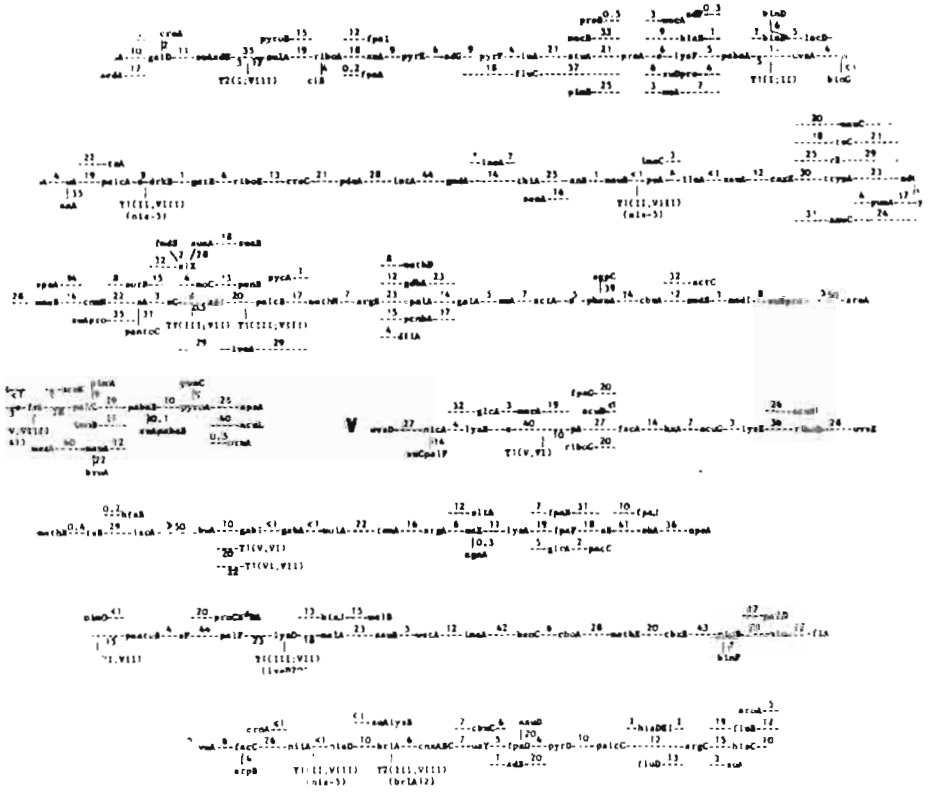
ASPERGILLUS NEWS LETTER

1981 **15**

Linkage map of ASPERGILLUS NIDULANS



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Department of Genetics
Glasgow University
Glasgow G11 5JS
Scotland U.K.

Dear Colleagues

I am again happy to acknowledge the generous financial help with the cost of this issue from:
I.C.I. Pharmaceuticals division
Pfizer Chemical Corporation
Cetus Corporation
E.R. Squibb & Sons.

I am less happy to have to repeat my apologies for the delay in production of the News Letter; time, typists and copy are never easy to bring together.

ANL 16 will be due in 1983. If contributors can send in their material early in that year, I will hope to be able to compile it in the summer.

Correction to ANL 14: p.24-26 "Products of A. nidulans" by D.S. Cole. The products of A. nidulans are listed on p.26, continuing on p.25, while the separate shorter list on p.24 is of the products of A.nidulans var. echinulatus.
In addition, the cordycepin producing isolate Birmingham No.23 belongs to A. nidulans, h-c group B, not A. nidulans var echinulatus.

Nomenclature. Compiling the locus list in this issue has revealed a few of the usual problems of clashing gene symbols. Since it is proposed to update the map and locus list biennially in "Genetic Maps" I would be grateful for notification of any new symbols or map data, and if anyone is willing to inform me of proposed new symbols before publication, I will be glad to operate the "clearing house" system to attempt to avoid future clashes.

Symbolic suggestions. 1. Addition of the letter A to unique symbols (xyzA1) may as well be done when the symbols are first used since someone else will have to add it later - that is unless the letter A is usurped by a later publication, leaving the original author with Z! The only reason for retaining the locus undefined (xyz-1) is in cases where a number of mutants have been isolated, but no allelic relationships have been worked out and no mutant has been singled out as a principal focus of attention.

2. Suppressors: The old system (e.g. suA1xyzA2) is very cumbersome. A simpler solution now being adopted by a number of authors is to use an ordinary three-letter symbol, possibly incorporating the letter s for suppressor, e.g.
suaA etc. = allele specific suppressor (Roberts et al., Mol. Gen. Genet. 177 (1979),57.)
sumA etc. = suppressor of mitochondrial gene cs-67 (csA?) (Waring & Scazzocchio, J. gen. Microbiol. 119 (1980), 297.
Alternatively, secondary properties of suppressors may provide a convenient symbol, e.g. drkB and galG mutants are suppressors of brlA12.

3. Regulatory regions: it would simplify matters for compilers of lists (i.e. me) if authors were willing to use standard locus symbols for regulatory regions, even if their function is still uncertain and may well not be coding. lacO mutants of E. coli provide a very good precedent.
Please note that if you are following my nomenclature proposals (Genet. Res. 21.291), locus symbols cannot include superscripts; these are reserved for individual mutants. A standard three-letter symbol can always be chosen to include a hint of the function of the region e.g. areA = ammonium regulation.

John Clutterbuck

Threonine, quinate and glucuronic acid utilization mutants of Aspergillus nidulans.

M.J. Hynes and J.M. Kelly, Department of Genetics, La Trobe University, Bundoora, Victoria 3083, Australia.

Due to our interests in regulation of the acetamidase enzyme (Hynes, M.G.G. 161: 59-65, 1978) and in pleiotropic catabolite repression mutations (Hynes and Kelly, M.G.G. 150: 193-204, 1977; Kelly and Hynes, M.G.G. 156: 87-92, 1977) we have isolated various mutants affected in the utilization of some carbon sources. Properties of these mutants are briefly reported here and all are available for other workers to use in either mapping or metabolic studies.

Threonine mutants. Since catabolism of L-threonine results in acetamidase induction (Hynes, J. Bact. 131: 770-775, 1977) mutants unable to use threonine as a nitrogen source or as a carbon source were isolated from a biA_1;$niiA_4$ strain after nitrosoguanidine mutagenesis. A very large number of new mutant genes were discovered, some of which were specific for threonine, glycine or serine utilization but many of which had pleiotropic effects. The results for specific mutants are summarized in Table 1 and are compatible with the pathway of L-threonine degradation via glyoxylate and acetyl-CoA suggested by Willets (J. gen. Microbiology 73: 71-83, 1972). Not very enthusiastic efforts to assay threonine dehydrogenase in A. nidulans have been unsuccessful.

Among the pleiotropic mutants, one large class which appear to be sensitive to glyoxylate were found. These have a phenotype of inhibition

Table 1: Properties of mutants affected in threonine utilization.

Locus	Mutant number	Linkage group	Properties
tutA	2	III	Specific for threonine utilization as either a nitrogen or carbon source; semi-dominant to wildtype; slightly less inducible for acetamidase by threonine.
tutB	3	II	Specific for threonine utilization; recessive; acetamidase non-inducible.
tutC	7	VII	Specific for threonine utilization; recessive.
tutD	9,35	V	Specific for threonine utilization; recessive; tutD35 inducible for acetamidase.
tutE	8,11,37, 45,46,49, 52,56,59, 60,72,75	VI	Specific for threonine utilization; recessive; tutE 11,37,45 non-inducible for acetamidase.
tguA	31	I	Leaky; specific for glycine and threonine utilization; recessive; inducible for acetamidase.
tguB	64	VIII	Specific for glycine and threonine utilization; recessive; inducible for acetamidase.
tguC	4,34	IV	Specific for glycine and threonine utilization; recessive; tutC4 inducible for acetamidase.
tgsA	40,62	II	Specific for threonine, glycine and serine; recessive; inducible for acetamidase.

by carbon sources such as acetate and ethanol utilized by the glyoxylate by-pass and are abnormal and inhibited on media with hypoxanthine, uric acid and allantoin as sole nitrogen sources. These compounds are degraded via glyoxylate. At least three genes are involved in this phenotype since haploidization of three mutants gave locations on I, IV and VI. Some acetate mutants were found - probably due to the agar effect described by Payton and Roberts (J. gen. Microbial 110: 475-478, 1979) and one creB allele (Hynes and Kelly, 1977) was found. New pleiotropic "cre-like" mutants were found and are being further studied.

D-quinate utilization mutants. D-quinate is a relatively good sole carbon source for A. nidulans. Since cre mutants were found to be defective in quinate utilization (Hynes and Kelly, 1977), nitrosoguanidine induced mutants defective in growth on quinate as the sole carbon source were isolated. Two pleiotropic mutants were found to have mutations in linkage group VII, lacked phospho-enol-pyruvate carboxy kinase activity and were allelic to acuF (Armitt et al., J. gen. Microbiol. 92: 263-282, 1976). Two mutants were affected in utilization of quinate and benzoate as carbon sources and had allelic mutations in a gene (bguA) in linkage group II. One mutant contained an acetate utilization lesion as well as a lesion resulting specifically in defective quinate utilization. This lesion (quaA7) was located in linkage group VIII. Three mutants had single mutations specifically resulting in defective quinate utilization and were closely linked in linkage group VIII but were not close to quaA7. Complementation tests revealed the presence of more than one gene and these mutations apparently define a group of genes analogous to the qua cluster in Neurospora crassa. Further characterization of these strains is in progress in the laboratory of Dr. Norman Giles by Jim Kinghorn.

D-glucuronate utilization mutants. These were selected beacuse of the effects of cre mutants on growth on this carbon source (Hynes & Kelly, 1977). One new creC allele was obtained. The following loci, mutation at which specifically affected glucuronate utilization, were identified - glrB (IV - 3 mutants), glrC (V - 2 mutants), glrD (VII - 3 mutants), glrE (VII - one very leaky mutant), glrF (VIII - one mutant) and glrG (VIII - one mutant). In addition several pleiotropic mutations, including acetate lesions were identified. No biochemical analysés have been performed.

Map order of ygA and adD

A.J.Clutterbuck, Genetics Department, Glasgow University, Glasgow G115JS, Scotland.

A new yg mutant has been induced with nitrous acid in a strain (G29) carrying cnxE and adD. This mutant failed to complement with ygA6 in a diploid and was therefore designated ygA8. The mutant strain was crossed with a pabaA1 yA2; tryA69 strain (G240) and the progeny selected for paba$^+$ ad$^+$. Four out of approximately 5000 such progeny were yg and three of these were try$^-$, cnx$^+$. One colony from an independent yg mutant in the same background gave only one paba$^+$ ad$^+$ yg colony which was again try$^-$. It is concluded that the map order is:

tryA---(23)---adD---0.1---ygA

An arabinose non-utilizing mutant araA1

A.J.Clutterbuck, Genetics department, Glasgow University, Glasgow G11 5JS, Scotland

Tests of various galactose mutants on assorted carbon sources revealed that the galB3 strain of C.F.Roberts (J. Gen. Microbiol. 31 (1963) 45-58) gave a much clearer negative phenotype on L-arabinose than on D-galactose. Roberts claimed that galB3 was located on linkage group II, but haploidisation and mapping of the arabinose determinant showed it to be on VIII, 9 units to the right of pyrD. During this mapping we were unable to detect the gal marker, so it is not certain whether the arabinose and galactose phenotypes reflect the same mutant; it seems sensible to give the arabinose determinant the designation araA1.

On arabinose agar the phenotype of araA1 is of the "non-leaky" type, that is, the residual spidery growth which occurs on plain agar (Payton et al. 1976 J. gen. Microbiol. 94;228-233.) is suppressed. In fact L-arabinose even inhibits, to some extent, growth on glucose, so it is possible that the original "galactose$^-$" designation was due to the contamination of the galactose medium with arabinose.

Report of a new mutation which modifies conidial colour in Aspergillus nidulans

Rosa Ana de la Torre and Silvia Díaz. Laboratorio de Genética de Hongos. Departamento de Genética y Evolución. Facultad de Biología. Universidad de la Habana. La Habana. Cuba.

In this report we describe a new morphological mutant in Aspergillus nidulans which dilutes the colour of the conidia and hence of the whole colony. Horn (1967) has pointed out the report by Jansen of a mutation with similar characteristics, which has been named dilA, mapping at the linkage group III. A characteristic of our new mutation, which has been named dilB, according to Clutterbuck's nomenclature (1973),is to diminish the normal colour of the colony, either the green wild type or any of the several colour mutations tested: fawn (fwA1 and fwA2), yellow(yA), chartreuse (chaA) and white (wA3). The mutation dilB arose after exposure to UV-light of the strain: adB;sA1;pyroA;chaA. We performed six crosses with different strains carrying the colour markers cited above and a green wild type strain. In all the cases it was found that the dilution characteristic behaved as a single nuclear gene mutation, giving typical segregation ratios of 1 dilute: 1 non-dilute colonies. All the colour markers and the green wild type were affected by the presence in its genome of the dilB mutation. Dilute colour recombinants were backcrossed, giving again the expected results if a single nuclear gene was involved.

The following diploid strain was synthesized, according to Roper's technique (1952), in order to assign the mutation to its linkage group:

adB	+	+	+	sA1	pyroA	+	+	+	chaA	(dilB)
+	pabaA	yA	acrA	+	+	nicA	lacA	choA	+	+

Analysis of haploid segregants induced by p-fluorophenylalanine (Lhoas,1961), showed that the dilB mutation had complete linkage with adB, so it was assigned to linkage group I.

Another characteristic of this new mutation is the appearance of

an intermediate colour zone when a strain carrying the dil3 mutation was growing very near to another strain which did not carry this marker. At this intermediate zone, the original colour of the strain carrying dil3 was observed. We picked out conidial heads at this intermediate zone, and when inoculated onto Complete Media, it grew again as a diluted colour colony. Genotype tests of the intermediate zones proved they were identical to the strains carrying the dil3 mutation. This finding suggests that the mutation dil3 complements in the heterocaryotic zone formed between two closely growing colonies. This argument could explain the observation and behaviour of the intermediate colour zone in all the paired colour combinations tested.

References

Clutterbuck A.J.1973. Genet. Res.21 p.291
Dorn G.L.1967.Genetics 56 p.619
Lhoas P. 1961. Nature 190 p. 744
Roper J.A.1952. Experientia 8 p.14

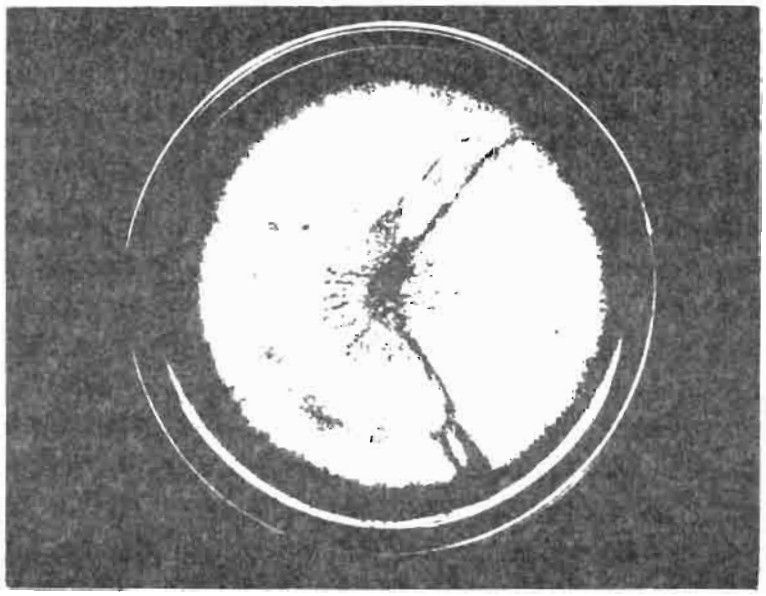

Figure 1. Two strains inoculated very near each other, growing at 37°C for six days. Left: strain dil3; chaA. Right: strain wA3. The intermediate chartreuse colour zone is observed between the 2 strains.

CLONING OF Aspergillus nidulans DNA. SELECTION OF A PLASMID CAPABLE OF COMPLEMENTING arg3 MUTATION IN Saccharomyces cerevisiae.

Brygida Berse
Department of Genetics, University of Warsaw, Al. Ujazdowskie 4, 00-478 Warsaw, Poland.

Dmochowska et al. /1980/ have constructed the Aspergillus nidulans gene library using pBR322 as a vector. Among recombinant plasmids selected in E. coli, one /pAD27/ was capable of complementing mutations in the ornithine carbamoyltransferase /OTCase/ structural genes /argIF/. After the DNA insert of pAD27 was transferred to a yeast vector, it was found to complement the arg3 mutation in the OTCase structural gene of Saccharomyces cerevisiae /unpublished data/.

The pAD27 insert hybridizes to both A. nidulans and E. coli DNA. This suggests that the cloned fragment has recombined with the host DNA during the cloning procedure. It is possible that such recombination events are necessary to allow expression of A. nidulans genes in the bacterial host. Therefore experiments were performed to clone the gene of interest directly in yeast.

Construction of the vector.

The chimaeric plasmid pJDB219 constructed by Beggs /1978/ served as a source of the yeast DNA sequences needed for replication and selection of a plasmid in yeast cells. pJDB219 carries the 2μ DNA of yeast and the yeast nuclear DNA fragment with the LEU2 gene cloned on bacterial plasmid pMB9.

DNAs of plasmids pBR322 and pJDB219 were digested with EcoRI, mixed together and ligated. The ligation mixture was used to transform E. coli strain JA221 /recA1 leuB6 trpΔE5 $hsdR^-$ $hsdM^+$ lacY C600/. Transformants were selected for ampicillin resistance and tested for leucine prototrophy. Plasmids were purified from $amp^r leu^+$ clones and restriction analysis with EcoRI was performed. One of the plasmids, named pBB8, was chosen for further experiments. It contains pBR322 and three of the four EcoRI fragments of pJDB219 bearing the complete sequence of 2μ DNA and the LEU2 gene. Plasmid pBB8 is capable of transforming E. coli to ampicillin and tetracycline resistance and to leuB prototrophy.

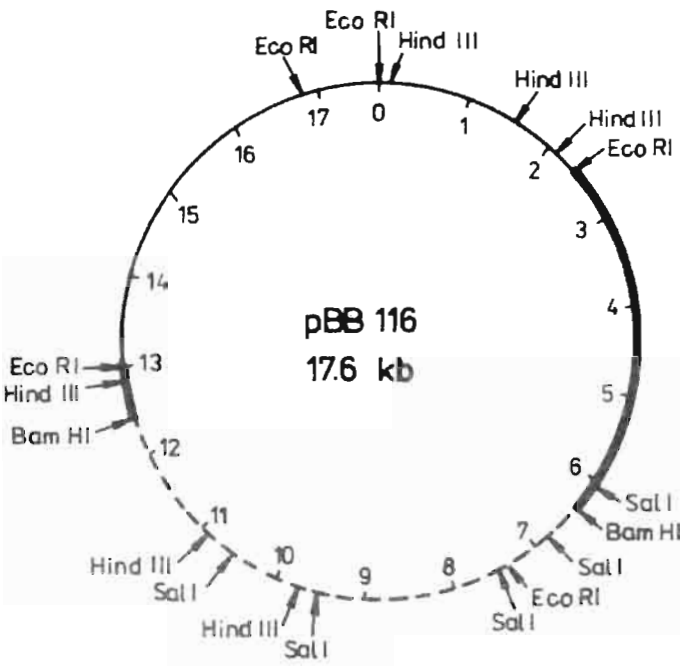

Fig. 1: Restriction map of pBB 116.
The thick line represents pBR 322 DNA, the thin line — yeast DNA sequences, the dotted line — the cloned fragment.

It also transforms S. cerevisiae leu2 strain to prototrophy with the efficiency of 10^4 colonies per μg DNA. pBB8 has unique cleavage sites for BamHI, SalI and PstI. The plasmid is 11.4 kb in size, i.e. it is 1 kb smaller than pJDB219.

Cloning experiments.

The A. nidulans DNA was cleaved with BamHI and ligated to the pBB8 DNA linearized with BamHI. The ligation mixture was used to transform S. cerevisiae strain SPU1 /leu2-3 leu2-112 his4-519 arg3 ade2/. Transformants were selected on medium lacking leucine and then tested for arginine prototrophy. One prototrophic leu^+arg^+ clone was obtained. DNA was purified from this clone and used to transform E. coli JA221. Several ampicillin-resistant transformants were obtained and in all cases they were leu^+ and

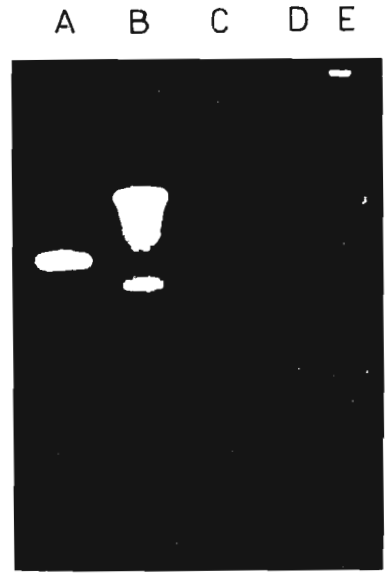

Fig. 2. Hybridization of ^{32}P-labelled insert of pBB116 to: A. pBB116 insert; B. A. nidulans DNA digested with BamHI; C. S. cerevisiae DNA digested with BamHI; D. E. coli DNA digested with BamHI; E. pAD27 insert.

contained plasmids of about 18 kb in size. Plasmid DNA was purified from one of the clones and its restriction map was established /fig. 1/. The plasmid, named pBB116, contains pBB8 sequences and a BamHI insert 6.2 kb in size. SPU1 strain was transformed with the purified plasmid which was again found to complement both leu2 and arg3 mutations.

In order to check whether the 6.2 kb insert is of A. nidulans origin, hybridization experiments were performed by the method of Southern /1975/. The results are presented /fig. 2/. ^{32}P-labelled 6.2 kb fragment of pBB116 was found to hybridize strongly to the A. nidulans DNA. However, it hybridized to two BamHI fragments about 5 and 9 kb in size, and not to a 6.2 kb fragment. Weak hybridization to S. cerevisiae DNA is due to homology between cellular 2μ DNA and the vector DNA contaminating the probe. No hybridization with E. coli DNA was observed.

Only very weak hybridization between the probe and the pAD27 insert was found. This indicates that there is little /or no/ homology between pAD27 and pBB116 inserts. Experiments are being performed to establish which plasmid /if any/ bears the structural gene for A. nidulans OTCase.

References

Beggs, J. D. Nature 275, 104-109 /1978/.

Dmochowska, A. T., Bal, J., Bartnik, E., Weglenski, P. Acta Microbiol. Polon. 29, 213-225 /1980/.

Southern, E. M. J. Mol. Biol. 98, 503-517 /1975/.

Growth of *Aspergillus nidulans* mycelia and conidia on acetate medium

D.R. Niffeneger-Souza, Dep. Bio. Geral, UnivFMG Belo Horizonte, Brazil.

and

J.L. Azevedo, Lab. Genetics and Evolution, Dep. Bio. An. Univ. Brasilia, Brazil.

From several deteriorated variants spontaneously produced by the duplication strain A of *Aspergillus nidulans* (Nga, B.H. & J.A. Roper, Genetics 58, 193 (1968), Azevedo, J.L. & J.A. Roper (1970), Genet. Res. 16, 79), one of them designated as V_{50}- was a mycelial mutant which started to produce conidia only after 72 hours of incubation at 37° on complete medium (CM). This variant and a derivative of it ($V_{50.1}$, which had probably lost the duplicated segment) when transferred from CM to AC (acetate medium, Apirion, D. (1962) Nature, Lond. 195, 959) before the formation of conidia behaved, as an acetate mutant and, in this way could not form colonies on AC medium. However, if transfer to AC medium is performed after 72 hours, when conidia are already present, both strains V_{50} and $V_{50.1}$, could develop on this medium. It was then hypothesized that this could be a general characteristic of *A. nidulans* strains, that is, that the start of growth on AC medium is only possible if conidia, and not hyphae fragments are transferred. To test this hypothesis, conidia from strains *proA1 pabaA6 yA2*, V_{50} and $V_{50.1}$, derived from colonies from CM, were transferred to CM, minimal medium plus requirements (MM) and AC medium plus requirements. In all these media conidia were able to germinate to form colonies. However, when hyphal fragments were transferred no colonies developed on AC.

In a second experiment, microcolonies (about 12 hours growth) from the same strains plus strain A, previously grown on CM, MM and AC were transferred to AC medium. No growth was observed when colonies originated from CM. However, growth was always observed when colonies derived from AC. In the case of colonies derived from MM, a variable percentage of growth was observed (table 1). A control, made by transferring microcolonies from all three media to CM and MM

Table 1. Growth of microcolonies from strains proA1 pabaA6 yA2, V_{50}, $V_{50.1}$ and strain A transferred to different media.*

STRAINS	ORIGINAL MEDIUM	PERCENTAGE OF GROWTH AFTER TRANSFERRING TO:		
		AC	MM	CM
V_{50}	AC	100	100	100
	MM	54	100	100
	CM	0	100	100
$V_{50.1}$	AC	100	100	100
	MM	81	100	100
	CM	0	100	100
proA1 pabaA6 yA2	AC	100	100	100
	MM	31	100	100
	CM	0	100	100
A	AC	100	100	100
	MM	42	100	100
	CM	0	100	100

*In all cases 26 microcolonies were used. MM and AC media were always supplemented with the necessary requirements to support growth of the strains used.

produced growth in all cases. Other strains carrying different genetic markers were also tested and behaved in the same way. Few exceptions were observed; for some strains microcolonies transferred from CM to AC could rarely form macroscopically visible colonies. These rare cases of growth were interpreted as due to the transferrence of microcolonies which were already in the stage of conidia formation when transferred to AC medium.

The results probably demonstrate differential use of acetate during germination phase, according to the developmental stage of the fungus. They also indicate the importance of morphological mutants to study aspects related to intermediate metabolism and gene regulation. From a technical point of view, these results also show that conidia and not only hyphae must be transferred from CM and MM when AC medium is used; that is the case in the analysis of segregants from crosses involving acetate markers.

The reasons for the inability of microcolonies transferred from CM to continue to develop on AC medium may be several ones, however, Clutterbuck and Glansdorff (see following note) suggest that the phenomenon is dependent on the very rapid uptake of acetate which induces a high osmotic pressure inside the hyphae.

Bursting of hyphal tips when transferred to acetate medium

A.J. Clutterbuck, Department of Genetics, Glasgow University.
and
N. Glansdorff, Inst. de Recherche, CERIA, Brussels.

We have found, as have Niffeneger-Souza and Azevedo, the authors of the preceding note, that when hyphae of a variety of A. nidulans strains are transferred from glucose medium to acetate (either Apirion's AM or MM in which the glucose is replaced by acetate) they fail to grow. Microscopic examination of the hyphae shows that this is because of bursting of the hyphal tips.

Conidia germinating on acetate medium, however, do not suffer from this problem, and it can be avoided for hyphae if they are transferred from glucose medium to acetate via medium containing both carbon sources. It is assumed either that rapid uptake of acetate by unadapted hyphae produces excessive turgor in the cells, or possibly that acetate weakens unadapted hyphal walls.

Apirion (Genetics 53 (1966),935-941) found that unlinked fac mutants failed to complement in heterokaryons, although they complemented satisfactorily in diploids. This can now be explained since diploid conidia would germinate successfully on acetate medium whereas hyphal inocula from heterokarons would fail. Repetition of these experiments showed that heterokaryons between unlinked unlinked fac mutants will show complementation if transferred via glucose + acetate medium.

Hyphal tip bursting is, however, subject to Sod's Law: a recent attempt to burst hyphae deliberately failed because the hyphae continued to grow happily when transferred to acetate. Armitt et al. (J. gen. Microbiol. 92 (1976), 263-282) also reported no difficulty in demonstrating growth of heterokaryons tested on acetate. The above note by Niffeneger-Souza and Azevedo suggests that the effect may depend on the vigour of the original growth since they found a difference in hyphae from CM and MM.

Mutagenic mycotoxins in lytic enzyme preparations?

E. Forbes, Genetics Department, Glasgow University, Glasgow G11 5JS, Scotland.

There is some evidence to suggest that the Trichoderma lytic enzyme preparation used to isolate protoplasts from Aspergillus nidulans is mutagenic.

Further work is proceeding and will be reported when complete, but in the meantime care should be taken with protoplast experiments in case the lytic enzyme is contaminated with mycotoxins. If these were proved to be mutagenic, they would probably also be carcinogenic.

lam A mutants of Aspergillus nidulans show decreased β lactamase activity but do not influence penicillin production

by John F. Makins, G. Holt[1] and K.D. Macdonald[2].

The lam A mutation in A.nidulans which results in the inability to utilise 2-pyrrolidone and 2 piperidone as precursors of γ amino butyric acid, (GABA) has been described by Arst, Penfold and Bailey (1978). This mutation has been located to linkage group VIII, is thought to specify the formation of a lactamase and is under the control of the integrator gene int A (ibid). We were interested in the possibility that the lam A gene product might influence penicillin production and more specifically that it may represent an alternative manifestation of mutation at the pen A locus which is also located on linkage group VIII and causes increased production of β lactam antibiotics (Ditchburn, Holt and Macdonald 1976). If isopenicillin N, 6 Aminopenicillamic (6-APA) acid or penicillin itself were susceptible to hydroloysis by the product of the lam A^+ gene then strains bearing lam A might be expected to overproduce penicillin.

Strains bearing lam A were tested for their ability to produce penicillin in submerged shaken culture according to the procedure of Holt and Macdonald (1968) and strains bearing pen A were tested for their ability to grow on media containing either 2 pyrrolidone or 6-APA as sole nitrogen source or 6-APA as sole sulphur source. A diploid strain heterozygous for lam A and pen A in trans was formed and tested for penicillin production and growth on the same media. These results are shown in Table 1.

Tests for linkage between pen A and lam A made in the normal manner (Pontecorvo et al, 1953), showed free recombination of these two markers. (lam A also recombines freely with cha A, sE and nir A. H. Arst personal communication).

The β lactamase activity present in lysates of protoplasts from strains bearing lam A, pen A or their wild type alleles was assessed from the rate of hydroloysis of benzylpenicillin. (See Fig.1.).

The ability of strains bearing pen A to grow on media containing 2 pyrrolidone as sole nitrogen source, the absence of significantly increased penicillin titres in strains bearing lam A and the free recombination between these two markers vitiated our original hypothesis that they may be alternative phenotypes of mutation at the same locus.

The β lactamase activities of strain G96 (pen A) and G (+) showed no significant difference which implies that the increased penicillin production associated with pen A does not result from a decreased ability to degrade the

β lactam molecule. Strain GA902 (lam A) did show lower levels of penicillin hydroloysis than the wild type strain implying that the lam A gene does control the formation of a lactamase. Strains bearing lam A are unable to utilise 2-pyrrolidone or 6-APA as nitrogen sources while the wild type strain and pen A mutants can do so. Strains bearing lam A can however utilise 6-APA as a source of sulphur which implies that there is more than one pathway by which lactam molecules can be catabolised in A. nidulans.

It was thought possible that the lam A^+ gene product might not be induced during growth under conditions suitable for penicillin production, and hence the penicillin titres shown in table 1. did not reflect the productivity in the presence of lactamase activity. Accordingly estimations of penicillin titre were made when β alanine, an inducer of lam A, 2-pyrrolidone a substrate for the lam A gene product, and GABA, the end product of the lam A initiated pathway, were added to penicillin production medium in 10 mM concentrations, prior to culture of wild type and mutant strains bearing pen A or lam A. On no occasion were the penicillin titres of any strain significantly different from cultures lacking the supplements.

It appears that lactam catabolism and β lactam anabolism are biochemically distinct and well separated within the metabolism of A. nidulans. It is possible however, that other mutations impairing the breakdown of β lactam molecules (for example mutants unable to use 6-APA as a source of sulphur) may have concomitant effects on penicillin yield.

Fig.1

Penicillin concentrations (after incubation of 1.8mls of Benzylpenicillin (5 units/ml^{-1}) with 0.2 mls of protoplast lysate ($0.5x10^7$ protoplasts) from strains GA902; (●); G96 (□); G96; (O)

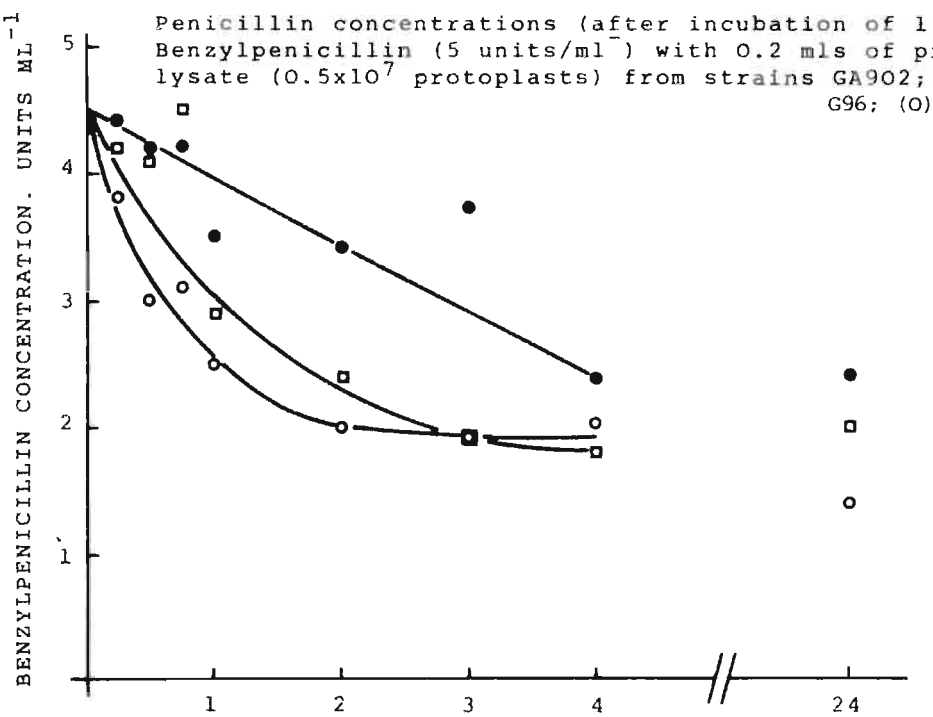

TABLE 1

GROWTH AND CHARACTERISTICS OF PENICILLIN PRODUCTION IN STRAINS BEARING

lam A and *pen A* MUTATIONS

STRAIN DOMESTIC CODE	RELEVANT GENOTYPE	CHARACTERISTIC GROWTH* ON MEDIA CONTAINING AS SOLE NITROGEN SOURCE		CHARACTERISTIC GROWTH* ON MEDIA CONTAINING AS SOLE SULPHUR SOURCE	PENICILLIN TITRE (UNITS ml^{-1}) FOLLOWING CULTURE IN PENICILLIN PRODUCTION MEDIUM WITH 10mM OF			
		2-pyrrolidone	6-APA	6-APA	NO ADDITION	β alanine	2-pyrrolidone	GABA
G	+	+	+	+	5.2	5.2	5.0	5.3
G96	penA	+	-	+	17.2	20.9	19.2	18.1
G52	penA	+	+	+	18.4	18.0	21	17.2
GA894	lamA	-	-	+	7.32	7.0	7.1	7.3
GA902	lamA	-	-	+	5.92	6.0	5.5	6.1
C500D	lamA + / + penA	+	+	+	8.8	10.2	9.1	10.8

* Growth scored by reference to that of wild type (G) + wild type growth - no growth.

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Arst., H.N., Penfold, H.A., and Bailey, C.R. (1978)
Molec. gen. Genet. 166 321-327.

Ditchburn, P., Holt, G. and Macdonald, K.D. (1976)
Proc. 2nd Int. Symp. Genet. Indust. Microorg.
Ed. Macdonald, K.D. p.213-227. Academic Press.

Holt, G. and Macdonald, K.D. (1968)
Antonie van Leeuwenhoek 34, 409-416.

Pontecorvo, G., Roper, J.A., Hemmons, L.A., Macdonald, K.D., Bufton, A.W.J. (1953)
Advanc. Genet. 5 141-238.

[1]The Polytechnic of Central London
School of Engineering and Science
115 New Cavendish Street
LONDON W1M 8JS

[2]D.M.D. C.D.E.
PORTON DOWN
Salisbury
Wilts.

Preparation of Polysomes from Germinating Conidia of Aspergillus nidulans

Lewis Stevens and Michael D Winther
Department of Biochemistry, University of Stirling, Scotland

Polysomes can be prepared from conidia by a procedure essentially similar to that described by Mirkes (1974). Approximately 10^8 to 5×10^9 conidia are harvested on Millipore filters and washed with 100 ml distilled water. They are first ground for 30 secs in a mortar with half their weight of sand plus 0.1 ml of buffer (0.03M triethanolamine HCl-0.01M KCl -0.01M $MgCl_2$ pH 7.5) and then mixed in the mortar for a further 30 secs

with 4 ml of buffer to form a suspension, which is centrifuged at 15000g X 10 min. Volumes of supernatant containing 4 to 15 OD units at 260nm are layered on a linear sucrose gradient (10-40% w/w) in 38 ml tubes and centrifuged for 3 hours at 85000g, 4°C. 50% sucrose (w/w) was pumped into the bottom of the gradients displacing the contents through a 0.5 cm flow cell continuously monitoring A_{260}. A typical polysome profile from 8 hr germinating conidia is shown in Fig.1. It was found that 30 secs to 1 min was the optimum time for grinding conidia. Longer times produced a higher proportion of monosomes. Cycloheximide (200 µg/ml) was normally added 5 mins before harvesting the conidia so that ribosomes did not "run off" by completing translation of mRNA. Treatment with pancreatic RNAse (80µg/ml for 15 min at 37°) or with 0.7mM puromycin - 0.01mM $MgCl_2$ degraded polysomes to monosomes and subunits respectively (Fig 1).

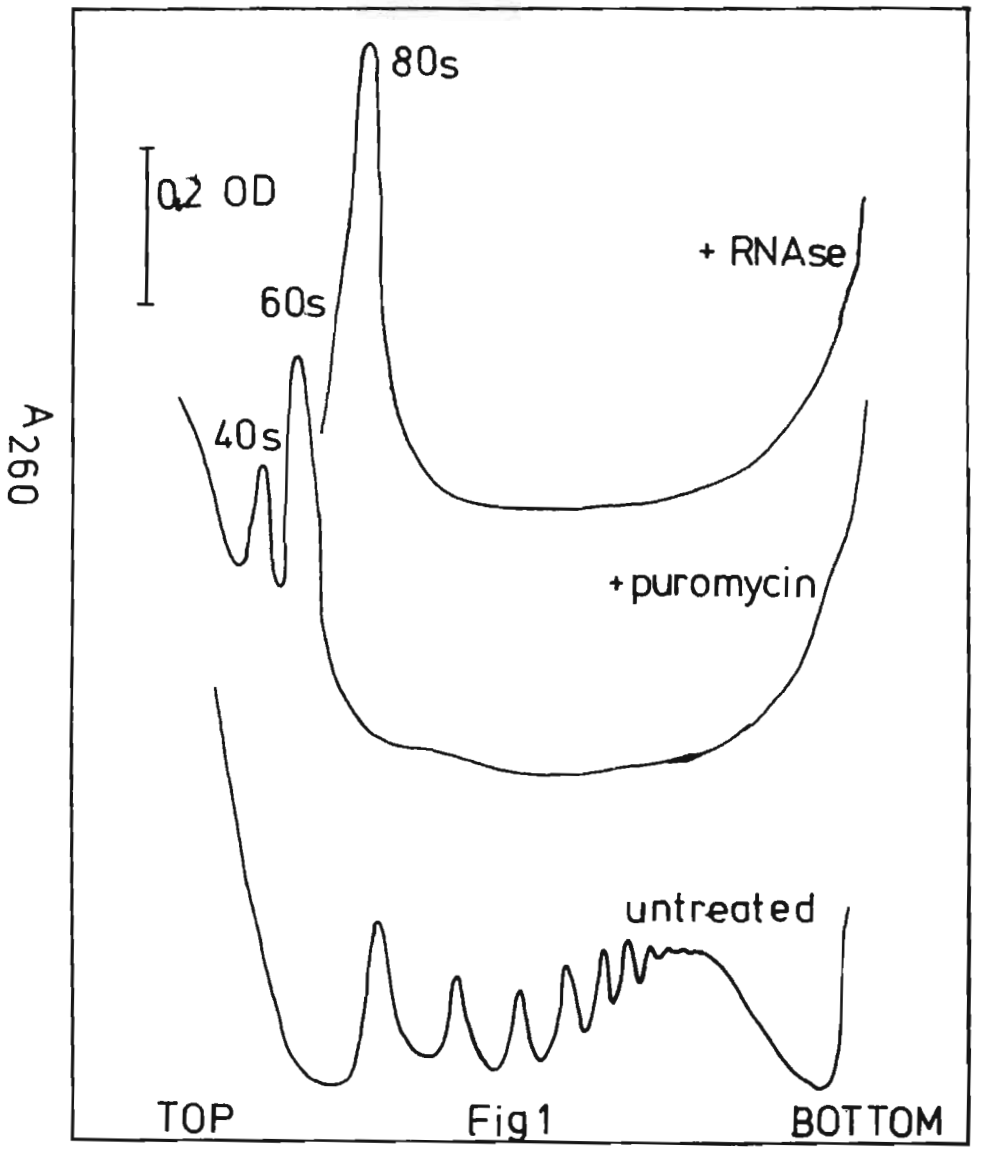

Fig 1

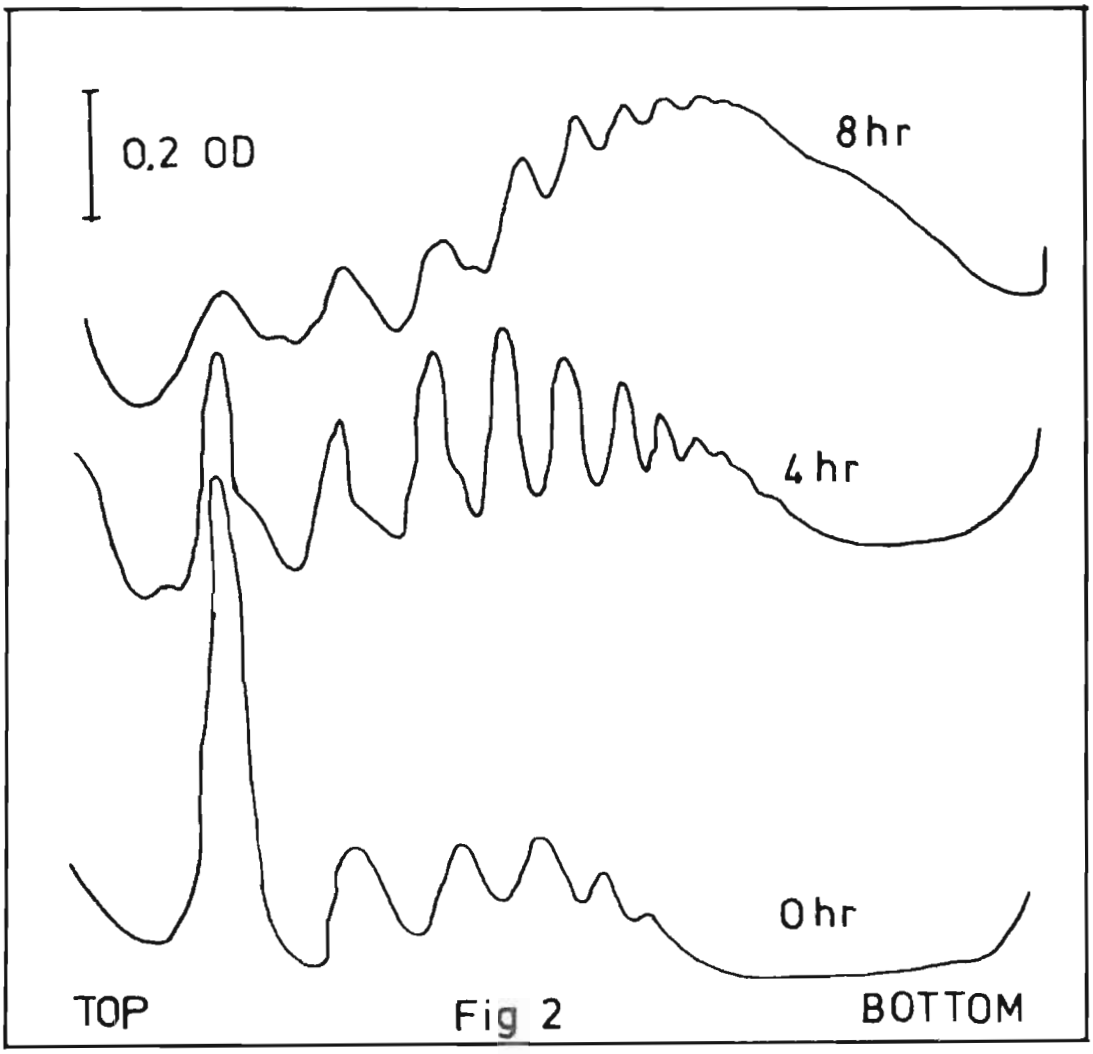

Fig 2

Treatment of the sucrose solutions by boiling with 0.02% diethyl pyrocarbonate to inhibit RNAse activity only made a marginal difference to the polysome profile. Polysomes were prepared from conidia which had been incubated in submerged culture (Stevens et al. 1976) for up to 8 hrs. An increasing proportion of polysomes compared with monosomes is found as germination proceeds (Fig 2), though some polysomes appear to be present at very early times.

Mirkes P.E. (1974) J Bacteriol 117, 196
Stevens L., McKinnon I. and Winther M.D. (1976) Biochem J 158, 235.

R.Maleszka, N.J.Pieniążek
Department of Genetics,University of Warsaw, 00 - 478 Warszawa

Modified replica plating technique of micro - colonies of Aspergillus nidulans using Triton X - 100

The selection of colonies of a desirable phenotypes is a difficult and long step in the isolation of auxotrophic mutants.Application of N - glycosyl - polifungin as the selective agent for obtaining specified classes of mutants has been described by Bal et al./1974/ originally for Aspergillus nidulans.The method is easy, effective and gives about 9000 - fold enrichment.However, under certain conditions / if many mutations within the same gene are selected / this procedure leads to selection of large number of isoallelic mutants.The value of replica plating technique with damp velveteen for the isolation of nutritionally exacting mutants is well known in genetics/ Lederberg and Lederberg, 1952/. This method in its original form has many limitations in the case of filamentous fungi like A.nidulans.Macintosh and Pritchard /1963/ described a modified method for replica plating using 0.08% sodium deoxycholate /SD/ which causes formation of microcolonies of A.nidulans and permits the use of higher plating densities than is normally possible / 150 - 300 colonies per plate /.These authors showed that only anionic detergents induced colonial growth of A.nidulans.Anionic detergents had already been shown to induce colonial growth of Neurospora and Syncephalastrun / Tatum et al.1949 /.In contrast to these data we have found that the non - ionic detergent Triton X - 100 induced compact growth of A.nidulans at a lower concentration than SD and produces micro - colonies of about 2 mm in diameter with no inhibition of conidiation and any mutagenic effect.In our experiments we obtained better results with Triton X - 100 than SD.

We used our method for isolation of auxotrophic mutants in ad A locus / adenylsuccinate lyase /.These mutants require adenine and are unable to utilize hypoxanthine. Six mls of heavy conidial suspension of pyro A4 bi A1 w A2 S A1 strain were incubated in 0.1 M phosphate buffer pH 7.0 with 1.5 ml NQO /4 - nitroquinoline - 1 - oxide/, Bal et al./1977/. After mutagenesis conidia were plated on minimal medium suplemented with adenine + detergent /Triton X - 100 or SD/. We have found that the optimal concentration of Triton X -100 is 0.01%.At lower concentration Triton X-100 does not produce colonies of restricted size and in higher concentration inhibited conidiation. At concentration 0.01 % we obtained almost full recovery with 400 colonies per plate.In contrast to SD, there were no precipitates in agar plates at Triton X - 100 concentration from 0.002 to 0.1%. This is very important for further photographical evaluation. After 3 days, master plates /minimal medium + adenine / were replicated with damp velveteen and replica plates / minimal medium + hypoxanthine / were incubated about 48 h at 37°C. The master plates and replicas were layered on black paper and photographed.The photographs were analysed by superposition of negatives and hypoxanthine requiring colonies were taken for further testing.All changes in colony pattern on replicas were easly detected on negatives. Most of selected colonies / 70% / were morphological mutants / poor conidiation or slow growth /. From among 60500 tested colonies, 651 /1%/ hypoxanthine requiring colonies were plated on master plates.After genetical analysis we found 11 mutants at ad A locus /0.016%/. The analysis of all 60500 colonies was performed in a short time of 11 days.

References

Bal,J.,Balbin,E.and Pieniążek,N.J. / 1974 / J.Gen.Microbiol.84, 111-116.

Bal,J.Kajtaniak,E.M. and Pieniążek N.J./1977 / Mut.Res. 56,153- 156.

Lederberg,J.,Lederberg,E.M. /1952/ J.Bacteriol. 63,399-404.

Mackintosh,M.E.,Pritchard,R.H. /1963 /Gent.Res.Camb. 4 , 320-322.

Tatum,E.L.,Barbatt,R.W. and Cutter,V.M./ 1949 / Science 109,509.

Purification and properties of arginase from Aspergillus nidulans.

Neeraja Ramakrishnan & E.R.B. Shanmuagasundaram

Department of Biochemistry, University of Madras, Madras-600 025, India

The activity of the enzyme arginase (L-arginine ureohydrolase, E.C. 3.5.3.1) is low in the wild strain of Aspergillus nidulans and is induced by the presence of arginine in the medium (Cybis et al. 1972). However, a significantly increased arginase activity is evident in the wild strain grown on minimal medium alone, on the first day of growth which then decreases on the second and successive days (Ramakrishnan & Shanmugasundaram 1979). It has also been observed that in A. nidulans arginine can serve as the sole source of nitrogen for growth (under publication) and under these conditions high levels of arginase are present. As part of our investigation of arginine metabolism in A. nidulans, the present study examines the physical and catalytic propertis of purified arginase.

Materials and methods: the wild strain of A. nidulans obtained from the Glasgow stock, bearing green conidia, was cultured in liquid minimal medium (Pontecorvo et al. 1953) supplemented with arginine (200mg/l). The mycelium obtained after 36 h of growth at 37°C was used in the purification of arginase.

The enzyme was extracted by grinding the mycelium with half its wieght of acid-washed glass powder, mixing with 2 vol. of 0.2M sodium glycinate buffer, pH 9.5, containing 0.5% Triton X-100, 0.5 mM $MnCl_2$ and 0.1 M KCl. The homogenate was centrifuged at 10,000g for 10 min and the supernatant used in the further steps of purification. The arginase activity of the supernatant was estimated as described earlier (Ramakrishnan & Shanmugasundaram 1979). To the supernatant was added an equal volume of distilled acetone at -10°C, and the precipitate was collected by centrifugation at 15,000g for 5 min. The precipitate was resuspended in 0.05M dipotassium hydrogen phosphate solution containing 0.5% Triton X-100. The suspension was stirred for an hour and homogenised in a Teflon homogeniser, it was then dialysed for 12h against 0.1M tris-HCl buffer (pH 7.5) containing 0.05M $MnCl_2$ and 0.001mM β-mercaptoethanol.

The above suspension was loaded onto a DEAE column and eluted with an L-arginine gradient (0 - 0.1M) buffered with 0.1M tris-HCl, pH 7.5. The enzyme appeared in the buffer fraction containing 0.1M arginine. The fractions containing the maximal enzyme activity were dialysed against 0.1M tris-HCl buffer (pH 7.5) containing 0.05M $MnCl_2$ and 0.001mM β-mercaptoethanol.

The enzyme fraction was then applied to a Sephadex G-200 column and the enzyme eluted.

Purification of Aspergillus nidulans arginase from a 36h old culture grown at 37°C.

Fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg protein)	Recovery (%)
Crude homogenate	635.1	889.2	1.4	100
Crude supernatant	280.0	844.8	3.0	95.0
Acetone precipitate	112.0	730.2	6.5	82.1
DEAE-cellulose chromatography	15.6	540.0	34.5	60.7
Sephadex G-200 chromatography	2.3	150.8	66.3	16.9

One unit of enzyme activity is defined as the amount of enzyme required to produce one μmol of ornithine per hour under incubation conditions.

The Km with respect to L-arginine was found to be 50 x 10^{-3}M and the Ki with respect to glutamic acid was 7.3 x 10^{-4}M. Glutamic acid was found to be an uncompetitive inhibitor of arginase. The pH optimum was 7.1 when activated by Co^{++} Divalent metal ions like Zn^{++}, Mg^{++}, Mn^{++}, Ni^{++} and Fe^{++} caused considerable decrease in enzyme activity, so did pCMB and EDTA. β-mercaptoethanol protected the enzyme from inactivation during dialysis. The molecular weight was determined to be 270,000.

References

Cybis, J., Piotrowska, M. & P.Weglenski (1972). Mol. Gen. Genet. 118, 272.
Ramakrishnan, N. & E.R.B. Shanmugasundaram (1979). J. Ind. Inst. Sci. 61(C),63.
Pontecorvo, G., J.A. Roper, L.M. Hemmons, K.D. MacDonald, & A.W.J. Bufton (1953) Adv. Genet. 5, 141.

Phenotype and Mapping of tubA1

C.E. Oakley and N.R. Morris, Department of Pharmacology, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854, U.S.A.

TubA1, a structural gene for the microtubule protein α-tubulin (Morris et al., 1979, Cell 16, 437-442) was isolated as an extragenic suppressor of the temperature sensitivity associated with the benA11 allele of benA - a structural gene for β-tubulin (Sheir-Neiss et al., 1978, Cell 15, 639-647). A strain carrying both benA11 and tubA1 shows not only suppression of temperature sensitivity but also a slight suppression of the benomyl resistance for which benA11 was originally isolated (Van Tuyl, Ph.D. thesis, Dept. of Genetics, Agricultural University, Wageningen, The Netherlands). A strain which carries tubA1 but is wild-type for benA however, is supersensitive to benomyl and several other antimicrotubule agents, showing 50% inhibition by benomyl at about 0.15 μg/ml as compared to 0.4 μg/ml for wild-type strains.

Genetic analysis shows tubA1 to be on linkage group VIII; recombination frequencies of 11.8% with chaA and 29% with sE show it to be about 10 map units to the left of chaA.