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Transport of basic amino acids in *Candida albicans*

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In *Candida albicans*, ATCC 46977, transport of basic amino acids is mediated by two systems (S_1 and S_2). Kinetic data and competitive inhibition studies of the different systems showed that transport of L-lysine, L-arginine and L-histidine have distinct specificities. System S_1 of L-lysine and L-arginine was highly specific for the respective single basic amino acid. However, S_2 of L-lysine and S_1 of L-histidine were shown to be specific systems for most of basic amino acids. S_2 of L-arginine was different from S_2 of L-lysine and S_1 of L-histidine. The effect of a thiol reagent, *N*-ethylmaleimide, revealed that S_2 of L-lysine and S_1 of L-histidine were sensitive to this reagent, while all other systems were insensitive. The transport activity of different systems of L-lysine, L-arginine and L-histidine was followed during the growth of *C. albicans*. It was observed that different basic amino-acid systems have maximum activity during different stages of *C. albicans* growth.

Introduction

The practically unidirectional transport of amino acids in baker's yeast proceeds against considerable concentration gradients. At least ten specific amino-acid transport systems with different specificities have been identified in *Saccharomyces cerevisiae* [1–7]. Generally, *S. cerevisiae* possesses two types of transport system for amino acids: one is specific for only one or a family of structurally related amino acids [1–4,8,9], and a general amino-acid permease is shared by a large number of amino acids [10,11]. The former type is not sensitive to ammonia, while the latter elicits repression or derepression in presence or absence of ammonia [10]. However, there are some specific amino-acid transport systems characterized in *S. cerevisiae* which are not mediated by general amino-acid permease, but demonstrate ammonia sensitivity [6,9,12].

Grenson and her group have characterized specific systems for basic amino acids, i.e., L-lysine, L-arginine and L-histidine, in *S. cerevisiae* [1,2,4]. For example, for L-lysine, three systems were identified: a highly specific lysine system [1], a system common to all basic amino acids [2] and a general amino-acid system which is shared by most of the neutral and the basic amino acids [10]. There is, however, no report available to demonstrate whether basic amino acids are transported by a single affinity system or by different affinity systems in *Candida albicans* cells.

As compared to *S. cerevisiae*, the transport systems of *C. albicans*, a strictly aerobic yeast, are less well characterized. *C. albicans* exhibits dimorphism depending upon its growth conditions and germ-tube phase is probably more pathogenic as compared to the yeast phase [13–16]. Among several factors responsible for its morphological transition, certain amino acids also help in in-

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ducing germ tubes in *C. albicans* cells [17–19]. We have been characterizing various amino-acid permeases of *C. albicans* cells to predict their involvement in regulation of growth and differentiation [20–24].

Our results have revealed that most of the amino acids are transported via an active mechanism and accumulate against a concentration gradient [20–22]. A specific and inducible proline transport is also characterized in *C. albicans* cells [23]. In contrast to *S. cerevisiae* cells, the transport of amino acids in *C. albicans* is insensitive to ammonia [24]. In the present study we have characterized the transport systems for L-lysine, L-arginine and L-histidine, and have measured their kinetic constants and specificities. Furthermore, variation in uptake during the course of growth, and its significance, are discussed.

Materials and Methods

Strain and culture conditions. C. albicans, ATCC 46977, was obtained from National Chemical Laboratory, Poona, India. Cells were grown in a synthetic growth media containing 0.5% glucose, 0.3% KH_2PO_4, 0.3% $(NH_4)_2SO_4$, 0.025% $CaCl_2$, 0.025% $MgSO_4$ and 0.001% biotin at 30°C as described earlier [20–24]. For uptake measurements, cells of mid-exponential phase were used.

Measurement of amino acid uptake. Transport assay methods were similar to those described earlier [20–24]. A reaction mixture containing *C. albicans* cells (200–250 μg protein/ml) in 50 mM citrate buffer (pH 5.0) was incubated at 30°C for 10 min with cycloheximide (200 μg/ml) to inhibit protein synthesis. The reaction was initiated by the addition of labelled amino acid. Final concentrations of amino acid for their uptake systems were 2–4-times more than their respective K_t values. At indicated time intervals, aliquots were removed with an Eppendorf pipette and immediately diluted in 5 ml chilled distilled water. The diluted suspension was rapidly filtered through 0.45 μm Maxflow filter discs (Maxflow, Bombay, India) and the radioactivity retained was counted in a LKB-Wallac (1217) Rack Beta liquid scintillation counter using a toluene-based scintillation fluid.

Protein measurement. Protein was determined according to Bradford [25], using bovine serum albumin as standard.

Chemicals. L-Amino acids, D-amino acids, cycloheximide, *N*-ethylmaleimide and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. L-[U-^{14}C]Lysine and L-[U-^{3}H]arginine were obtained from Bhabha Atomic Research Centre, Bombay, India. L[U-^{14}C]Histidine was procured from Amersham International, Amersham, U.K. All other chemicals were obtained from commercial sources and were of the highest purity.

Results

Kinetic studies of basic amino-acid transport

To test the transport activities for basic amino acids, i.e., L-lysine, L-arginine and L-histidine in *C. albicans,* ATCC 46977, various external concentrations of different amino acids ranging from 1 μM to 100 mM were used. The uptake kinetics for these three basic amino acids show a deviation from simple Michaelis-Menten kinetics. Biphasic curves of the Lineweaver-Burk plot (Fig. 1) and concave upward curves of the Hofstee plot (not shown) revealed the existence of two kinetic sys-

TABLE I

APPARENT K_t AND J_{max} VALUES OF HIGH- AND LOW-AFFINITY SYSTEMS FOR BASIC AMINO ACIDS

K_t and J_{max} values were calculated from the Lineweaver-Burk plots shown in Fig. 1.

Basic amino acid	High-affinity system (S_1)		Low-affinity system (S_2)	
	$K_{t,1}$ ($1 \cdot 10^{-6}$ M)	$J_{max,1}$ (nmol/mg protein per s)	$K_{t,2}$ ($1 \cdot 10^{-6}$ M)	$J_{max,2}$ (nmol/mg protein per s)
L-Lysine	2.01 ± 0.10	0.035 ± 0.001	14.3 ± 0.60	0.94 ± 0.05
L-Arginine	2.5 ± 0.12	0.034 ± 0.005	200 ± 7	1.8 ± 0.10
L-Histidine	13.3 ± 0.60	0.600 ± 0.063	500 ± 10	4.1 ± 0.12

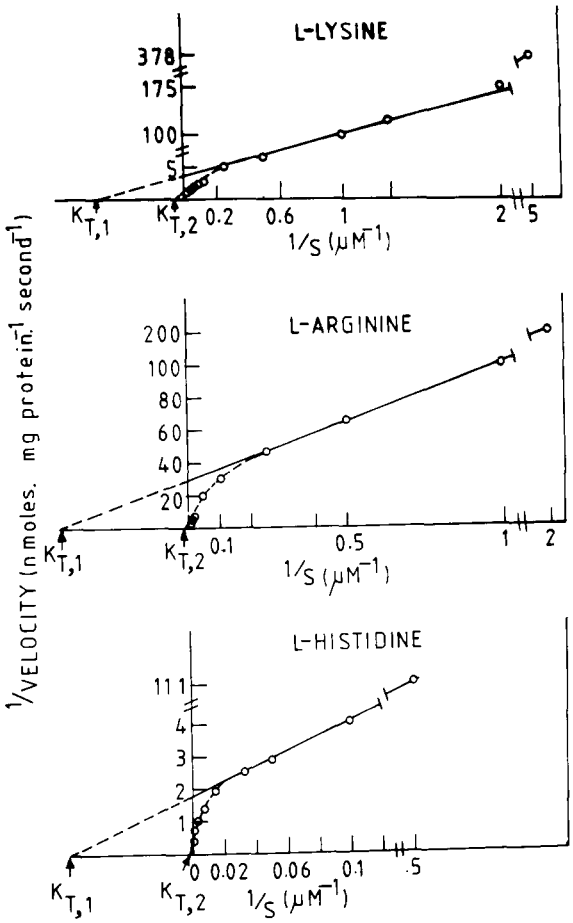

Fig. 1. Lineweaver-Burk plots of basic amino acid uptake. Kinetic studies were carried out in 0.5 ml reaction mixture containing cells (100 μg protein) and 50 mM citrate buffer (pH 5.0) with various external concentrations of amino acids (range 1 μM to 100 mM). A 0.1 ml aliquot was taken out every 30 s for 2 min. Velocities of accumulation were determined from the linear plots of the time-course of accumulation. The reciprocal of the velocity is shown as a function of the reciprocal of the amino-acid concentrations. All Lineweaver-Burk plots are shown at lower concentration range. Since the reciprocals of velocities of accumulation at higher concentrations are also linear, with those at lower concentration, the accumulation at higher concentrations is not shown in the graph.

tems of all the three basic amino acids. One system (S_1) has high affinity for a particular amino acid, with a low half-saturation constant ($K_{t,1}$) and low maximum velocity ($J_{max,1}$). The other system (S_2) has rather low affinity, with high $K_{t,2}$ and $J_{max,2}$. These two uptake systems of each basic amino acid were clearly detectable at low concentration ranges. The values of $K_{t,1}$, $K_{t,2}$, $J_{max,1}$ and $J_{max,2}$ for L-lysine, L-arginine and L-histidine are listed in Table I.

The $K_{t,1}$ values of L-lysine ($2 \cdot 10^{-6}$ M) and L-arginine ($2.5 \cdot 10^{-6}$ M) were quite similar; however, these two high-affinity systems were different in their specificities and characteristics. There was, on the other hand, no such system with same K_t value for L-histidine transport. The K_t values for S_1 and S_2 of L-histidine were $13.3 \cdot 10^{-6}$ M and $500 \cdot 10^{-6}$ M, respectively. It was interesting that the K_t value of S_1 of L-histidine was close to that of S_2 of L-lysine ($14.3 \cdot 10^{-6}$ M). Further results obtained from both systems would indicate that the high-affinity system of L-histidine (S_1) and the

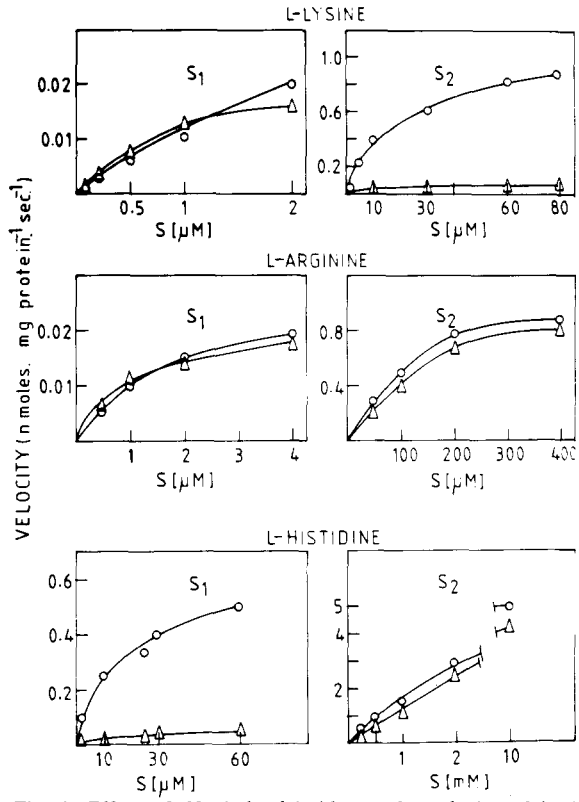

Fig. 2. Effect of *N*-ethylmaleimide on the velocity of basic amino acid uptake at various external concentrations. Cells were preincubated with *N*-ethylmaleimide (500 μM) for 10 min and labelled basic amino acid was added. Aliquots were removed every 30 s up to 2 min. The velocity (nmol/mg protein per s) of each basic amino acid uptake at various external concentrations near to its K_t, was measured (△). Simultaneously, velocity was also determined in the absence of *N*-ethylmaleimide (○).

TABLE II

SPECIFICITY OF BASIC AMINO ACID UPTAKE SYSTEMS

Cells were incubated with and without 100-fold concentration of inhibitor indicated in the table, along with labelled basic amino acid. After 2 min the whole mixture was filtered and radioactivity was counted. The values represent an average of three or four separate experiments. The variation of the inidividual values was not more than 10%.

Inhibitors	Percentage of inhibition					
	L-Lysine		L-Arginine		L-Histidine	
	S_1 (1 [a])	S_2 (20 [a])	S_1 (1 [a])	S_2 (3 [a])	S_1 (3 [a])	S_2 (100 [a])
L-Lysine	90	95	16	57	95	56
L-Arginine	18	96	87	84	93	67
L-Histidine	15	79	15	9	92	69
L-Ornithine	20	94	33	56	91	46
L-Citrulline	7	65	0	46	76	40
D-Arginine	19	85	15	25	85	62
D-Lysine	22	81	12	35	90	39
D-Histidine	12	75	17	30	91	63
L-Valine	10	30	14	25	31	43
L-Glutamine	12	28	12	18	30	52
L-Methionine	10	19	17	16	18	56
L-Serine	14	20	16	19	35	49
L-Threonine	–	25	12	25	31	44
L-Tryptophane	16	22	13	18	20	55
L-Phenylalanine	8	35	0	12	16	33
L-Proline	9	15	0	18	16	42

[a] Inhibitor concentration in mM.

low-affinity system of L-lysine (S_2) are probably a part of one common basic amino-acid transport system.

Specificity of the high- and low-affinity systems

In order to investigate the characteristics of the different uptake systems for L-lysine, L-arginine

TABLE III

EFFECT OF *N*-ETHYLMALEIMIDE (NEM) ON VELOCITY, K_t and J_{max} OF BASIC AMINO-ACID UPTAKE SYSTEMS OF *C. ALBICANS*

The K_t and J_{max} values of the various basic amino acid uptake systems were calculated from the velocity values taken from Fig. 2. All values are an average of four separate experiments.

Basic amino acid	High-affinity system (S_1)			Low-affinity system (S_2)		
	Velocity (nmol/mg protein per s)	$K_{t,1}$ (μM)	$J_{max,1}$ (nmol mg protein per s)	Velocity (nmol/mg protein per s)	$K_{t,2}$ (μM)	$J_{max,2}$ (nmol/mg protein per s)
L-Lysine						
– NEM	0.02	2.01	0.35	0.6	14.3	0.94
+ NEM	0.017	2.01	0.34	0.003	1.45	0.03
L-Arginine						
– NEM	0.015	2.5	0.034	1.0	200	1.8
+ NEM	0.015	2.7	0.032	1.28	215	1.5
L-Histidine						
– NEM	0.4	13.3	0.6	1.5	500	4.1
+ NEM	0.03	1.33	0.065	1.4	520	4.0

and L-histidine, their specificities were ascertained. The uptake of each system was measured in presence of a high concentration of unlabelled amino acids (100-fold). Results listed in Table II show that the S_1 systems of L-lysine and L-arginine are very specific, because their uptakes were not significantly inhibited, either by basic amino acids or by other amino acids. However, the S_2 systems of L-lysine and L-arginine were inhibited by other basic and structurally related amino acids. In contrast to L-lysine and L-arginine, uptake of L-histidine through its high-affinity system (S_1) was inhibited between 90 and 95% by basic amino acids and other structurally related amino acids. Furthermore, high concentrations of amino acids other than basic ones had no effect on either system of the basic amino acids. However, the S_2 system of L-histidine is an exception, since other amino acids could also inhibit its uptake between 40 and 60%. Therefore, S_1 of L-histidine and S_2 of L-lysine and of L-arginine are specific for basic amino acids. The S_2 system of L-arginine needs special mention, since L-arginine uptake is inhibited by L-lysine and L-ornithine, but L-histidine has no effect. The D-isomers of basic amino acids also had no significant effect on L-arginine uptake, while they were effective inhibitors for S_2 of L-lysine and S_1 of L-histidine.

Effect of N-ethylmaleimide on basic amino acid transport systems

The effect of the thiol reagent, *N*-ethylmaleimide on the velocity of different transport systems corroborates above results. *N*-Ethylmaleimide effectively inhibited the velocity of the S_2 system of L-lysine. This sensitivity to *N*-ethylmaleimide was observed from 6 to 100 μM external L-lysine concentration (Fig. 2). Results obtained by above experiment have shown that $K_{t,2}$ and $J_{max,2}$ of S_2 of L-lysine decreased in presence of *N*-ethylmaleimide (Table III). However, S_1 of L-lysine was insensitive to *N*-ethylmaleimide. This insensitivity could also be seen at very low external concentrations of L-lysine, from 0.2 to 2 μM (Fig. 2). Similar to S_2 of L-lysine, the velocity of S_1 of L-histidine was also inhibited. In presence of *N*-ethylmaleimide, $K_{t,1}$ and $J_{max,1}$ of L-histidine were decreased (Table III). The S_2 system of L-histidine as well as the two systems of L-arginine were insensitive to *N*-ethylmaleimide (Fig. 2).

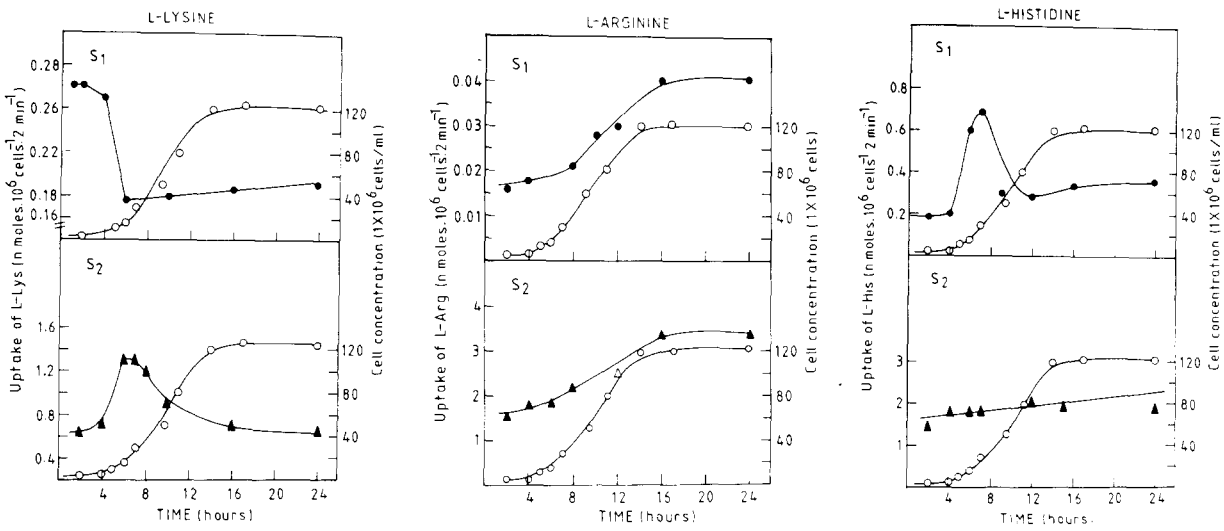

Fig. 3. Transport activity of basic amino-acid uptake systems during different growth stages. Cells were grown as described in Materials and Methods. The assay conditions for uptake studies were same as described earlier. Cell growth expressed in cell number/ml, was estimated by direct microscopic counting with a hemocytometer. ●, Uptake of amino acid by S_1 system; ▲, uptake of amino acid by S_2 system; ○, cell growth.

Influence of growth stage on transport of basic amino acids

It is known that solute uptake undergoes significant variation during the course of growth. Study of growth-dependent variation has been of interest in detecting cryptic activities. Robinson and coworkers [26] reported a transient increase of the acidic amino-acid permease during germination of *Aspergillus nidulans*. Jayakumar [27] has observed a growth-dependent variation of proline uptake in *C. albicans* cells.

To ascertain how different uptake systems of basic amino acids respond to different growth conditions, we have measured the transport activity of different systems during growth stages of *C. albicans*. Maximum activity of different systems of basic amino acids was observed at different stages. The transport activity of S_1 of L-lysine was high in the lag phase, whereas for the S_2 system it was high in the exponential phase. Maximum transport activity of S_1 of L-histidine was observed in exponentially growing cells, while the activity did not change significantly throughout the growth for the S_2 system. The maximum activity of the L-arginine transport by both systems was observed after exponential stage (Fig. 3).

Discussion

Based on kinetic data, competitive inhibition, and sensitivity to thiol reagent (*N*-ethylmaleimide), it appears that there are very specific transport S_1 systems for L-lysine and L-arginine. In addition, both amino acids have S_2 systems, which are rather less specific, with high K_t and J_{max} values. However, systems S_2 of L-lysine and L-arginine are different from each other (Table III, Fig. 2). In contrast to L-arginine and L-lysine, the L-histidine uptake is not mediated via a very specific permease. S_1 of L-histidine is competitively inhibited by other basic amino acids, and hence does not appear to be as specific as S_1 of L-arginine and L-lysine. S_2 of L-histidine is of the nonspecific type, since most of the other amino acids are able to inhibit its uptake.

The existence of a general amino-acid permease and its sensitivity to ammonia is not demonstrable in *C. albicans* cells [24]. However, the competitive inhibition by both D and L stereoisomers of S_2 of L-lysine, and S_1 and S_2 of L-histidine suggests that their permeases, too, can mediate the uptake of both stereoisomers. General amino-acid permease of *S. cerevisiae* is sensitive to ammonia, which mediates the uptake of both D and L stereoisomers [10,11]. Based on competition by the D and L stereoisomers of the amino acids, the basic permeases of *C. albicans* resemble general amino-acid permease characteristics, but in the absence of its sensitivity towards ammonia, the possibility of general amino-acid-type permease does not exist [24].

The basic amino acid systems specific for L-arginine, L-lysine and L-histidine have been characterized in *S. cerevisiae* cells by Grenson and her group [1,2,4]. Specific permeases for basic amino acids are also known to exist in other fungi [28,29]. However, this report constitutes the first detailed study of basic amino-acid transport systems in *C. albicans* cells. The multiplicity of amino-acid transport is a well-known characteristic of *S. cerevisiae*, which is also evident in this pathogenic yeast. What is the exact role of these multiple systems remains to be elucidated. The different responses of basic amino-acid transport to various growth stages of *C. albicans* cells do suggest that the multiple systems behave differently at the different growth stages.

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References

1 Grenson, M. (1966) Biochim. Biophys. Acta 127, 339–346
2 Grenson, M., Moussett, M., Wiame, J.M. and Bechet, J. (1966) Biochim. Biophys. Acta 127, 325–338
3 Gits, J.J. and Grenson, M. (1967) Biochim. Biophys. Acta 135, 507–516
4 Crabeel, M. and Grenson, M. (1970) Eur. J. Biochem. 14, 197–204
5 Lasko, P.F. and Brandriss, M.C. (1981) J. Bacteriol. 148, 241–247

6 Verma, R.S., Rao, T.V.G. and Prasad, R. (1984) Biochim. Biophys. Acta 778, 289–297
7 Sato, T., Ohsumi, Y. and Anraku, Y. (1984) J. Biol. Chem. 259, 11505–11508
8 Ramos, E.H., De Bongioanni, L.C. and Stoppani, A.O.M. (1980) Biochim. Biophys. Acta 599, 214–231
9 Darte, C. and Grenson, M. (1975) Biochem. Biophys. Res. Commun. 67, 1028–1033
10 Grenson, M., Hou, C. and Crabeel, M. (1970) J. Bacteriol. 103, 770–777
11 Rytka, J. (1975) J. Bacteriol. 121, 562–570
12 Woodward, J.R. and Cirillo, V.P. (1977) J. Bacteriol. 130, 714–723
13 Shepherd, M.G. and Sullivan, P.A. (1976) J. Gen. Microbiol. 93, 361–370
14 Shepherd, M.G., Chiew, Y.Y., Ram, S.P. and Sullivan, P.A. (1980) Can. J. Microbiol. 26, 21–26
15 Manning, M. and Mitchell, T.G. (1980) J. Bacteriol. 144, 258–273
16 Mattia ,E. and Cassone, A. (1979) J. Gen. Microbiol. 113, 439–442
17 Lee, K.L., Buckely, H.R. and Campbell, C.C. (1975) Sabouraudia 13, 148–158
18 Dabrowa, N. and Howard, D.H. (1981) J. Gen. Microbiol. 127, 391–397
19 Dabrowa, N., Taxer, S.S.S. and Howard, D.H. (1976) Infect. Immun. 13, 830–835
20 Jayakumar, A., Singh, M. and Prasad, R. (1978) Biochim. Biophys. Acta 514, 348–355
21 Singh, M., Jayakumar, A. and Prasad, R. (1978) Arch. Biochem. Biophys. 191, 680–686
22 Verma, R.S. and Prasad, R. (1983) Ind. J. Biochem. Biophys. 20, 104–109
23 jayakumar, A., Singh, M. and Prasad, R. (1979) Biochim. Biophys. Acta 556, 144–150
24 Verma, R.S. and Prasad, R. (1983) Biochem. Int. 7(6), 707–717
25 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
26 Robinson, J.H., Anthony, C. and Drabble, W.T. (1973) J. Gen. Microbiol. 79, 65–85
27 Jayakumar, A. (1979) Ph.D. thesis, School of Life Sciences, Jawaharlal Nehru University, New Delhi
28 Piotrowsks, M., Stepien, P.P., Bartnik, E. and Zakrzewska, E. (1976) J. Gen. Microbiol. 92, 89–96
29 Hunter, D.R. and Segel, I.H. (1971) Arch. Biochem. Biophys. 144, 168–183