Fungal Genetics Reports

Volume 6 Article 4

Effect of nitrogen source and pH on the growth of a glutamine requiring strain (glm)

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Abstract

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Barratt, R. W. and W. N. Ogata. Effect of nitrogen source and pH on the growth of a glutamine requiring strain (glm).

Reich and Silagi (1963 Proc. Intern. Congr. Genet. IIth, The Hague, 1:49) reported a number of allelic mutants of independent origin which require L-glutamine (500 mg/l) for growth. glm strains are not leaky on minimal, are very sensitive to L-amino acids, especially methionine, and lack the enzyme glutamine synthetase (Reich, personal communication).

The results reported below were obtained on glm allele 1015 (FGSC#1115). FGSC #1115 is the double mutant glm, inos, carrying inos allele 89601. All media were supplemented with inositol (25 mg/l). L-glutamine was sterilized by filtration. Wild type strain STA4 (FGSC#262) was used for comparative purposes. During routine testing it was observed that the glm strain grows on minimal synthetic agar slants with little or no delay either in growth or conidiation, and grows especially well on Neurospora Culture Agar (Difco Laboratories, Detroit, Michigan). Neurospora Culture Agar contains proteose peptone, yeast extract, maltose and agar and has a final pH of 6.7. Reich and Silagi reported delayed growth on all media tested; no such delay was observed on Neurospora Culture Agar. Thus, it would appear that this medium would be ideal for the routine culture of glm strains. Reich and Silagi used Vogel's medium N throughout their investigations. Our data confirm that the glm strain fails to grow in minimal medium N even after long periods of incubation (see Figure I and Table I).

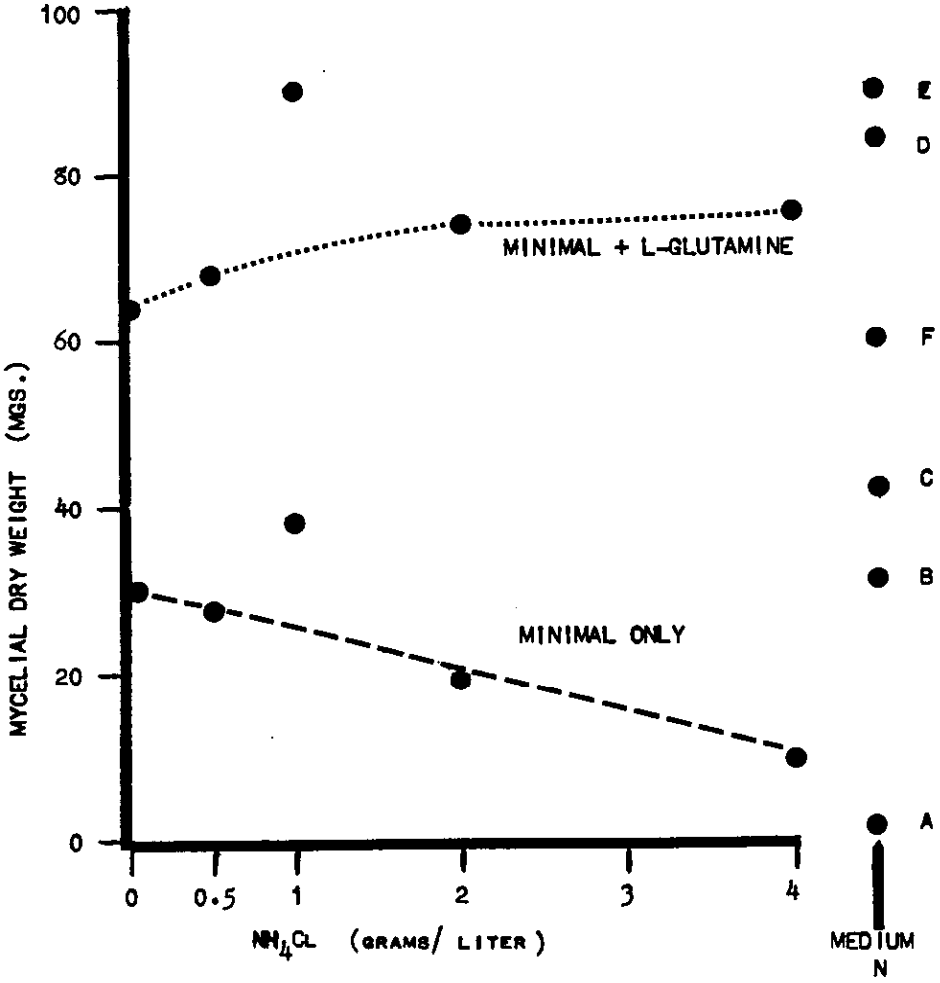

FIGURE 1. EFFECT OF NH_4Cl ON THE GROWTH OF THE glm STRAIN. THE MEDIUM USED WAS AMMONIUM ION FREE MODIFIED SYNTHETIC CROSS (SEE TEXT). GROWTH CONDITIONS: 20 ML MEDIUM IN 125 ML FLASKS; STILL CULTURE; 32 C; 70 HOURS; INOCULUM ONE DROP FILTERED CONIDIAL SUSPENSION. LOWER CURVE - MINIMAL ONLY; UPPER CURVE - MINIMAL SUPPLEMENTED WITH L-GLUTAMINE (1 MG/ML). DATA ON RIGHT HAND SIDE ARE DRY WEIGHTS OBTAINED AS FOLLOWS:

A. glm STRAIN IN MEDIUM N
B. glm STRAIN IN MEDIUM N + L-GLUTAMINE
C. WILD TYPE STRAIN IN MEDIUM N
D. WILD TYPE STRAIN IN MEDIUM N + L-GLUTAMINE
E. WILD TYPE STRAIN IN MEDIUM N + DL-ASPARAGINE
F. WILD TYPE STRAIN IN MODIFIED SYNTHETIC CROSS MEDIUM + L-GLUTAMINE

Nitrogen in synthetic cross medium is entirely in the form of nitrate ions, while medium N contains both ammonium and nitrate ions (supplied as NH_4NO_3 at 2 g/l). Medium N also contains citrate ions at a concentration of 3 g/l. In flask assays substantial growth of the glm strain was obtained in media free from ammonium ions. Mycelial growth on a modified minimal synthetic cross (containing only 0.2 g/l $MgSO_4$ instead of 0.5 g/l), and adjusted to an initial pH of 6.5 was equal to that obtained in medium N supplemented with L-glutamine (1 g/l) (compare Figure 1, point B with minimal only in the absence of

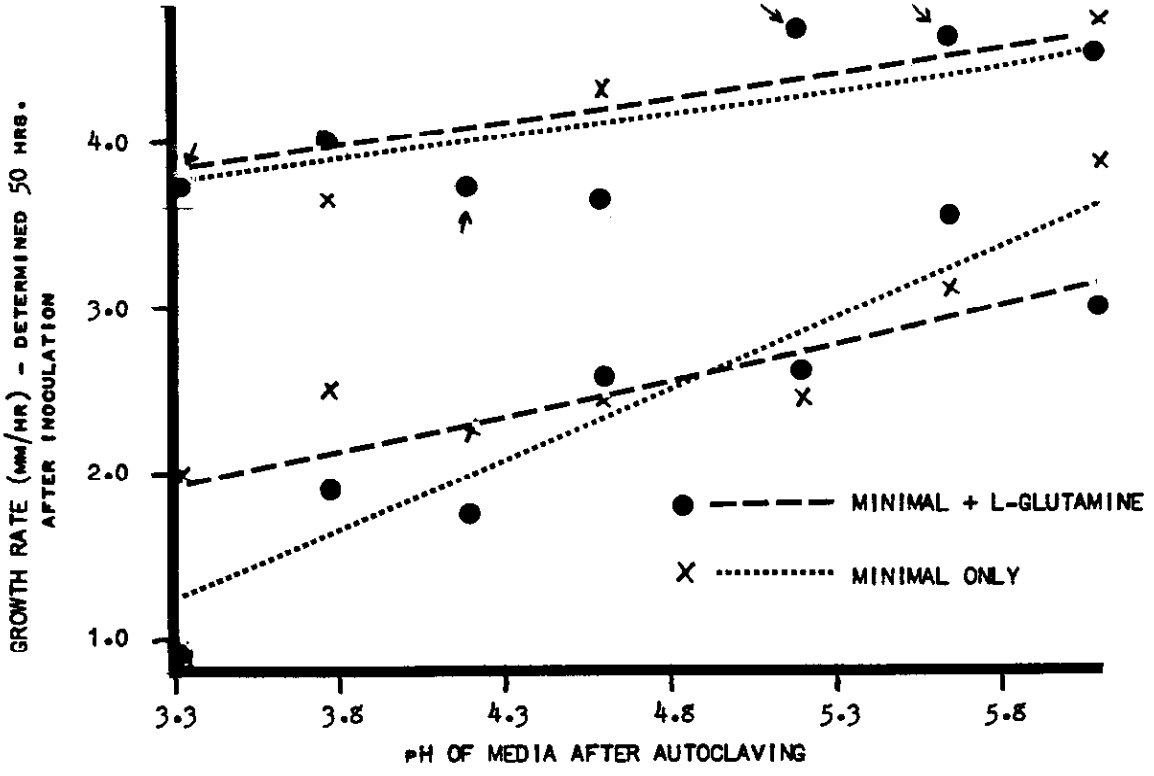

FIGURE 2. RELATION BETWEEN pH OF CULTURE MEDIA AND GROWTH RATE OF glm AND WILD TYPE, WITH AND WITHOUT L-GLUTAMINE. MEDIUM USED WAS MODIFIED SYNTHETIC CROSS (SEE TEXT) SOLIDIFIED WITH 2 PER CENT AGAR. pH WAS ADJUSTED WITH Na CITRATE-CITRIC ACID BUFFER WHICH WAS ADDED AFTER AUTOCLAVING. GROWTH CONDITIONS: GROWTH TUBES; INCUBATION TEMPERATURE 32 C; INOCULUM ONE LOOPFUL OF DENSE CONIDIAL SUSPENSION. UPPER CURVES WILD TYPE STA4 ; LOWER CURVES glm. DATA FROM SAME EXPERIMENT AS THOSE IN TABLE 1. THE GROWTH RATE OF glm ON MINIMAL MEDIUM N (pH 5.95) 50 HOURS AFTER INOCULATION WAS 0.0 MM/HR.

NH_4Cl). Further, growth of the glm strain was found to be progressively inhibited by increasing concentrations of NH_4Cl (Figure I, lower curve). Ammonium ion inhibition does not occur in media supplemented with L-glutamine (Figure I, upper curve). Either in NH_4Cl supplemented (2 g/l) or unsupplemented media, increasing amounts of citrate were without effect except at the highest concentration (4 g/l). It is perhaps worthy of note that the glm strain grown in media supplemented with 4 g/l of citrate plus L-glutamine showed evidence of a colonial growth habit and altered carotenoid pigmentation. The dry weight of glm mycelia grown in ammonium ion-free media supplemented with L-glutamine is over twice that when grown on medium N supplemented with L-glutamine (Figure I, point B), approaches that of wild type grown on medium N supplemented with L-glutamine (Figure I, point D), and exceeds that of wild type grown on the ammonium ion-free medium supplemented with L-glutamine (Figure I, point F). When grown in medium N, even the wild type strain is markedly stimulated by glutamine or asparagine (Figure I, points C, D, E). Apparently additional inhibitory components other than ammonium ions exist in medium N for the glm strain.

Experiments using flask cultures to investigate the effect of pH on the glm strain were inconclusive because it was impossible to control the pH changes which occur during growth. However, using the growth tube technique in which the growing mycelial frontier is constantly exposed to fresh media, results were obtained (Figure 2 and Table I). Under these conditions the growth rate of the glm strain on minimal, arbitrarily plotted 50 hours after inoculation, shows a marked pH dependence. This effect is largely obliterated in the presence of added L-glutamine. In contrast, the wild type strain is relatively insensitive to pH

Table 1

Effect of pH on 'lag' period. Experimental conditions given in legend to figure 2.

pH of media	Hours for initial 20 mm growth			
	glm		wild type	
	minimal	+ L-glutamine	minimal	+ L-glutamine
3.3	63.0	33.0	19.0	21.5
3.75	36.5	25.5	20.5	19.5
4.2	38.5	25.0	19.5	18.5
4.6	26.0	24.0	19.5	19.0
5.2	35.0	23.5	20.0	20.0
5.65	35.0	24.0	20.0	20.0
6.1	30.5	18.5	15.5	20.0
medium N				
5.95	>103.0	22.5	19.0	19.0

All cultures were sampled at the end of the growth period and showed no change in requirement.

over the range tested, and identical results were obtained with or without added L-glutamine. The effect of pH on the glm strain was minifested not only in growth rates but also in the duration of the 'lag' period prior to linear growth after inoculation (Table I). The effect was less marked in the presence of L-glutamine but persisted at pH 3.3. No 'lag' was noted for the wild type strain either with or without added L-glutamine (Table I).

In summary, the glm strain is inhibited by components in minimal medium N. One of these is ammonium ions. Ammonium ion inhibition can be overcome by L-glutamine. Growth in ammonium ion-free minimal synthetic cross medium equals that in medium N supplemented with L-glutamine. However, even in ammonium ion-free medium L-glutamine is markedly stimulatory for growth. Addition of L-glutamine to this medium results in growth of the glm strain nearly equal to wild type on medium N containing L-glutamine, and somewhat greater than wild type on synthetic cross medium supplemented with L-glutamine. In growth tubes the glm strains shows a sensitivity to low pH which is largely overcome by L-glutamine. Over the pH range investigated, the growth rate of the glm strain is less than that of wild type, but the final mycelial weight is equal. The mechanism of action of these phenomena has not been established. For optimal growth glm strains should be grown on Neurospora Culture Agar. To score isolates from crosses segregating for glm, medium N should give the clearest results. - - - Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire.