation, because the total complement of the amidase in the cell could be measured without disrupting cells as judged by preliminary experiments using a number of bacteria, e.g. *Escherichia coli* (3 strains), *Proteus vulgaris*, *Serratia marcescens*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *B. cereus*, *B. pumilus*, *B. brevis*,

Table 1. *Distribution of* L-*asparaginase and* L-*glutaminase among bacteria*

Bacterial strain	Number of strains: L-Asparaginase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01	L-Glutaminase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01
Pseudomonadaceae								
Pseudomonas aeruginosa	2	81	—	—	2	81	—	—
P. chlororaphis	—	2	—	—	—	2	—	—
P. schuylkilliensis	1	—	—	1	1	—	—	1
P. synxantha	—	2	1	—	—	2	1	—
P. fluorescens	1	6	—	—	1	6	—	—
P. pavonacea	2	—	—	—	2	—	—	—
P. iodinum	1	—	—	—	1	—	—	—
P. putida	3	—	—	—	3	—	—	—
P. ovalis	3	1	—	—	3	1	—	—
P. teatrolens	1	—	—	—	1	—	—	—
P. mildenbergii	1	1	—	—	2	—	—	—
P. fragi	1	—	—	—	1	—	—	—
P. putrefaciens	3	2	—	—	5	—	—	—
P. dacunhae	2	4	—	—	2	4	—	—
P. stetzeri	1	—	—	—	1	—	—	—
P. riboflavina	1	—	—	—	1	—	—	—
P. coronafaciens	1	—	—	—	1	—	—	—
P. tabaci	1	—	—	—	1	—	—	—
P. polycolor	—	1	—	—	1	—	—	—
P. marginalis	—	1	—	—	—	1	—	—
P. solanacearum	1	—	—	—	1	—	—	—
P. aureofaciens	—	5	1	—	—	6	—	—
P. multophile	1	—	—	—	1	—	—	—
P. diminuta	1	—	—	—	1	—	—	—
P. nitroreducens	1	—	—	—	1	—	—	—
P. vendrelli	—	1	—	—	—	1	—	—
P. melanogenum	2	—	—	—	2	—	—	—
P. azotoformans	—	1	—	—	—	1	—	—
P. trifolii	1	—	—	—	1	—	—	—
P. alkanolytica	1	—	—	—	1	—	—	—
P. desmolytica	1	—	—	—	1	—	—	—
P. graveolens	—	1	—	—	1	—	—	—
P. convexa	—	1	—	—	—	1	—	—
Aeromonas hydrophila	3	—	—	—	3	—	—	—
A. liquefaciens	1	—	—	—	1	—	—	—
Protaminobacter alboflavus	1	—	—	—	1	—	—	—
Spirillaceae								
Spirillum lunatum	—	1	—	—	1	—	—	—
S. metamorphum	—	1	—	—	1	—	—	—
Chlamydobacteriaceae								
Sphaerotilus natans	1	2	—	—	2	1	—	—
Azotobacteraceae								
Azotobacter chroococcum	1	—	—	—	1	—	—	—
A. agilis	1	—	—	—	1	—	—	—
A. indicum	1	—	—	—	1	—	—	—
Rhizobiaceae								
Agrobacterium tumefaciens	2	—	—	—	2	—	—	—
A. radiobacter	3	—	—	—	3	—	—	—
Chromobacterium violaceum	1	—	—	—	1	—	—	—
Achromobacteraceae								
Alcaligenes faecalis	1	2	1	—	3	1	—	—
Achromobacter liquidum	—	1	—	—	1	—	—	—

Table 1 (*continued*)

Bacterial strain	Number of strains: L-Asparaginase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01	L-Glutaminase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01
A. delmarvae	—	1	—	—	1	—	—	—
Flavobacterium flavescens	2	—	—	—	2	—	—	—
F. suaveolens	1	—	—	—	1	—	—	—
F. esteroaromaticum	1	—	—	—	1	—	—	—
F. aquatile	2	—	—	—	2	—	—	—
F. heparinum	1	—	—	—	1	—	—	—
F. gasgenes	1	—	—	—	1	—	—	—
F. meningosepticum	1	—	—	—	1	—	—	—
Enterobacteriaceae								
Enterobacter cloacae	—	1	—	—	—	1	—	—
Escherichia coli	—	11	5	28	38	6	—	—
Klebsiella aerogenes	6	6	—	—	10	2	—	—
K. pneumoniae	—	1	—	—	1	—	—	—
Erwinia carotovora	—	1	—	—	1	—	—	—
E. aroideae	1	1	—	—	2	—	—	—
E. amylolytica	—	1	—	—	1	—	—	—
E. herbicola	—	1	—	—	1	—	—	—
Serratia marcescens	—	7	—	—	7	—	—	—
S. piscatorum	—	1	—	—	1	—	—	—
S. plymuthicum	1	—	—	—	1	—	—	—
S. indica	—	1	—	—	1	—	—	—
Proteus vulgaris	—	—	4	—	4	—	—	—
P. morganii	1	—	1	—	1	1	—	—
P. mirabilis	—	—	2	—	2	—	—	—
Shigella paradysenteriae	1	—	—	—	1	—	—	—
Brucellaceae								
Brucella bronchiseptica	2	—	—	—	1	1	—	—
Micrococcaceae								
Micrococcus lutens	1	—	—	—	1	—	—	—
M. lysodeikticus	1	—	—	—	1	—	—	—
M. roseus	1	—	—	—	1	—	—	—
M. variabilis	1	—	—	—	1	—	—	—
M. flavus	1	—	—	—	1	—	—	—
M. varians	1	—	—	—	1	—	—	—
M. subflavus	1	—	—	—	1	—	—	—
M. marginata	1	—	—	—	1	—	—	—
M. ureae	1	—	—	—	—	1	—	—
M. rubens	1	—	—	—	1	—	—	—
M. glutamicus	2	—	—	—	2	—	—	—
M. cerificans	—	1	—	—	—	1	—	—
Staphylococcus aureus	3	—	—	—	3	—	—	—
S. epidermidis	1	—	—	—	1	—	—	—
Sarcina lutea	1	—	—	—	1	—	—	—
S. aurantiaca	1	—	—	—	1	—	—	—
Brevibacteriaeceae								
Brevibacterium flavum	1	—	—	—	1	—	—	—
B. ammoniagenes	4	1	—	—	4	1	—	—
B. protomorphiae	2	—	—	—	2	—	—	—
B. saperdae	1	—	—	—	1	—	—	—
B. divaricatum	3	—	—	—	3	—	—	—
B. vitarumen	1	—	—	—	1	—	—	—
B. stationis	2	—	—	—	2	—	—	—
B. insertum	1	—	—	—	1	—	—	—
B. leucinophagum	—	1	—	—	—	1	—	—

Table 1 (*continued*)

Bacterial strain	Number of strains: L-Asparaginase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01	L-Glutaminase (i.u./ml) <0·10	0·11–0·50	0·51–1·00	>1·01
B. roseum	1	—	—	—	1	—	—	—
B. saccharolyticum	1	—	—	—	1	—	—	—
B. chang-fua	1	—	—	—	1	—	—	—
B. glutamicus	1	—	—	—	1	—	—	—
B. immariophilium	1	—	—	—	1	—	—	—
B. taipei	1	—	—	—	1	—	—	—
B. alkanolyticum	1	—	—	—	1	—	—	—
B. tesaceum	1	—	—	—	1	—	—	—
B. citreum	1	—	—	—	1	—	—	—
B. fuscum	—	1	—	—	1	—	—	—
B. linens	3	1	—	—	4	—	—	—
B. imperiale	1	—	—	—	1	—	—	—
B. pusillum	1	—	—	—	1	—	—	—
B. sulfureum	1	—	—	—	1	—	—	—
B. helvorum	—	1	—	—	—	1	—	—
Kurthia zopfii	1	—	—	—	1	—	—	—
Corynebacteriaceae								
Corynebacterium equi	1	—	—	—	1	—	—	—
C. insidiosum	1	—	—	—	1	—	—	—
C. nephridii	2	—	—	—	2	—	—	—
C. vesiculare	1	—	—	—	1	—	—	—
C. paurometabolum	2	—	—	—	2	—	—	—
C. sepedonicum	1	—	—	—	1	—	—	—
C. flaccumfaciens	1	—	—	—	1	—	—	—
C. hydrocarboclatus	1	—	—	—	1	—	—	—
C. diphtheriae	1	—	—	—	1	—	—	—
C. tritici	1	—	—	—	1	—	—	—
C. xerosis	1	—	—	—	1	—	—	—
C. faccians	2	—	—	—	2	—	—	—
Arthrobacter simplex	2	—	—	—	2	—	—	—
A. globiformis	2	—	—	—	2	—	—	—
A. pascens	1	—	—	—	1	—	—	—
A. aurescens	1	—	—	—	1	—	—	—
A. atrocyaneus	1	—	—	—	1	—	—	—
A. citreus	1	—	—	—	1	—	—	—
A. ramosus	1	—	—	—	1	—	—	—
Microbacterium lacticum	1	—	—	—	1	—	—	—
Bacillaceae								
Bacillus megaterium	6	—	—	—	6	—	—	—
B. cereus	13	—	—	—	13	—	—	—
B. anthracis	2	—	—	—	2	—	—	—
B. licheniformis	6	3	—	—	9	—	—	—
B. subtilis	53	3	—	—	56	—	—	—
B. pumilus	7	3	11	2	22	1	—	—
B. firmus	1	—	—	—	1	—	—	—
B. circulans	1	—	—	—	1	—	—	—
B. brevis	2*	—	—	—	2	—	—	—
B. sphaericus	6†	—	—	—	6	—	—	—

* One of two strains showed L-asparaginase activity at pH 5·6.
† One of six strains showed L-asparaginase activity at pH 5·6.

B. licheniformis and *B. megaterium*. Wade *et al.* (1971) also pointed out that asparaginase activity in bacterial cells could be assayed without disruption.

Thus 464 bacteria were cultivated and L-asparaginase and L-glutaminase activities were measured at pH 5·6 and 8·4. The results, summarized in Table 1, show the distribution of the pH 8·4 activities/ml of culture. The activities at pH 5·6 were generally less than at pH 8·4, but the pattern of distribution was almost identical. The distribution pattern obtained when using the specific activities was more or less the same as that shown in Table 1.

A large proportion of strains of Pseudomonas species showed L-asparaginase and L-glutaminase simultaneously. These observations confirm the wide distribution of L-asparaginase and L-glutaminase activities in this genus which has been reported previously by de Groot & Lichtenstein (1960), Greenberg *et al.* (1964), Ramadan *et al.* (1964), El-Asmar & Greenberg (1966), Peterson & Ciegler (1969*b*) and Arima *et al* (1972). Two *Pseudomonas putrefaciens* strains, like organisms of the Enterobacteriaceae, showed L-asparaginase with little L-glutaminase activities. In this point they differ from other species of *Pseudomonas*. It was also found that they differ from other Pseudomonas species in possessing nucleoside *N*-ribosyl transfer activity which occurred rarely in Pseudomonadaceae but was common in Enterobacteriaceae (Imada & Igarasi, 1967). We suspect some relationship between these red *Pseudomonas putrefaciens* and the Enterobacteriaceae, especially Serratia species.

Two Spirillum strains showed L-asparaginase activity. Two strains of *Sphaerotilus natans* showed L-asparaginase activity and one of the two strains showed L-glutaminase activity. In the family Achromobacteraceae strains of Alcaligenes and Achromobacter species showed L-asparaginase or L-glutaminase activity, while Flavobacterium species showed little activity. Arima *et al.* (1972) indicated the extracellular production of L-asparaginase by Alcaligenes species, Wade *et al.* (1971) showed the L-asparaginase activities of Alcaligenes species, and Roberts *et al.* (1972) reported the properties of glutaminase-asparaginase of members of the Achromobacteraceae having antitumour activity.

Most members of the Enterobacteriaceae were active in L-asparaginase but not so active in L-glutaminase. *Escherichia coli* strains were extraordinarily active in L-asparaginase, and Proteus species also showed strong L-asparaginase activity.

As compared with the above-mentioned Gram-negative bacteria, Gram-positive bacteria were usually less active in the amidases. A few strains among Micrococcus and Brevibacterium species showed these activities, but none was shown among the Staphylococcus, Corynebacterium and Arthrobacter species.

Several strains of *Bacillus licheniformis*, *B. subtilis* and *B. pumilus*, which are included in the *B. subtilis* group, showed L-asparaginase activity, and for many strains of *B. pumilus* the level was high. *Bacillus megaterium*, *B. cereus* and other Bacillus species showed little activity.

Bacteria which showed comparatively high amidase activities were selected and cultivated for 16 and 40 h in 40 ml of CSL-medium in 200 ml Erlenmeyer flasks and the amidase activities in cells and culture filtrates were examined (see Table 2). The activities were always higher in cells cultivated in 200 ml Erlenmeyer flasks than in those cultivated in test tubes. Strains of *Escherichia coli*, *Proteus morganii*, *P. vulgaris*, *P. mirabilis*, *Pseudomonas fluorescens*, *P. schuylkilliensis*, *Alcaligenes faecalis* and *Bacillus pumilus* formed more than 1 i.u./ml of L-asparaginase within the bacteria. Strains of *Pseudomonas aureofaciens*, *P. schuylkilliensis* and *Alcaligenes faecalis* possessed a little L-asparaginase or L-glutaminase activity in their culture filtrates.

Table 2. *L-Asparaginase and L-glutaminase activities of selected bacterial strains*

	16 h culture					40 h culture				
		L-Asparaginase* (i.u./ml)		L-Glutaminase* (i.u./ml)			L-Asparaginase (i.u./ml)		L-Glutaminase (i.u./ml)	
Bacterial strain	Growth (E_{600} nm × 10^3)	Bacteria	Culture filtrate	Bacteria	Culture filtrate	(E_{600} nm × 10^3)	Bacteria	Culture filtrate	Bacteria	Culture filtrate
Escherichia coli F-221 (IFO Iijima)	340	5·23	0	—†	—	300	4·85	0	—	—
Proteus vulgaris IFO3167	330	3·31	0	—	—	340	2·00	0	—	—
P. morganii IFO3848	240	1·15	0	—	—	280	1·46	0	—	—
P. mirabilis IFO12255	310	2·23	0	—	—	320	1·39	0	—	—
Serratia indica IFO3759	280	0·62	0	—	—	270	0·62	0	—	—
S. piscatorum IFO12527	270	0·77	0	—	—	200	0·92	0	—	—
Alcaligenes faecalis IFO12624	145	0·85	trace	—	—	240	2·15	0·26	—	—
Pseudomonas fluorescens IFO3461	240	2·54	0	1·23	0	340	2·39	0	1·92	0
P. aureofaciens IFO3522	260	0·89	trace	0·35	0	290	0·85	0·25	0·69	0·26
P. schuylkilliensis IFO12055	275	2·39	0	1·08	0	340	2·31	0·07	1·77	0·11
Spirillum metamorphum IFO12012	60	0	0	0	0	200	0·15	0	0·22	0
Micrococcus cerificans IFO12522	240	0·55	0	—	—	270	0·31	0	—	—
Brevibacterium sp. IFO12147	290	0·42	0	0·18	0	200	0·31	0	0·31	0
Bacillus pumilus IFO12093	205	1·54	0	—	—	225	1·62	0	—	—

* Activities at pH 8·4.

† Not examined.

Table 3. L-*Asparaginase and* L-*glutaminase activities of Streptomyces species*

Strain	L-Asparaginase (i.u./ml)	L-Glutaminase (i.u./ml)
Streptomyces californicus IFO3386	0·20	0·18
S. globiformis IFO12208	0·18	0·17
S. griseoflavus IFO3428	0·09	0·15
S. griseolus IFO3403	0·09	0·12
S. netropsis IFO3723	0·40	0·44
S. olivochromogenes IFO3178	0·11	0·14
S. rimosus IFO3226	0·22	0·18
S. roseochromogenes IFO3363	0·11	0·09

Table 4. *Fungal strains producing* L-*asparaginase and* L-*glutaminase in culture filtrates*

Fungal strain	L-Asparaginase (i.u./ml)	L-Glutaminase (i.u./ml)
Ascomycetes		
Anixiella reticulata IFO5814	0·42*	0*
Microascus desmosporus IFO7021	0·56	Not examined
	0·28*	0·24*
Dichotomyces albus var. *spinosus* IFO8655	0·11	0
Nectria haematococca IFO6891	0·46	0
N. elegans IFO7187	0·20	0
N. cinnabarina IFO6821	1·05	0
Hypomyces solani IFO7707	0·66	0
H. solani var. *xanthoxyli* IFO7710	0·55	0
H. haematococcus IFO5980	0·28	0
Fungi Imperfecti		
Fusarium solani IFO5899	0·29	0
F. solani var. *rasinfetum* IFO4473	0·12	0
F. oxysporum f.2 IFO5264	0·40	0
F. roseum IFO5421	0·10	0
Tilachlidium humicola IFO5696	0·29	0·31
Verticillium malthoasei IFO6624	0·42	0·38
Penicillium urticae IFO4633	1·32	0·06
P. claviforme IFO4676	0·22	0
P. expansum IFO5453	0·11	0
	0·83*	0*
P. aculeatum IFO7840	0·29	0
P. granulatum IFO5737	0·44*	0*

* The activities in culture filtrates of 5-day culture in DP-medium in shaking flasks. Others are the activities in culture filtrates of 5-day culture in M-medium in test tubes.

L-Glutaminase activity was also found in cells of Pseudomonas, Spirillum and Brevibacterium species. L-Glutaminase activity was stronger in 40 h-culture bacteria than in 16 h-culture bacteria, while L-asparaginase activity remained unchanged or was slightly weaker in 40 h-culture bacteria. Therefore, it might be suggested that there exist more than two enzymes which deamidate L-asparagine or L-glutamine. Nikolaev, Evseev, Tyul'panova & Abdumalikov (1969) pointed out the presence of isoenzymes in a pseudomonad. One of the isoenzymes deamidated L-asparagine selectively and another deamidated L-asparagine and L-glutamine.

We have observed that the amidases from various bacteria differ from each other with respect to their pH-activity relationship, heat-stability and substrate specificity.

Table 5. *Distribution of* L-*asparaginase and* L-*glutaminase activities among yeasts*

Genus and species	Number of strains: Examined	L-Asparaginase active (A/B)*	L-Asparaginase and L-glutaminase active (A/B)*	L-Glutaminase active (A/B)*	Remarks
Eremascus	1	0	0	0	—
Wickerhamia	1	0	0	0	—
Endomyces	5	0	0	0	—
Endomycopsis	19	0	0	0	—
Schizosaccharomyces	29	1	0	0	—
Schizosacch. octosporus	—	(1/2)	—	—	—
Saccharomyces	239	5	0	0	—
S. peka	—	(2/2)	—	—	—
S. mangini	—	(1/1)	—	—	—
S. cerevisiae	—	(1/57)	—	—	—
S. logos	—	(1/3)	—	—	—
Zygosaccharomyces	80	0	0	0	—
Chlamydozyma	3	0	0	0	—
Torulaspora	17	0	0	0	—
Schwanniomyces	2	0	0	0	—
Debaryomyces	89	4	0	0	—
D. kloeckeri	—	(1/14)	—	—	V†
D. hansenii	—	(2/18)	—	—	V
D. nicotianae	—	(1/3)	—	—	V
Hansenula	101	29	2	0	—
H. saturnus	—	(3/8)	—	—	VI
H. anomala	—	(4/28)	—	—	VI
H. suaveolens	—	(2/2)	—	—	VI
H. subpelliculosa	—	(1/1)	—	—	VI
H. schneggii	—	(1/2)	—	—	VI
H. wingei	—	(5/11)	—	—	—
H. canadensis	—	(2/3)	—	—	—
H. jadini	—	(1/2)	(1/2)	—	VI‡
H. beijerinckii	—	(4/5)	(1/5)	—	VI
H. pettersonii	—	(2/2)	—	—	—
H. fabiani	—	(2/2)	—	—	—
H. miso	—	(2/3)	—	—	—
Pichia	90	2	0	0	—
P. polymorpha	—	(1/4)	—	—	—
P. etchelsii	—	(1/1)	—	—	—
Hanseniospora	10	1	0	0	—
Hanseniospora valbyensis	—	(1/4)	—	—	I
Saccharomycodes	4	0	0	0	—
Nadsonia	3	0	0	0	—
Naganishia	1	0	1	0	CRYP†
Naganishia globosus	—	—	(1/1)	—	—
Metschnikowia	1	0	0	0	—
Nematospora	2	0	0	0	—
Candida	237	19	0	5	—
C. utilis	—	(9/9)	—	—	VI
C. robusta	—	(1/9)	—	—	II§
C. pelliculosa	—	(3/3)	—	—	VI
C. scottii ‖	—	—	—	(5/10)	—
C. humicola	—	(1/3)	—	—	—
C. melnii	—	(1/4)	—	—	III
C. curvata	—	(1/2)	—	—	CRYP†
C. fabiani	—	(2/2)	—	—	—
C. tropicalis	—	(1/10)	—	—	I

Table 5 (*continued*)

Genus and species	Number of strains: Examined	L-Asparaginase active (A/B)*	L-Asparaginase and L-glutaminase active (A/B)*	L-Glutaminase active (A/B)*	Remarks
Lipomyces	4	0	0	0	—
Ashbya	2	0	0	0	—
Petasospora	3	0	0	0	—
Eremothecium	6	0	0	0	—
Sporobolomyces	29	15	0	0	—
S. coralliformis	—	(2/2)	—	—	—
S. holsaticus	—	(2/3)	—	—	—
S. coprophilus	—	(1/1)	—	—	—
S. roseus	—	(6/7)	—	—	—
S. ruber	—	(1/1)	—	—	—
S. pararoseus	—	(2/3)	—	—	RHO†
S. carnicolor	—	(1/1)	—	—	—
Bullera	4	0	2	0	—
B. alba	—	—	(2/4)	—	—
Cryptococcus	44	9	12	7	—
C. laurentii	—	(4/11)	(2/11)	(1/11)	CRYP
C. nitens	—	(1/1)	—	—	—
C. albidus	—	—	(8/16)	(6/16)	CRYP
C. neoformans	—	(1/10)	(2/10)	—	CRYP
C. luteolus	—	(2/2)	—	—	—
C. diffluens	—	(1/1)	—	—	CRYP
Torulopsis	93	6	0	0	—
T. capsuligenes	—	(1/1)	—	—	—
T. aeria	—	(2/2)	—	—	CRYP
T. bacillaris	—	(1/6)	—	—	SCH†
T. colliculosa	—	(1/5)	—	—	VII
T. candida	—	(1/3)	—	—	V
Pityrosporum	3	0	0	0	—
Kluyveromyces	1	0	0	0	—
Pachysolen	1	0	0	0	—
Brettanomyces	10	0	0	0	—
Mycoderma (*Candida*)	19	0	0	0	—
Kloeckera	28	0	0	0	—
Trichosporon	21	1	1	0	—
T. cutaneum	—	—	(1/9)	—	—
T. pullulans	—	(1/3)	—	—	—
Rhodotorula	108	30	8	0	—
R. flava	—	(2/5)	(1/5)	—	—
R. glutinis	—	(10/27)	—	—	—
R. pilimanae	—	(1/1)	—	—	—
R. rubra	—	(16/39)	(6/39)	—	RHO
R. infirmoniata	—	—	(1/1)	—	—
R. macerans	—	(1/1)	—	—	—
Others	16	1	0	0	—
'Aspergillus Hefe'¶	—	(1/1)	—	—	—

The yeast strains which showed more than 0·03 i.u./ml of the activity were regarded as active ones.

* (A/B) shows active number (A)/tested number (B).

† Roman number, CRYP (Cryptococcus), RHO (Rhodotorula) and SCH (Schizosaccharomyces) indicate the serological group.

‡ Spore former of *Candida utilis*.

§ Non-spore former of *Saccharomyces cerevisiae*.

‖ Heterobasidiomycetous stage is present.

¶ An Aspergillus sp. grows like a yeast.

L-Asparaginase and L-glutaminase activities of Streptomyces and Nocardia

L-Asparaginase activity was not detected in the culture filtrates of 261 strains of actinomycetes. Since these strains grow well on media which contain L-asparagine as a single nitrogen source, L-asparagine-metabolizing activity was presumed to be present. When 25 strains of Streptomyces species were cultivated in 40 ml of ST-2 medium in 200 ml Erlenmeyer flasks for 3 days with shaking, L-asparagine and L-glutamine deamidating activities were found in the sonicated preparations from such cultures. Some of the examples are shown in Table 3. The only other reference to the presence of L-asparaginase in Streptomyces is that by Campbell & Mashburn (1969), who showed its presence in *Streptomyces griseus*.

L-Asparaginase and L-glutaminase activities of fungi

Culture filtrates of 518 organisms of Basidiomycetes, 440 strains of Phycomycetes, 660 strains of Ascomycetes and 2540 strains of Fungi Imperfecti were examined. Amidase-active strains are listed in Table 4.

Among strains of Fungi Imperfecti, all strains of Fusarium species formed L-asparaginase. L-Asparaginase activity was also detected in culture filtrates of a number of Penicillium species. In the culture filtrates of strains of *Penicillium claviforme* and *P. expansum*, L-asparaginase occurred frequently. Arima *et al.* (1972) also reported the presence of L-asparaginase in the culture filtrate of *P. claviforme*.

Strains of *Tilachlidium humicola* and *Verticillium malthousei*, organisms of Fungi Imperfecti formed L-asparaginase and L-glutaminase.

Among strains of Ascomycetes, the genera *Hypomyces* and *Nectria*, which are the perfect stage of the genus *Fusarium*, formed L-asparaginase. Therefore the ability of extracellular formation of L-asparaginase is a common property in *Fusarium* and its related genera. Several other species of Ascomycetes also formed L-asparaginase or L-glutaminase.

Basidiomycetes and Phycomycetes showed little growth under our culture conditions and enzyme activities were not detected in their culture filtrates.

L-Asparaginase and L-glutaminase activities of yeasts

Among 1326 yeasts, L-asparaginase or L-glutaminase activity occurred in about 12% of them. The distribution of the activities was closely related to the classification, especially to the serological classification by Tsuchiya (1967). The results are summarized in Table 5.

L-Asparaginase activity occurred frequently in yeasts which are serologically grouped into the VI or Hansenula group, and included *Candida utilis*, *C. pelliculosa* and many strains of Hansenula, the Cryptococcus group and the Rhodotorula group. Several yeasts of the Cryptococcus and Rhodotorula groups possessed L-glutaminase together with L-asparaginase. Sporobolomyces species which are serologically related to Rhodotorula species frequently showed L-asparaginase activity.

That *Hansenula jadini* is regarded as the perfect stage of *Candida utilis* was reflected in the fact that all the strains of these two species possessed L-asparaginase.

Yeast strains forming L-glutaminase alone were found in *Candida scottii*, which has a heterobasidiomycetous stage named *Leucosporidium* (Fell, Statzell, Hunter & Phaff, 1969), in *Cryptococcus albidus* and *C. laurentii*.

Extracellular formation of L-asparaginase by *Candida utilis* and *Rhodotorula rosa* was reported by Arima *et al.* (1972). We have also observed L-asparaginase in culture filtrates of *Hansenula jadini*, *Rhodotorula rubra*, *Cryptococcus albidus*, *Sporobolomyces roseus* and some other strains as well as in the culture filtrate of *Candida utilis*.

Saccharomyces cerevisiae is known to possess L-asparaginase (Gorr & Wagner, 1932; Grassmann & Mayr, 1933; Greenberg *et al.* 1964), but under our experimental conditions the activity was detected in only one of 57 strains.

In spite of the presence of a number of investigations on microbial L-asparaginase and L-glutaminase, the correlation between their distribution and microbial classification has not been discussed. Having studied systematically the occurrence of the activities in a wide variety of micro-organisms we have observed that the amidase activities are concentrated in certain taxonomic groups of bacteria, fungi and yeasts. Such evidence may be valuable in understanding to what extent the current classification, which is mainly based on morphology and so some extent on physiology, is correlated with biochemical characteristics. It may also help in selecting microbial sources of enzymes.

We thank Drs S. Tatsuoka and R. Takeda, Central Research Division, Takeda Chemical Industries Ltd, for their continued interest and encouragement throughout the course of our work. We also thank Drs T. Hasegawa, K. Tubaki and I. Banno, Institute for Fermentation, Osaka, for supplying micro-organisms and for rewarding discussions. The technical assistance of Mr H. Ono, Miss H. Sotoma and Miss Y. Nakamura is also appreciated.

REFERENCES

ALTENBERN, R. A. & HOUSEWRIGHT, R. D. (1954). Stereospecific asparaginases in smooth *Brucella abortus*, strain 19. *Archives of Biochemistry and Biophysics* **49**, 130–137.

ARIMA, K., SAKAMOTO, T., ARAKI, C. & TAMURA, G. (1972). Production of extracellular L-asparaginases by microorganisms. *Agricultural and Biological Chemistry* **36**, 356–361.

BACH, D. (1928). Les conditions d'action de l'asparaginase de l'*Aspergillus niger*. *Comptes rendus* **187**, 955–956.

BOYD, J. W. & PHILLIPS, A. W. (1971). Inhibition of lymphoma 6C3HED by L-asparaginase from *Serratia marcescens*. *Journal of National Cancer Institute* **46**, 1271–1276.

BROOME, J. D. (1961). Evidence that the L-asparaginase activity of guinea pig serum is responsible for its antilymphoma effects. *Nature, London* **191**, 1114–1115.

BROOME, J. D. (1963*a*). Evidence that the L-asparaginase of guinea pig serum is responsible for its antilymphoma effects. I. Properties of the L-asparaginase of guinea pig serum in relation to those of the antilymphoma substance. *Journal of Experimental Medicine* **118**, 99–120.

BROOME, J. D. (1963*b*). Evidence that the L-asparaginase of guinea pig serum is responsible for its antilymphoma effects. II. Lymphoma 6C3HED cells cultured in a medium devoid of L-asparagine lose their susceptibility to the effects of guinea pig serum *in vivo*. *Journal of Experimental Medicine* **118**, 121–148.

CAMPBELL, H. A. & MASHBURN, L. T. (1969). L-Asparaginase EC-2 from *Escherichia coli*. Some substrate specificity characteristics. *Biochemistry* **8**, 3768–3775.

DE-ANGELI, L. C., POCCIARI, F., RUSSI, S., TONOLO, A., ZURITA, V. E., CIARANFI, E. & PERIN, A. (1970). Effect of L-asparaginase from *Aspergillus terreus* on ascites sarcoma in the rat. *Nature, London* **225**, 549–550.

DOX, A. W. (1909). The intracellular enzymes of lower fungi, especially those of *Penicillium camemberti*. *Journal of Biological Chemistry* **6**, 461–467.

EL-ASMAR, F. A. & GREENBERG, D. M. (1966). Studies on the mechanism of inhibition of tumor growth by the enzyme glutaminase. *Cancer Research* **26**, 116–122.

EREMENKO, V. V., EVSEEV, L. P. & NIKOLAEV, A. YA. (1968). Asparaginase of *Bacterium cadaveris*. *Mikrobiologiya* **37**, 207–212.

FELL, J. W., STATZELL, A. C., HUNTER, I. L. & PHAFF, H. J. (1969). *Leucosporidium* gen.n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie van Leeuwenhoek* **35**, 433–462.

GORR, G. & WAGNER, J. (1932). Über das Amidspaltungsvermögen der Hefen. *Biochemische Zeitschrift* **254**, 1–4.

GRASSMANN, W. & MAYR, O. (1933). Zur Kenntnis der Hefeasparaginase. *Zeitschrift für physiologische Chemie* **214**, 185–210.

GREENBERG, D. M., BLUMENTHAL, G. & RAMADAN, M-E. A. (1964). Effect of administration of the enzyme glutaminase on the growth of cancer cells. *Cancer Research* **24**, 957–963.

DE GROOT, N. & LICHTENSTEIN, N. (1960). The action of *Pseudomonas fluorescens* extracts on asparagine and asparagine derivatives. *Biochimica et biophysica acta* **40**, 99–110.

HASKEL, C. M., CANELLOS, G. P., LEVENTHAL, B. G., CARBONE, P. P., BLOCK, J. B., SERPICK, A. A. & SELAWRY, O. S. (1969). L-Asparaginase. Therapeutic and toxic effects in patients with neoplastic disease. *New England Journal of Medicine* **281**, 1028–1034.

HILL, J. M., ROBERTS, J., LOEB, E., KHAN, A., MACLELLAN, A. & HILL, R. W. (1967). L-Asparaginase therapy for leukemia and other malignant neoplasms. Remission in human leukemia. *Journal of American Medical Association* **202**, 882–888.

IMADA, A. & IGARASI, S. (1967). Ribosyl and deoxyribosyl transfer by bacterial enzyme systems. *Journal of Bacteriology* **94**, 1551–1559.

KIDD, J. G. (1953). Regression of transplanted lymphomas induced *in vivo* by means of normal guinea pig serum. I. Course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum, or rabbit serum. *Journal of Experimental Medicine* **98**, 565–581.

LAW, A. S. & WRISTON, J. C., JUN. (1971). Purification and properties of *Bacillus coagulans* L-asparaginase. *Archives of Biochemistry and Biophysics* **147**, 744–752.

MANNING, G. B. & CAMPBELL, L. L., JUN. (1957). The asparagine deamidase of *Bacillus coagulans* and *Bacillus stearothermophilus*. *Canadian Journal of Microbiology* **3**, 1001–1009.

MASHBURN, L. T. & WRISTON, J. C., JUN. (1964). Tumor inhibitory effect of L-asparaginase from *Escherichia coli*. *Archives of Biochemistry and Biophysics* **105**, 450–452.

NIKOLAEV, A. YA., EVSEEV, L. P., TYUL'PANOVA, E. S. & ABDUMALIKOV, A. KH. (1969). Isoenzymes of asparaginase in *Pseudomonas* sp. *Biokhimiya* **34**, 352–355.

OETTGEN, H. F., OLD, L. J., BOYSE, E. A., CAMPBELL, H. A., PHILLIPS, F. S., CLARKSON, B. D., TALLAL, L., LEEPER, R. D., SCHWARTZ, M. K. & KIM, J. H. (1967). Inhibition of leukemias in man by L-asparaginase. *Cancer Research* **27**, 2619–2631.

OLD, L. J., BOYSE, E. A., CAMPBELL, H. A. & DARIA, G. M. (1963). Leukemia-inhibiting properties and L-asparaginase activity of sera from certain South American rodents. *Nature, London* **198**, 801.

PETERSON, R. E. & CIEGLER, A. (1969*a*). L-Asparaginase production by *Erwinia aroideae*. *Applied Microbiology* **18**, 64–67.

PETERSON, R. E. & CIEGLER, A. (1969*b*). L-Asparaginase production by various bacteria. *Applied Microbiology* **17**, 929–930.

RAMADAN, M. E-D. A., EL-ASMAR, F. & GREENBERG, D. M. (1964). Purification and properties of glutaminase and asparaginase from a pseudomonad. I. Purification and physical chemical properties. *Archives of Biochemistry and Biophysics* **108**, 143–149.

REDDY, V. V. S., JAYARAM, H. N., SIRSI, M. & RAMAKRISHNAN, T. (1969). Inhibitory activity of L-asparaginase from *Mycobacterium tuberculosis* on Yoshida ascites sarcoma in rats. *Archives of Biochemistry and Biophysics* **132**, 262–267.

ROBERTS, J., BURSON, G. & HILL, J. M. (1968). New procedures for purification of L-asparaginase with high yield from *Escherichia coli*. *Journal of Bacteriology* **95**, 2117–2123.

ROBERTS, J., HOLCENBERG, J. S. & DOLOWY, W. C. (1970). Antineoplastic activity of highly purified bacterial glutaminases. *Nature, London* **227**, 1136–1137.

ROBERTS, J., HOLCENBERG, J. S. & DOLOWY, W. C. (1972). Isolation, crystallization, and properties of *Achromobacteraceae* glutaminase-asparaginase with antitumour activity. *Journal of Biological Chemistry* **247**, 84–90.

ROWLEY, B. & WRISTON, J. C., JUN. (1967). Partial purification and antilymphoma activity of *Serratia marcescens* L-asparaginase. *Biochemical and Biophysical Research Communications* **28**, 160–165.

SAKAMOTO, T., ARAKI, C., TAMURA, G., ARIMA, K., YAMAMOTO, T., MATSUZAWA, A. & HORI, S. (1970). On the extracellular asparaginases of microorganisms. *Proceedings of the Society of Agricultural Chemistry*, p. 159.

SCHEETZ, R. W., WHELAN, H. A. & WRISTON, J. C., JUN. (1971). Purification and properties of an L-asparaginase from *Fusarium tricinctum*. *Archives of Biochemistry and Biophysics* **142**, 184–189.

SCHMALFUSS, K. & MOTHES, K. (1930). Über die fermentative Desamidierung durch *Aspergillus niger*. *Biochemische Zeitschrift* **221**, 134–153.

SCHWARTZ, J. H., REEVES, J. Y. & BROOME, J. D. (1966). Two L-asparaginases from *E. coli* and their action against tumors. *Proceedings of the National Academy of Sciences of the United States of America* **56**, 1516–1519.

TCHAN, Y. T., ASANO, J. & KOBAYASHI, M. (1971). Study of L-asparaginase production by *Rhodopseudomonas capsulatus*. (1) A model for the induction of L-asparaginase. *Journal of Fermentation Technology* **49**, 733–739.

TOSA, T., SANO, R., YAMAMOTO, K., NAKAMURA, M., ANDO, K. & CHIBATA, I. (1971). L-Asparaginase from *Proteus vulgaris*. *Applied Microbiology* **22**, 387–392.

TSUCHIYA, T. (1967). Serological classification of yeasts. In *Kobo-gaku* (*Yeast studies*), pp. 100–135. Edited by Y. Hashitani. Tokyo: Iwanami Shoten.

TSUJI, Y. (1957). Amidase. *Naika-Hokan* **4**, 222.

WADE, H. E., ELSWORTH, R., HERBERT, D., KEPPIE, J. & SARGEANT, K. (1968). A new L-asparaginase with antitumour activity. *Lancet* **ii**, 776–777.

WADE, H. E., ROBINSON, H. K. & PHILLIPS, B. W. (1971). Asparaginase and glutaminase activities of bacteria. *Journal of General Microbiology* **69**, 299–312.

YAMADA, K. (1970). Antileukemia activity of L-asparaginase. *Igaku no Ayumi* **72**, 112–113.

YELLIN, T. O. & WRISTON, J. C., JUN. (1966*a*). Purification and properties of guinea pig serum asparaginase. *Biochemistry* **5**, 1605–1612.

YELLIN, T. O. & WRISTON, J. C., JUN. (1966*b*). Antagonism of purified asparaginase from guinea pig serum toward lymphoma. *Science, New York* **151**, 998–999.