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The specific transport system for lysine is fully inhibited by ammonium in *Penicillium chrysogenum*: An ammonium-insensitive system allows uptake in carbon-starved cells

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Abstract

The regulation exerted by ammonium and other nitrogen sources on amino acid utilization was studied in swollen spores of *Penicillium chrysogenum*. Ammonium prevented the L-lysine, L-arginine and L-ornithine utilization by *P. chrysogenum* swollen spores seeded in complete media, but not in carbon-deficient media. Transport of L-[^{14}C]lysine into spores incubated in presence of carbon and nitrogen sources was fully inhibited by ammonium ions (35 mM). However, in carbon-derepressed conditions (growth in absence of sugars, with amino acids as the sole carbon source) L-[^{14}C]lysine transport was only partially inhibited. Competition experiments showed that L-lysine (1 mM) inhibits the utilization of L-arginine, and vice versa, L-arginine inhibits the L-lysine uptake. High concentrations of L-ornithine (100 mM) prevented the L-lysine and L-arginine utilization in *P. chrysogenum* swollen spores. In summary, ammonium seems to prevent the utilization of basic amino acids in *P. chrysogenum* spores by inhibiting the transport of these amino acids through their specific transport system(s), but not through the general amino acid transport system that is operative under carbon-derepression conditions.

Introduction

The lysine biosynthetic pathway in *Penicillium chrysogenum* provides α-aminoadipate for the synthesis of δ(L-α-aminoadipyl)-L-cysteinyl-D-valine, the first intermediate of the penicillin biosynthetic pathway (Díez et al. 1990; Martín 1998; Martín et al. 1997). It is known that the intracellular concentration of α-aminoadipate limits the biosynthesis of penicillin in *P. chrysogenum*, since the increment in the intracellular pool of α-aminoadipate results in a higher rate of penicillin production (Casqueiro et al. 1998, 1999; Hönlinger & Kubicek 1989; Lu et al. 1992). Reduction of the flux of intermediates through the lysine biosynthetic pathway, via feedback inhibition/repression of the homocitrate synthase (first enzyme of the lysine biosynthetic pathway), decreases the penicillin production (Jaklitsch & Kubicek 1990; Luengo et al. 1980). High concentrations of extracellular lysine are required either to inhibit (Luengo et al. 1980) or to repress the homocitrate synthase (Bañuelos et al. 1999) apparently due to a low efficiency of the lysine transport systems, although this inefficient uptake may be due to interference by other nitrogen sources. In addition, lysine is catabolized to α-aminoadipic acid in *P. chrysogenum*, although the contribution of this catabolic pathway to penicillin production is yet unclear (Esmahan et al. 1994). It was, therefore, of great interest to study lysine transport and its regulation in *P. chrysogenum*.

P. chrysogenum appears to have nine different amino acid transport systems, namely: system I for L-methionine (Benko et al. 1967); II for L-cystine (Skye & Segel 1970); III for neutral and basic amino acids (Benko et al. 1969); IV for acidic amino acids; V for L-proline; VI for L-lysine and L-arginine; VII for L-arginine; VIII for L-lysine and IX for L-cysteine (Hunter & Segel 1971). Three of the systems, namely III, VI and VIII may be involved in lysine transport.

Amino acid transport systems I and II are expressed under sulfur starvation and systems III, IV and V are derepressed under nitrogen and carbon starvation. All other transport systems (VI, VII, VIII and IX) are expressed constitutively, and do not seem to be affected by starvation (Horák 1986). Transport systems are also regulated by feedback inhibition (so-called transinhibition), metabolic inhibitors and competence between substrates (Hillenga et al. 1996; Hunter & Segel 1973a,b).

The nitrogen source is one of the most important regulators of the amino acid transport in fungi. One of the preferred nitrogen sources, ammonium, exerts repression of the general amino acid transport system (system III) in *P. chrysogenum* (Horák 1986; Hunter & Segel 1971, 1973a) and in *Saccharomyces cerevisiae* (Grenson et al. 1970).

The swollen spores system constitutes an excellent homogeneous system to study transport in fungi (Martín et al. 1974). Most studies about amino acid transport in *P. chrysogenum* have been performed with mycelium, but there is a scarce knowledge about amino acid transport in other development stages. Auxotrophic mutants have been reported to be an excellent tool to study amino acid transport (Greasham & Moat 1973); uptake of amino acids by auxotrophs and its regulation can be studied easily by testing the growth in minimal medium supplemented with the corresponding amino acid and the effector molecules. In this work we describe the use of auxotrophic mutants as an easy method to study the regulation of transport of L-lysine and other basic amino acids by ammonium ions in the spore stage of *P. chrysogenum* Wisconsin 54-1255.

Materials and methods

Strains

P. chrysogenum Wisconsin 54-1255 is a low penicillin production strain containing a single copy of the penicillin gene cluster (Fierro et al. 1995). Its auxotrophic derivatives 1255-LYS (for lysine), 1255-ARG (for arginine), 1255-MET (for methionine), 1255-MC (for methionine or cysteine) and 1255-PO (for proline or ornithine) were isolated after nitrosoguanidine treatment (see Results, Table 1).

Media and growth conditions

All the strains were grown on Power medium (a 1:1 mixture of PM1 (Lopez-Nieto et al. 1985) and modified Czapek medium (sucrose 30 g/l, $NaNO_3$ 3 g/l, K_2HPO_4 0.5 g/l, $MgSO_4$ 0.5 g/l, $FeSO_4$ 0.01 g/l, 0.7 M KCl and agar 2.5 g/l, pH 6.5), supplemented with the appropriate filter-sterilized amino acid(s) at a final concentration of 1.75 mM. Spores were collected from Power medium plates after incubation during 4 days at 28°C. Growth tests were performed on (i) Czapek minimal medium without nitrogen source (MM) (containing sucrose 30 g/l, K_2HPO_4 0.5 g/l, $MgSO_4$ 0.5 g/l, $FeSO_4$ 0.01 g/l, agar 2 g/l, pH 7.5), (ii) Czapek minimal medium with 35 mM sodium nitrate as nitrogen source (MMNitrate) and (iii) Czapek minimal medium with 35 mM ammonium chloride as nitrogen source (MMAmmonium). Media were supplemented with the appropriate amino acids at a final concentration of 1.75 mM. Competition/inhibition tests with *P. chrysogenum* 1255-LYS, 1255-ARG and 1255-PO auxotrophic mutants were done on MMNitrate supplemented with L-lysine, L-arginine or L-ornithine at a final concentration of 0.1 mM.

Auxotroph mutant isolation

P. chrysogenum Wisconsin 54-1255 spores (10^6/ml) were incubated in 0.1 M Tris–maleate buffer, pH 9.0, for 12–15 h. To induce germination which correlated with DNA synthesis (Martín et al. 1974), glucose and $(NH_4)_2SO_4$ were added at a final concentration of 15 and 1 g/l, respectively. The mutation was carried out during 90 min with 0.5 mM nitrosoguanidine in the same buffer, pH 9.0, to obtain a mortality rate of 90%.

Serial dilutions of mutated spores were plated on Power medium plus 10% peptone. Auxotrophic mutants were screened by replicating each colony on MMNitrate and Power medium plus peptone. The auxotrophic clones were characterized by the Holliday test (Holliday 1956). Reversion rates of the mutations were determined for all the auxotrophic mutants by plating spore suspensions (with known viable spore concentrations) on MMNitrate, and counting the revertant prototrophic colonies.

Lysine transport assay

Spores of *P chrysogenum* 1255-LYS mutant were incubated for 12–15 h in 100 ml of 0.025 M phosphate buffer, pH 7.0, supplemented with glucose and $NaNO_3$ (or NH_4Cl) at a final concentration of 166 and 35 mM,

Table 1. Amino acid auxotrophic mutants of *P. chrysogenum* Wisconsin 54-1255

Mutant no.	Auxotrophy	Reversion rate (%)	Mutant designation
1347	Lysine	$<2.6\times10^{-7}$	
8322	Lysine	$<5.34\times10^{-8}$	
8821	**Lysine**	$<\mathbf{3.89\times10^{-8}}$	**1255-LYS**
9749	Lysine	$<4.31\times10^{-8}$	
6266	Arginine	$<9.12\times10^{-8}$	
8245	**Arginine**	$<\mathbf{4.15\times10^{-8}}$	**1255-ARG**
12654	Arginine	$<7.96\times10^{-8}$	
13565	Arginine	$<2.27\times10^{-7}$	
2480	**Proline or ornithine**	$<\mathbf{3.77\times10^{-8}}$	**1255-PO**
2951	Methionine or cysteine	2.35×10^{-8}	
8427	**Methionine or cysteine**	$<\mathbf{2.42\times10^{-8}}$	**1255-MC**
1128	Methionine	2.42×10^{-6}	
12 915	**Methionine**	$<\mathbf{3.8\times10^{-8}}$	**1255-MET**
5740	Methionine	2.0×10^{-7}	
7472	Methionine	3.0×10^{-7}	

Mutants selected for uptake studies are marked in bold.

respectively. The swollen spores (10^9) were harvested by centrifugation, washed twice with 0.025 M phosphate buffer, pH 7.0, and resuspended in 10 ml of 0.025 M phosphate buffer, pH 7.0, supplemented with glucose and $NaNO_3$ (or NH_4Cl) at a final concentration of 166 and 35 mM, respectively, and incubated during 1 h.

L-[^{14}C]lysine solution for transport assays was prepared by adding L-[^{14}C]lysine (uniformly labeled, 300 μCi/μmol, The Radiochemical Center, Amersham, UK) to a solution of 1 mM carrier L-lysine in 0.025 M phosphate buffer, pH 7.0, to give an activity of 10^6 cpm/μmol. After addition of 1 ml of L-[^{14}C]lysine solution to the spore suspension, samples were taken at 2-min intervals and the transport of L-[^{14}C]lysine was chased by dilution with 9 ml of 0.1 M L-lysine in 0.025 M phosphate buffer, pH 7.0. The samples were rapidly filtered through Whatman GF/C (2.5 cm) membranes on a multifilter apparatus (Millipore Corp., Bedford, MA) and washed with 50 ml of 0.025 M phosphate buffer, pH 7.0. The filters were dried and the radioactivity was determined in a liquid scintillation counter (Beckman, LS6000TA).

Results

Selection of lysine, arginine, proline/ornithine, methionine and cysteine/methionine auxotrophic mutants

After nitrosoguanidine treatment of *P. chrysogenum* Wisconsin 54-1255 spores, 26 auxotrophic mutants were isolated from 14 000 viable spores tested, which represent a frequency of about 0.2% auxotrophic mutants. The nutrient requirement of 15 of the mutants was established by the Holliday tests (Table 1).

In order to study the transport of lysine, arginine, proline, ornithine, methionine and cysteine in *P. chrysogenum* spores, five mutants were selected for transport studies: namely namely 1255-LYS, 1255-ARG, 1255-PO, 1255-MC and 1255-MET which were shown to be lysine, arginine, proline/ornithine, methionine/cysteine, and methionine auxotrophs, respectively.

The selected auxotrophic mutants allowed us to investigate the activity of the general amino acid transport system (system III) and six of the eight specific amino acid transport systems described in *P. chrysogenum*. Mutants 1255-ARG, 1255-LYS and 1255-PO were used to investigate the uptake of arginine, lysine and ornithine; the three substrates of the specific basic amino acid transport system VI. Mutant 1255-PO was able to grow also with proline, known to be transported through the system V. Mutants 1255-MET and 1255-MC were used to study the uptake of methionine by the system I and cysteine by the system IX.

Nitrogen source regulation of the basic amino acid uptake

To study the regulation of the amino acid transport systems by the nitrogen source in *P. chrysogenum* spores, the ability of the auxotrophic mutants spores

Table 2. Effect of the nitrogen source on growth of *P. chrysogenum* Wisconsin 54-1255 and the auxotrophic mutants

Strain	Single amino acid added	GROWTH ON				
		MM Nitrate	MM Ammonium	MM Ammonium $NaNO_3$	MM Adenine[a]	MM +required[a] amino acid
Wis 541255		Yes	Yes	Yes	Yes	Yes
1255-LYS	L-Lysine	Yes	**No**	**No**	Yes	Yes
1255-ARG	L-Arginine	Yes	**No**	**No**	Yes	Yes
1255-PO	L-Ornithine	Yes	**No**	n.d.	Yes	Yes
1255-PO	L-Proline	Yes	Yes	n.d.	Yes	Yes
1255-MC	L-Methionine	Yes	Yes	n.d.	Yes	Yes
1255-MC	L-Cysteine	Yes	Yes	n.d.	Yes	Yes
1255-MET	L-Methionine	Yes	Yes	n.d.	Yes	Yes

Mutants were seeded on MMNitrate (35 mM), MMAmmonium (35 mM) or MM (without nitrogen source) supplemented with the appropiate amino acid at a final concentration of 1.75 mM. [a] Adenine or the appropiate amino acid (in the last column) were added at 20 mM. Note that ammonium inhibits uptake of arginine, lysine and ornithine (bold). n.d., not determined.

to grow on MM with different nitrogen sources was tested (Table 2). *P. chrysogenum* Wis 54-1255 and all the auxotrophic mutants selected (Table 1) were able to grow on MMNitrate or MMAdenine (with their required amino acids at 1.75 mM) as well as on MM supplemented with their required amino acids (20 mM) as sole nitrogen sources. However, mutants 1255-LYS and 1255-ARG were unable to grow on MMAmmonium (with NH_4Cl or $(NH_4)_2SO_4$ as nitrogen source) supplemented with L-lysine or L-arginine at 1.75 mM, respectively (Figure 1A). Similarly, mutant 1255-PO grew on MMAmmonium supplemented with L-proline but was unable to grow on MMAmmonium supplemented with L-ornithine (Figure 1B). To establish the minimal ammonium concentration that prevented growth of the auxotrophic mutants, 1255-LYS, 1255-ARG and 1255-PO were plated on MM (without nitrogen source) supplemented with L-lysine, L-arginine or L-ornithine at 0.1 mM, and with ammonium chloride ranging from 10 nM to 10 mM. Mutants 1255-LYS, 1255-ARG and 1255-PO grew in presence of low ammonium concentration (10 nM to 0.1 mM) but they were unable to grow on 10 mM and higher concentrations of ammonium chloride.

The failure of the basic amino acid auxotrophs to grow on NH_4Cl or $(NH_4)_2SO_4$ as nitrogen source could be due to an inhibition of ammonium transport exerted by the amino acids, or vice versa, to an inhibition of amino acid transport exerted by ammonium (Hunter & Segel 1973b). To elucidate this point, the ability of the parental strain Wisconsin 54-1255 and the mutants 1255-LYS and 1255-ARG to grow on MMAmmonium with 35 mM $NaNO_3$ as additional nitrogen source (plus L-lysine or L-arginine at 1.75 mM, respectively) was tested (Table 2). Both 1255-LYS and 1255-ARG mutants, but not the parental strain, failed to grow in these conditions, in spite of the alternative nitrogen source ($NaNO_3$) present in the medium (Figure 1A), indicating that the failure to grow is not due to the lack of a nitrogen source, but to the inability to take up the basic amino acids in presence of high ammonium concentrations.

Ammonium inhibits L-*[^{14}C]lysine transport in* P. chrysogenum *swollen spores*

To confirm that inhibition of the basic amino acid utilization was due to inhibition of amino acid uptake, L-[^{14}C]lysine transport was measured in mutant 1255-LYS. Swollen spores incubated in phosphate buffer supplemented with glucose (control), at a final concentration of 166 mM, and with glucose and $NaNO_3$, at a final concentration of 166 and 35 mM, respectively, showed a linear rate of L-[^{14}C]lysine transport during at least 16 min. The highest rate of L-[^{14}C]lysine transport was observed in spores incubated only in presence of glucose (without nitrogen source). On the contrary, 1255-LYS mutant spores incubated in the same conditions but with 166 mM glucose and 35 mM NH_4Cl, showed almost no L-[^{14}C]lysine transport (89% inhibition respect to transport observed in presence of glucose and $NaNO_3$ and 98% inhibition respect to the transport observed in presence of only glucose) (Figure 2).

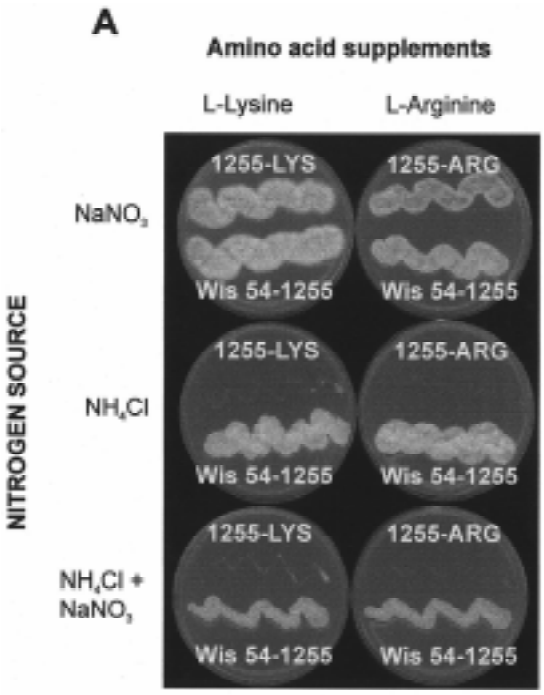

Figure 1. Effect of the nitrogen source on uptake of L-lysine, L-arginine and L-ornithine in *P. chrysogenum* spores. Amino acid uptake was evidenced by the growth ability of auxotrophic mutants on MM with the corresponding amino acids supplemented with different nitrogen sources (35 mM). (A) 1255-LYS and 1255-ARG auxotrophic mutants were plated on MMNitrate, MMAmmonium and MMAmmonium plus $NaNO_3$ supplemented with 1.75 mM L-lysine or L-arginine, respectively. (B) 1255-PO auxotrophic mutant was plated on MMNitrate and MMAmmonium supplemented with L-ornithine or L-proline at 1.75 mM.

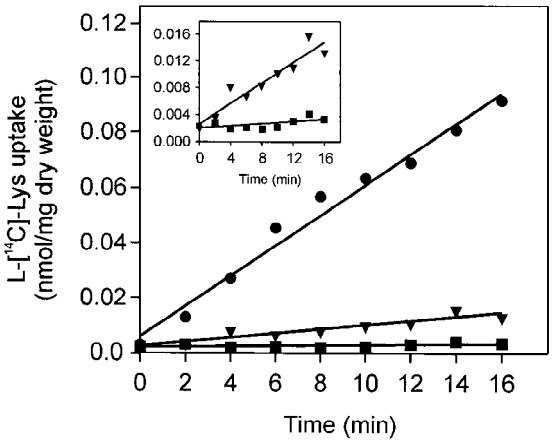

Figure 2. Effect of ammonium on L-[^{14}C]lysine transport in 1255-LYS swollen spores. Assays were performed with 10^9 spores suspended in 10 ml of 0.025 M phosphate buffer, pH 7.0, supplemented with 166 mM glucose (●), with 166 mM glucose and 35 mM $NaNO_3$ (▼) or with 166 mM glucose and 35 mM NH_4Cl (■). Inset: enlarged scale of L-[^{14}C]lysine transport in presence of 166 mM glucose and 35 mM $NaNO_3$ (▼) or 166 mM glucose and 35 mM NH_4Cl (■). L-[^{14}C]Lysine solution (10^6 cpm/μmol) was added to give a final concentration of 0.1 mM.

Effect of ammonium on L-*lysine,* L-*arginine and* L-*ornithine uptake on carbon-starved spores*

As shown above, ammonium inhibits the L-lysine transport, and it could be the cause of the inhibition of basic amino acid utilization by *P. chrysogenum* spores. Transport of these amino acids is affected by the composition of the growth media. Basic amino acids can be transported by specific transport systems (VI, VII and VIII), expressed constitutively, or by the general amino acid transport system (III), active under carbon or nitrogen starvation (Benko et al. 1967; Hunter & Segel 1971).

To observe the effect of ammonium on the basic amino acid utilization under carbon starvation, spores of 1255-LYS, 1255-ARG and 1255-PO mutants were plated on MM, MMNitrate and MMAmmonium, each with the amino acid required (10 mM) as sole carbon source. Under these conditions, all mutants, as well as the parental strain Wisconsin 54-1255, were able to grow using the amino acids as carbon source in media with ammonium, nitrate or the amino acid(s) as nitrogen source (Figure 3).

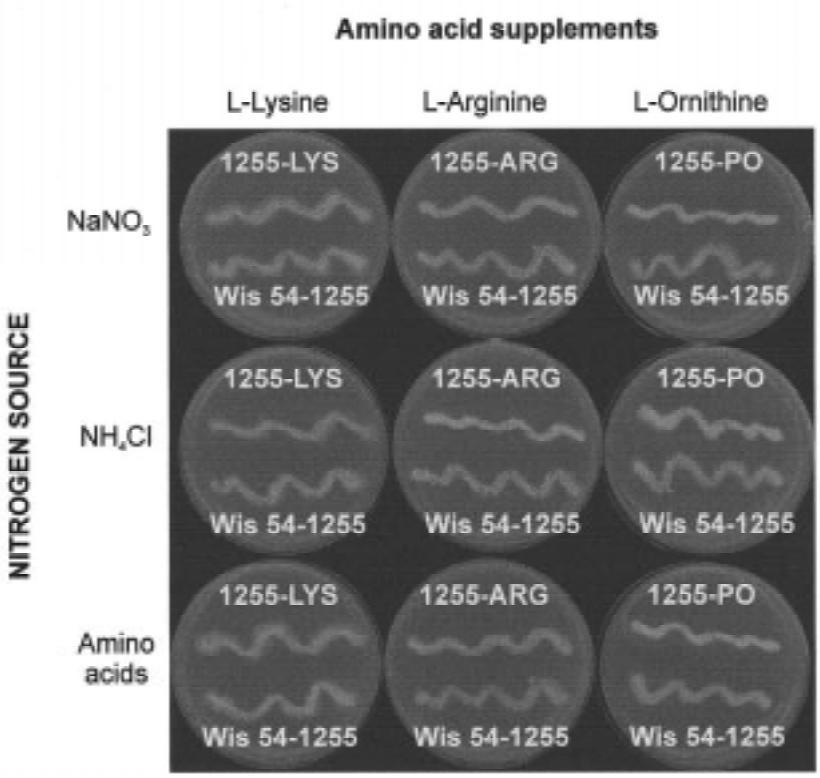

Figure 3. Effect of carbon starvation on utilization of the basic amino acid in *P. chrysogenum* spores. Mutants 1255-LYS, 1255-ARG and 1255-PO were tested for growth ability on MM without sugar and supplemented with 10 mM L-lysine, L-arginine or L-ornithine, respectively, and $NaNO_3$ (35 mM), NH_4Cl (35 mM) or amino acids as nitrogen sources. Note that under carbon-starvation conditions, growth (using the amino acids as carbon source) is lower than in sucrose-supplemented medium (compare with Figure 1).

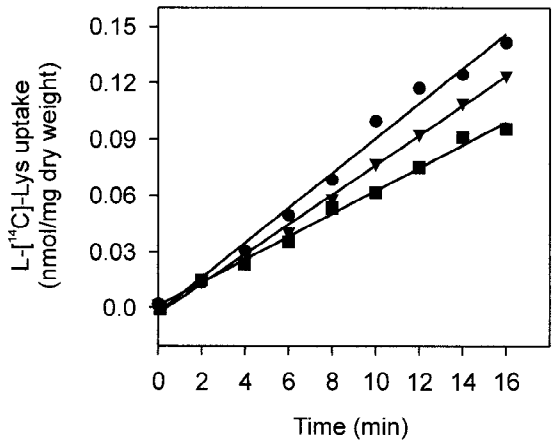

Figure 4. Effect of ammonium on L-[^{14}C]lysine transport in carbon-starved swollen spores of 1255-LYS. Spores (10^9) were incubated in 10 ml of 0.025 M phosphate buffer, pH 7.0 (●), and in phosphate buffer supplemented with 35 mM $NaNO_3$ (▼) or 35 mM NH_4Cl (■), L-[^{14}C]lysine solution (10^6 cpm/μmol) was added to give a final concentration of 0.1 mM.

Ammonium does not inhibit lysine transport by carbon-starved spores

L-[^{14}C]lysine transport was measured in carbon-starved swollen spores of the 1255-LYS mutant, by incubating the spores in phosphate buffer (control), in phosphate buffer supplemented with $NaNO_3$ (35 mM) and in phosphate buffer with NH_4Cl (35 mM). The L-[^{14}C]lysine transport rate was linear during the assay in all conditions. The rate of transport under carbon and nitrogen starvation (control) was higher than under only carbon starvation. On the other hand, and in contrast to the results observed in carbon-rich medium, lysine was still transported (with a 66% efficiency respect to the transport under carbon and nitrogen starvation) in carbon-starved spores in presence of high ammonium concentrations (Figure 4).

Table 3. Competitive effect of amino acids on uptake of L-lysine, L-arginine and L-ornithine

Amino acid	Inhibition of uptake of		
	L-Lysine (1255-LYS)	L-Arginine (1255-ARG)	L-Ornithine (1255-PO)
L-Lysine	–	**Yes**[b]	**Yes**[a]
L-Arginine	**Yes**[b]	–	–
L-Ornithine	**Yes**[d]	**Yes**[d]	–
DL-Histidine	No[d]	No[d]	**Yes**[d]
L-Glutamate	No[c]	No[c]	No[c]
L-Proline	No[d]	No[d]	–
L-Serine	No[d]	No[d]	No[d]
L-Threonine	No[d]	No[d]	No[d]

Transport inhibition was determinated by growth inhibition of auxotrophic mutants. Mutants were seeded on MMNitrate supplemented with L-lysine, L-arginine or L-ornithine at 0.1 mM. Competitor amino acids concentration was: (a) 1 mM; (b) 10 mM; (c) 50 mM; (d) 100 mM. Positive cases of competition uptake are shown in bold.

Effect of competitors on the L*-lysine,* L*-arginine and* L*-ornithine transport*

Amino acids that might share its transport system(s) with L-lysine, L-arginine and L-ornithine, or act as inhibitors of its transport, were tested on MMNitrate under different concentrations of competitor/basic amino acids, to study if they prevented the growth of 1255-LYS, 1255-ARG and 1255-PO mutants (Table 3).

Results showed that L-arginine at concentrations higher than 1 mM prevented growth of the 1255-LYS mutant (Figure 5A). 1255-ARG mutant was able to grow on MMNitrate with 1 mM, but not with 10 mM of L-lysine (Figure 5B). L-Ornithine prevented growth of 1255-LYS and 1255-ARG mutants, but at a high concentration (100 mM) (Figure 5C). L-Proline, L-serine, L-threonine and DL-histidine at 100 mM, and L-glutamate at 50 mM did not prevent growth of 1255-LYS and 1255-ARG mutants on the same medium (Table 3).

The growth of the PO mutant was completely inhibited by L-lysine at 1 mM and DL-histidine at 100 mM, while L-serine and L-threonine at 100 mM only produced a small reduction of its growth.

Discussion

Ammonium plays an important role in the amino acid transport regulation in some fungi (Grenson et al. 1970; Horák 1986; Hunter & Segel 1971, 1973a; Pateman et al. 1974; Robinson et al. 1973). Our results show that ammonium strongly inhibits the uptake of L-lysine in *P. chrysogenum* spores incubated in carbon-rich media. Ammonium inhibition of the lysine transport has been reported in *Saccharomycopsis lipolytica* grown on nitrogen-poor medium (Beckerich & Heslot 1978). In this yeast, extracellular ammonium appears to be a competitive inhibitor of the L-lysine uptake, while intracellular ammonium may act as an inhibitor/repressor of the permease (Beckerich & Heslot 1978).

The strong inhibition by ammonium of L-[^{14}C]lysine transport in swollen spores, incubated in phosphate buffer plus glucose and ammonium, confirms the results obtained by growth assays. Taken together these results indicate that ammonium prevents the growth of 1255-LYS, 1255-ARG and 1255-PO mutants, by inhibition of basic amino acid (L-lysine, L-arginine and L-ornithine) uptake, whereas transport of L-proline, L-methionine and L-cysteine (neutral amino acids) into 1255-PO, 1255-MET and 1255-MC mutants is not affected.

Ammonium seems to inhibit the basic amino acid transport through the specific transport system(s). In presence of glucose and nitrogen source (nitrate or ammonium) the general amino acid transport system is repressed (Benko et al. 1967; Hunter & Segel 1971). As shown in this work, ammonium strongly inhibits the uptake of lysine and the growth of the auxotrophic mutants, whereas nitrate allows L-[^{14}C]lysine transport (possibly through the specific transport systems) and growth of basic amino acid auxotrophic mutants.

On the other hand, in absence of carbon source, ammonium does not inhibit completely the L-lysine uptake. Under carbon starvation, the general amino acid transport system (III) is derepressed (Benko et al. 1967; Hunter & Segel 1971) being able to transport basic amino acids. Under these conditions, the observed reduction in the L-[^{14}C]lysine uptake in presence of ammonium is possibly due to the inhibition of specific L-lysine transport system(s) VI and VIII but not of the general amino acid transport system (III). The lack of ammonium inhibition of the general transport systems also explains the growth of basic amino acid auxotrophic mutants in presence of ammonium on carbon deficient media.

Inhibition by ammonium of the specific basic amino acid transport system(s) in *P. chrysogenum* had not been described so far, in spite of previous studies on amino acid transport (Hillenga et al. 1996; Hunter & Segel 1971, 1973a,b; Hunter et al. 1973). Amino

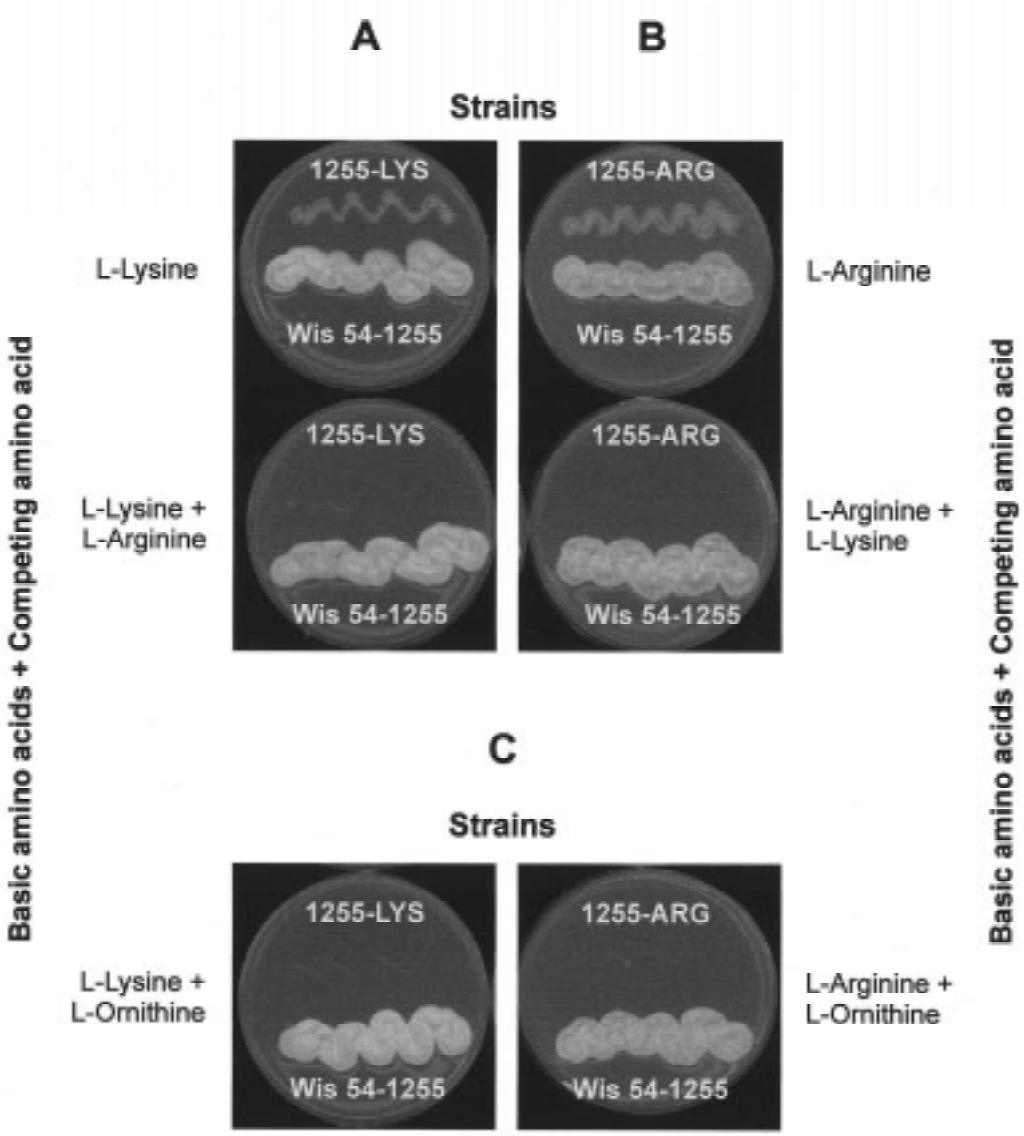

Figure 5. Effect of competition on L-lysine and L-arginine utilization exerted by some amino acids. (A) 1255-LYS mutant and parental strain plated on MMNitrate with L-lysine (0.1 mM) and L-arginine as competitor (10 mM). (B) 1255-ARG mutant and parental strain plated on MMNitrate with L-arginine (0.1 mM), and L-lysine as competitor (10 mM). (C) Parental strain, 1255-LYS and 1255-ARG mutants plated on MMNitrate supplemented with L-lysine or L-arginine (0.1 mM) and L-ornithine as competitor (100 mM).

acid transport studies have been done mostly using mycelium, but other developmental stages of fungi may exhibit differences in activity, regulation and specificity of their amino acid transport systems (Horák 1986; Tisdale & De Dusk 1970).

The competition tests showed that the addition of L-lysine or L-arginine prevents the uptake of L-arginine or L-lysine respectively in *P. chrysogenum* spores. Similar results were described previously using *P. chrysogenum* mycelium (Hillenga et al. 1996; Hunter & Segel 1971) and in other fungi, including *Penicillium cyclopium* (Roos 1989), *S. cerevisiae* (Morrison & Lichstein 1976), *A. nidulans* (Piotrowska et al. 1976) and *S. lipolytica* (Beckerich & Heslot 1978). L-Ornithine uptake differs in some aspects with the L-lysine and L-arginine transport. As shown in this work, ammonium inhibits the L-ornithine uptake in *P. chrysogenum* spores, indicating that this amino acid is also transported by the specific basic amino acid transport system(s). However, high concentrations of

L-ornithine are necessary to prevent the L-lysine and L-arginine utilization by spores. It is possible that L-ornithine is transported by the same transport system than L-lysine and L-arginine, but with a lower affinity, as occurs in *Aspergillus nidulans* (Piotrowska et al. 1976) and *S. lipolytica* (Beckerich & Heslot 1978).

Our results showed that L-lysine or L-arginine uptake is not significantly affected by a high excess of neutral amino acids that could compete with them to be transported by the general amino acid transport system. L-Leucine has been described to inhibit partially the L-lysine uptake in nitrogen-starved mycelium of *P. chrysogenum*, preventing the transport by the general, but not by the specific transport system(s) (Hunter & Segel 1971). In conclusion, the predominant transport system(s) for basic amino acids in *P. chrysogenum* spores incubated in complete media seems to be the specific transport system(s), and therefore it seems the logic target for ammonium inhibition.

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