PHYSIOLOGICAL COMPARISON OF A NEWFOUNDLAND AND A NORTH CAROLINA ISOLATE OF *SAPROLEGNIA AUSTRALIS*

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SUMMARY

Two isolates of *Saprolegnia australis,* one from Newfoundland (NFLD) and one from North Carolina (NC), were compared physiologically. The NFLD isolate had a temperature optimum of 26 C for mycelial growth, and the NC isolate grew best at 29 C. The optimum *p*H ranges for growth were 4.75 to 5.7 for the NFLD isolate and 5.3 to 5.7 for the NC isolate. Any effects of various *p*H indicators on dry-weight yield were not detected. The optimum concentrations for phosphate were 10 to 15 mM for the NFLD isolate and 2.5 to 12.5 mM for the NC isolate. The optimum concentrations of vitamin-free casamino acids were 4 g/liter for the NFLD isolate and 12 g/liter for the NC isolate. In the presence of glucose, both isolates utilized L-aspartic acid, L-threonine, L-serine, L-proline, L-glutamic acid, glycine, L-alanine, L-α-amino-n-butyric acid, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine, L-histidine and L-arginine from casamino acids. The NC isolate was unique in producing L-1-methylhistidine and in utilizing L-ornithine. Both isolates produced ammonia and spermidine and utilized glucose during growth.

Representatives of *Saprolegnia australis* Elliott have been found in only three localities: New Zealand (Elliott, **1968**), North Carolina (Padgett, **1976**, **1978a**) and Newfoundland (Nolan and Maestres, **1978**; Maestres and Nolan, **1978**). Padgett (**1978a**) investigated, over a 2-mo period, the distribution of *S. australis* within estuarine systems based upon the ability to colonize sterilized hemp seeds placed at locations with different salinities; Maestres and Nolan (**1978**) studied, over a 1-yr period, the quantitative occurrence of *S. australis* in relation to 19 physical and chemical parameters in a river. Padgett (**1978b**) studied the pure-culture asexual response of *S. australis* growing on hemp seeds and the tolerance of zoospores to fluctuating and fixed salinity.

The purpose of the present study was to assess the degree of physiological similarity between two *S. australis* isolates from distinctly different habitats and separate geographical locations.

MATERIALS AND METHODS

Origin and maintenance of isolates.—The North Carolina isolate (W-10b; Padgett, **1976**) of *S. australis* was obtained from the American Type Culture Collection (ATCC 32940). The Newfoundland isolate (7–16D) was selected from amongst 332 isolates of *S. australis* obtained from Broadcove River near St. John's (Nolan and Maestres, **1978**; Maestres and Nolan, **1978**). Stock cultures of both isolates were maintained at 17 C on Emerson's yeast-extract, soluble-starch medium (EYPSS; Emerson, **1941**) in Petri dishes (9 cm) and transferred every wk.

Inoculum preparation and yield determination.—For both isolates Petri dishes (9 cm) containing 30 ml of EYPSS were inoculated in the center with a 4-mm plug (no. 1 cork borer) of material from a stock culture and incubated at 17 C. After 3 da the outer limits of growth were marked, and the culture allowed to grow for an additional 48 h. Plugs were then removed from along the 3-da growth line using the cork borer, and one plug was used as the inoculum for each flask in an experiment. The dry-weight variation of the plugs used as inocula was 1.1 ± 0.7 mg for the Newfoundland (NFLD) isolate and 1.8 ± 0.6 mg for the North Carolina (NC) isolate based upon 31 determinations for each isolate.

Culture yields were determined on a dry-weight basis in the same manner as in a previous study (Nolan and Lewis, **1974**). The mean dry-weight values for different treatments within an experiment were compared using Ducan's new multiple-range test (5% level; Steel and Torrie, **1960**).

Chemicals and stock solutions.—The *p*H indicators were obtained from Matheson, Coleman and Bell (Norwood, Ohio). The vitamin-free casamino acids were obtained from Difco Laboratories (Detroit, Michigan). The micronutrients were 'Baker Analysed' reagent grade (J. T. Baker Chemical Co., Phillipsburg, New Jersey). All stock solutions were prepared with double-distilled water and stored at 4 C in glass-stoppered bottles.

Preparation of glassware and media.—All glassware was chemically cleaned (concentrated sulphuric acid saturated with potassium dichromate) after having been washed. The glassware was then rinsed several times with double-distilled water. The combined volume of the media components was 50 ml. The components were mixed before

autoclaving at 120 C for 15 min. Experimental cultures were grown in 125-ml Bellco flasks with stainless steel caps (Bellco Glass, Inc., Vineland, New Jersey). The inoculated flasks were incubated on a horizontal, gyrotary shaker (150 rpm; Psycrotherm, Model G26, New Brunswick Scientific Co., New Brunswick, New Jersey) in darkness. For incubation temperatures see text.

The basic medium (medium A) contained: glucose, 5 g/liter; vitamin-free casamino acids, 1 g/liter; KH_2PO_4 plus K_2HPO_4, 10 mM; *p*H indicator (see text), 2 mg/liter; and a standard micronutrient solution plus disodium ethylenediaminetetraacetic acid (Na_2EDTA). The concentrations and sources of the elements were those given by Nolan (**1970**) as modified from Machlis (**1953**; medium B), and the Na_2EDTA concentration was from Nolan (**1970**).

p*H adjustments*.—The *p*H of the cultures was adjusted just prior to inoculation by the addition of either sterile 0.5 N potassium hydroxide or sterile 0.5 N hydrochloric acid from a calibrated syringe.

Determination of ninhydrin-positive substances in media.—The media from certain harvests were analyzed for the levels of ninhydrin-positive substances according to the technique given in Nolan and Lewis (**1974**). The polyamine determinations were made in a similar manner except that a column using a Beckman polystyrene resin (Beckman PA-35) and two eluting concentrations of sodium citrate buffer (*p*H 5.2; 0.35 and 2.35 N Na^+) were used.

Glucose determinations.—The media from various harvests were stored by freezing, and later Statzyme (Worthington Biochemical Corp., Freehold, New Jersey) determinations were made of the amount of glucose in the media and in the controls as given in the procedures in Nolan and Lewis (**1974**).

RESULTS

Optimum temperatures for vegetative growth.—The optimum temperatures were determined using standing cultures of medium A adjusted to *p*H 6.2 and incubated at 4, 10, 13, 16, 19, 23, 26, 29 and 32 C (FIG. 1). After 7 da of incubation, no growth had occurred at 32 C for the NFLD isolate; little growth occurred at 4 C for either the NFLD isolate (2 mg) or the NC isolate (1 mg). The yields were significantly better at 29 C for the NC isolate and at 26 C for the NFLD isolate (FIG. 1) than at any other temperatures [5% level; error degrees

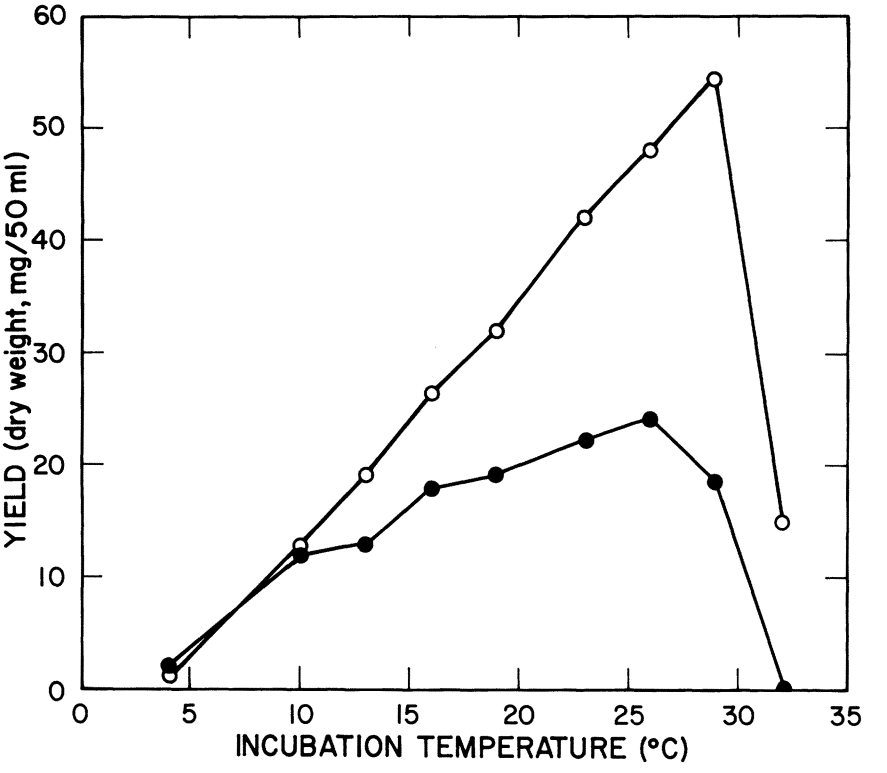

FIG. 1. The effects of various temperatures on mycelial dry weight yields for the Newfoundland (●—●) and North Carolina (○—○) isolates of *Saprolegnia australis* after 7 da of incubation. Each value represents the average of five determinations.

of freedom (d.f.) = 36 for each isolate]. In subsequent experiments, all flasks for both isolates were incubated at 25 C. The use of a single temperature, which was suboptimal for both isolates, allowed for the use of one incubator shaker without subsequent adjustment.

Effects of p*H indicators.*—The effects of several *p*H indicators (2 mg/liter) on the growth of the NFLD and NC isolates were studied using shake cultures of medium A adjusted to *p*H 6.2 and incubated at 25 C (for rationale, see Nolan, 1970). The results obtained after 3 da of growth in the presence of no added indicator (control), bromophenol blue, bromocresol green, methyl red, bromocresol purple, bromothymol blue or metacresol purple indicated that the yields of all treatments were not significantly different (5% level; error d.f. = 28) for each isolate.

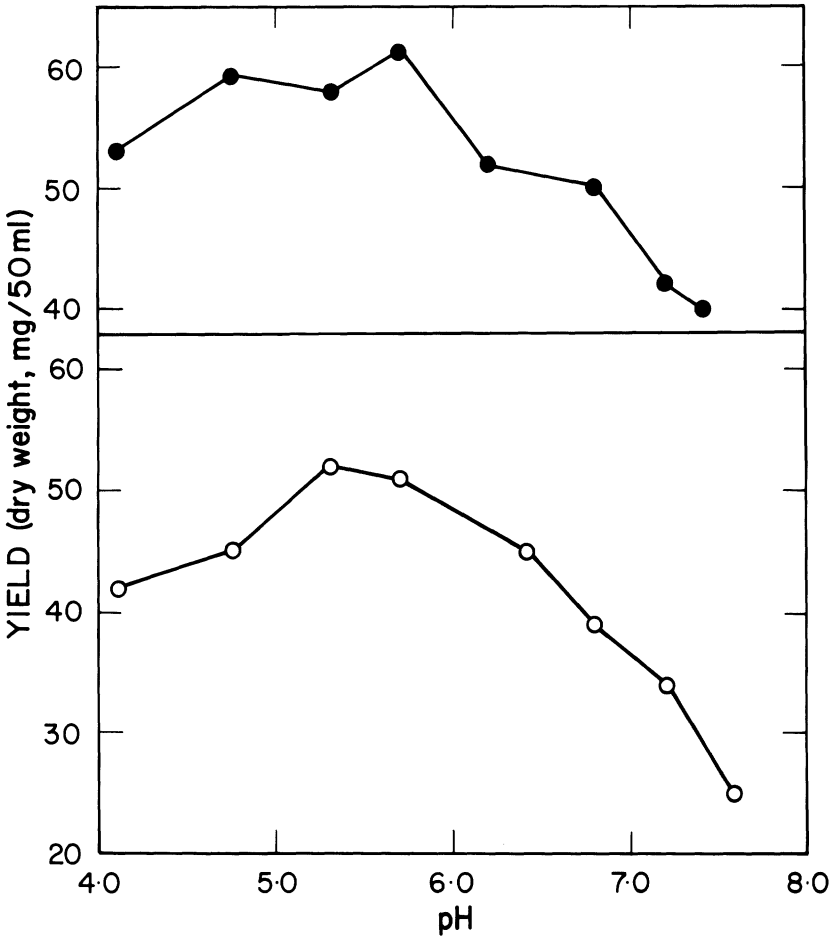

FIG. 2. The effects of various *p*H levels on mycelial dry weight yields for the Newfoundland (●—●) and North Carolina (○—○) isolates of *Saprolegnia australis* after 2 da of incubation. Each value represents the average of five determinations.

Optimum p*H for growth.*—The effects of *p*H on the growth of the NFLD and NC isolates were determined using shake cultures of a modified medium A containing either 20 mM phosphate (NC isolate, see next text section) or 10 mM phosphate (NFLD isolate, see next text section) and incubated at *p*H 4.1, 4.75, 5.3, 5.7, 6.2 (NFLD) and 6.4 (NC), 6.8, 7.2 and 7.4 (NFLD) and 7.6 (NC) at 25 C for 2 da. The results (FIG. 2) indicated that the *p*H range 4.75 to 5.7 was optimal for the NFLD isolate and that the *p*H range 5.3 to 5.7 was

optimal for the NC isolate. In subsequent experiments, media for the NFLD isolate were initially adjusted to *p*H 5.2 and media for the NC isolate were initially adjusted to *p*H 5.5.

Effects of phosphate concentration.—Two separate studies were made of the effects of total phosphate concentration on yield and *p*H control. The first study was conducted prior to determining the optimum *p*H for growth of the isolates. This initial study led to the adoption of a

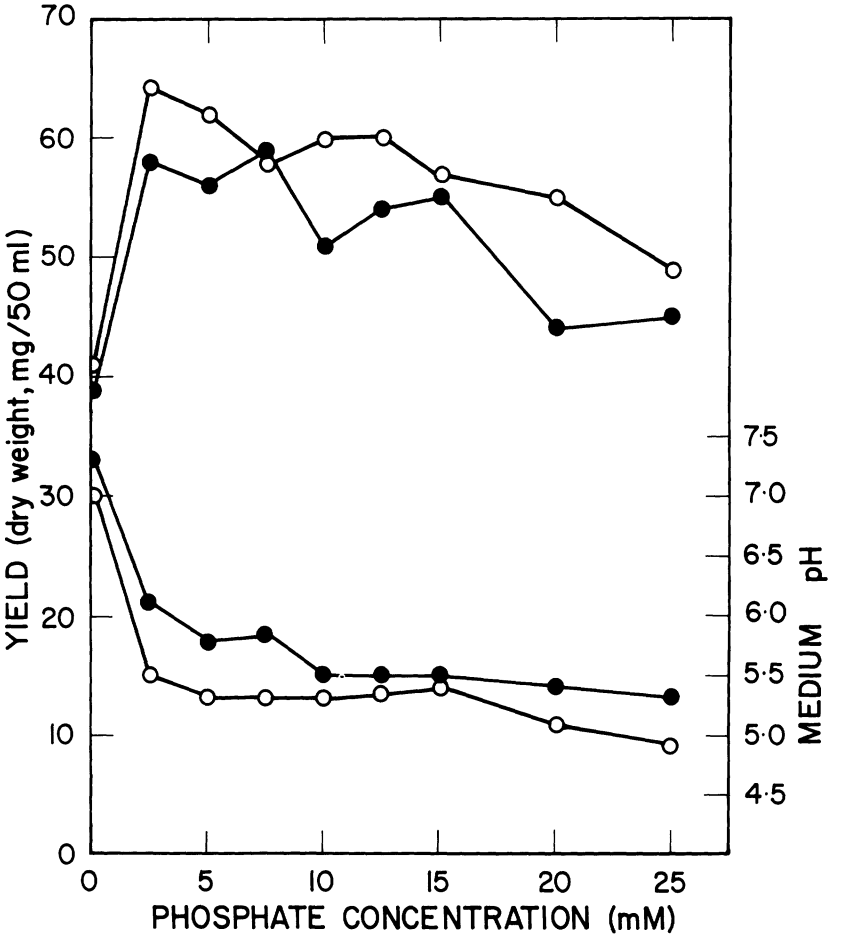

FIG. 3. The effects of phosphate concentration on mycelial dry weight yields and final medium *p*H for the Newfoundland (●—●) and North Carolina (○—○) isolates of *Saprolegnia australis* after 2 da of incubation. Each value represents the average of five determinations.

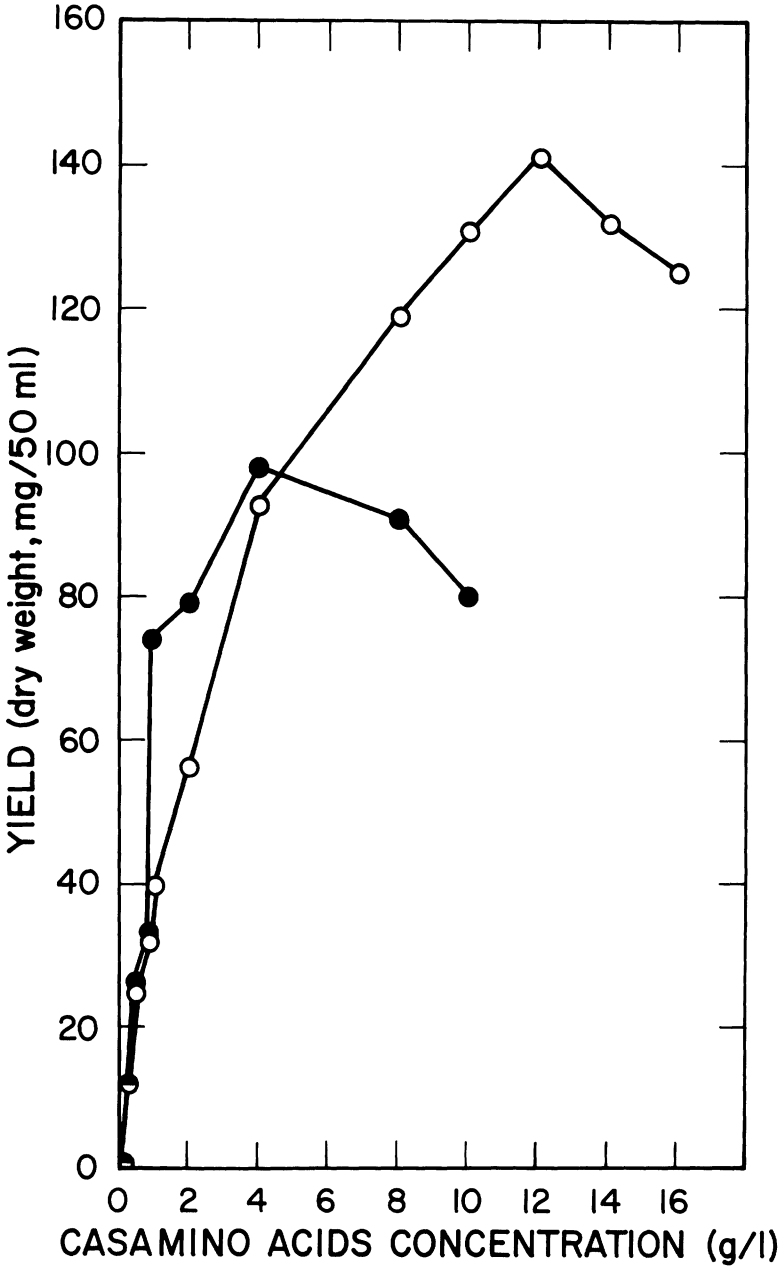

FIG. 4. The effects of vitamin-free casamino acids concentration on mycelial dry weight yields for the Newfoundland (●—●) and North Carolina (○—○) isolates of *Saprolegnia australis* after 2 da of incubation. Each value represents the average of five determinations.

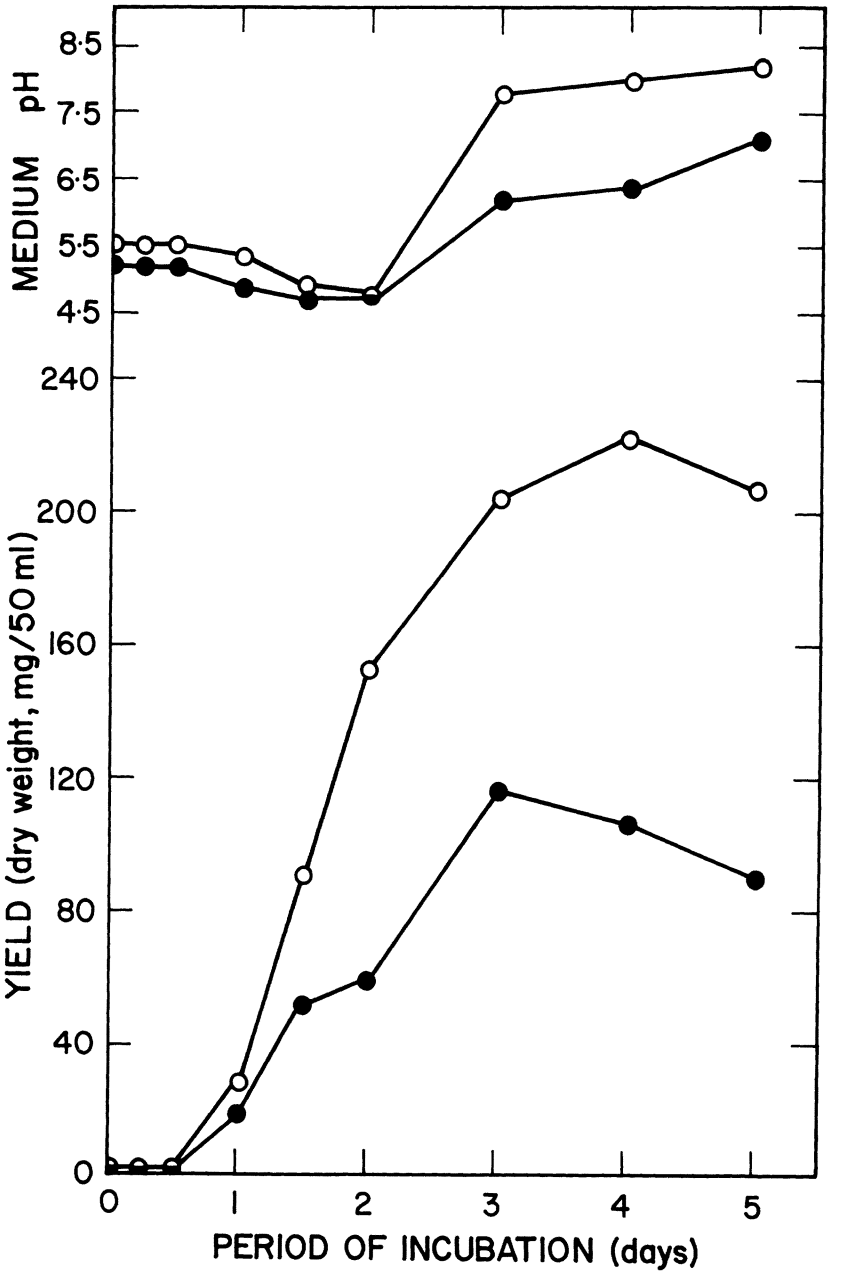

FIG. 5. The effects of period of incubation on mycelial dry weight yields and final medium *p*H for the Newfoundland (●—●) and North Carolina (○—○) isolates of *Saprolegnia australis*. Each value represents the average of five determinations.

20-mM total phosphate level for the NC isolate and of a 10-mM total phosphate level for the NFLD isolate.

The second study used shake cultures of modified medium A adjusted to either *p*H 5.2 (NFLD isolate) or *p*H 5.5 (NC isolate) and incubated at 25 C. The results obtained after 2 da of growth (FIG. 3) indicated that the optimum concentration ranges were 10 to 15 mM for the NFLD isolate and 2.5 to 12.5 mM for the NC isolate. In subsequent experiments, media for both isolates incorporated 12.5 mM phosphate as a level adequate for good mycelial growth and which also provided adequate buffering.

Optimum concentration of casamino acids.—The effects of the concentration of casamino acids were determined using shake cultures of a modified medium A adjusted to either *p*H 5.2 (NFLD isolate) or *p*H 5.5 (NC isolate) and containing 12.5 mM phosphate. The cultures were incubated at 25 C for 2 da. The study was conducted twice. In the first experiment, the casamino-acid concentrations ranged from 0 (control) to 8 g/liter. This concentration range was adequate to include the apparent optimum for the NFLD isolate; however, the optimum concentration for the NC isolate was in excess of 8 g/liter. Therefore, the experiment was run a second time with casamino-acid concentrations of 0 (control) to 10 g/liter for the NFLD isolate and concentrations of 0 (control) to 16 g/liter for the NC isolate (FIG. 4). The results indicated that the optimum concentrations were 4 g/liter for the NFLD isolate (5% level; error d.f. = 36) and 12 g/liter for the NC isolate (5% level; error d.f. = 48).

Glucose utilization and ninhydrin-positive-substance utilization from casamino acids.—Both studies were carried out during the same experiment. The experiment used shake cultures of a modified medium A containing either 12 g casamino acids/liter (NC isolate) or 4 g casamino acids/liter (NFLD isolate), 12.5 mM phosphate and adjusted to either *p*H 5.5 (NC isolate) or *p*H 5.2 (NFLD isolate). The growth curves for 5 da of incubation are given in FIG. 5 and indicate an initial lag period of 12 h for both isolates. The peak of the dry weight yields was achieved on da 3 for the NFLD isolate and on da 4 for the NC isolate. Both isolates produced increasingly more basic conditions after da 2 (FIG. 5) through the release of ammonia into the media (TABLES I and II). Of the substances originally detected in the growth media, only taurine, half cystine and L-ornithine were neither utilized nor produced by the NFLD isolate (TABLE I); whereas, only taurine and

TABLE I

UTILIZATION OF NINHYDRIN-POSITIVE SUBSTANCES BY *Saprolegnia australis* (NEWFOUNDLAND ISOLATE) AFTER INCUBATION IN MODIFIED MEDIUM A (CASAMINO ACIDS 4 G/LITER; 12.5 MM PHOSPHATE; *p*H 5.2)

Substance	Period of incubation (h)								
	0	6	12	24	36	48	72	96	120
Cysteic acid	23.2[a]	25.1	23.9	26.2	57.9	29.1	28.4	30.8	25.3
Taurine	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
L-Aspartic acid	1,082.2	1,270.3	1,208.4	1,212.1	980.5	981.2	424.9	405.7	6.1
L-Threonine	606.4	617.9	602.5	482.3	349.0	281.8	8.2	7.5	Trace
L-Serine	1,001.1	993.9	974.1	830.1	619.3	480.8	35.5	29.5	13.3
L-Glutamine	—[b]	—	—	—	—	—	—	20.0	Trace
L-Proline	1,797.7	1,766.5	1,662.3	1,185.3	135.6	61.4	Trace	Trace	Trace
L-Glutamic acid	2,371.2	2,656.6	2,599.7	2,553.7	2,357.7	1,819.2	434.5	129.8	50.1
Glycine	508.0	564.1	533.7	546.1	548.9	437.8	96.0	30.2	2.0
L-Alanine	712.9	748.2	739.5	758.7	725.4	587.5	77.4	67.1	23.7
L-α-Aminoadipic acid	—	—	—	—	—	—	—	3.7	7.3
L-α-Amino-n-butyric acid	8.0	5.9	5.9	5.0	10.9	10.0	Trace	—	—
Half cystine	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
L-Valine	626.7	659.5	618.2	604.0	719.6	506.6	43.5	12.1	6.5
DL-Cystathionine	15.1	16.7	5.9	7.5	7.4	7.6	5.0	5.2	6.6
L-Methionine	160.9	169.9	136.9	22.9	15.7	11.2	—	—	—
L-Isoleucine	240.1	244.9	219.4	206.8	200.9	152.4	6.0	—	—
L-Leucine	371.1	390.8	360.7	321.0	230.2	88.9	7.0	—	—
L-Tyrosine	41.4	42.6	39.0	40.0	11.0	4.0	Trace	—	—
L-Phenylalanine	185.6	200.9	194.4	40.0	15.4	5.0	Trace	—	—
γ-Aminobutyric acid	Trace	Trace	Trace	12.5	203.9	278.7	20.0	Trace	Trace
L-Ornithine	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Ammonia	434.8	458.5	463.1	598.0	1,398.0	1,935.6	4,156.5	6,387.5	8,639.8
L-Lysine	855.5	864.7	840.0	807.9	823.3	638.9	114.5	13.0	Trace
L-Histidine	195.6	187.1	182.6	126.6	50.7	26.3	—	—	—
L-Arginine	303.3	315.2	303.3	146.3	3.0	—	—	—	—

[a] Each value is the average of duplicate determinations and is given in nanamoles/ml.
[b] Not detected.

TABLE II

UTILIZATION OF NINHYDRIN-POSITIVE SUBSTANCES BY *Saprolegnia australis* (NORTH CAROLINA ISOLATE) AFTER INCUBATION IN MODIFIED MEDIUM A (CASAMINO ACIDS 12 G/LITER; 12.5 MM PHOSPHATE; *p*H 5.5)

Substance	Period of incubation (h)								
	0	6	12	24	36	48	72	96	120
Cysteic acid	25.0[a]	25.0	25.0	38.8	91.9	42.6	25.0	25.0	25.0
Taurine	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
L-Aspartic acid	4,117.7	3,994.1	4,222.0	4,245.7	4,176.8	4,211.3	40.8	34.1	22.7
L-Threonine	1,894.5	1,856.1	1,829.3	1,664.9	1,185.3	929.1	10.3	9.0	8.1
L-Serine	3,216.6	3,089.9	3,092.4	2,858.1	2,142.1	1,628.3	107.3	56.1	49.1
L-Glutamine	—[b]	—	—	9.0	53.0	53.0	20.4	15.2	7.9
L-Proline	5,311.8	5,332.1	5,303.8	4,258.0	1,101.8	131.1	—	—	—
L-Glutamic acid	8,580.1	8,384.1	8,661.8	8,636.2	7,472.4	6,342.8	198.7	124.1	122.9
Glycine	1,677.9	1,674.2	1,684.4	1,712.3	1,600.8	1,404.3	104.3	13.4	6.8
L-Alanine	2,291.3	2,290.0	2,272.4	2,361.8	2,729.8	2,061.3	207.2	152.6	108.6
L-α-Aminoadipic acid	—	—	—	—	—	—	105.2	47.3	50.7
L-α-Amino-n-butyric acid	23.2	20.8	22.4	22.4	53.2	65.6	20.8	10.0	—
Half cystine	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
L-Valine	1,875.0	1,625.9	1,795.7	1,816.7	1,946.1	1,901.3	138.1	15.2	9.5
DL-Cystathionine	6.4	6.4	11.5	8.7	33.2	13.7	8.0	8.0	8.0
L-Methionine	474.7	465.4	472.6	264.8	108.3	38.1	4.3	2.0	Trace
L-Isoleucine	749.8	748.0	770.5	742.0	784.4	766.6	15.1	3.5	—
L-Leucine	1,178.1	1,173.3	1,262.1	1,094.6	1,026.1	904.6	12.9	4.5	1.5
L-Tyrosine	160.0	159.9	160.0	177.6	209.0	107.8	15.8	8.0	—
L-Phenylalanine	570.6	561.1	582.8	404.3	113.9	23.9	—	—	—
γ-Aminobutyric acid	Trace	Trace	Trace	19.2	890.0	1,675.4	—	—	—
L-Ornithine	Trace	Trace	Trace	Trace	Trace	Trace	—	—	—
Ammonia	1,426.4	1,431.8	1,567.9	1,421.8	1,819.3	3,154.6	22,557.7	28,268.2	32,330.7
L-Lysine	2,714.4	2,686.1	2,693.2	2,616.2	2,396.6	2,304.1	258.7	8.0	—
L-Histidine	568.1	570.5	548.9	527.3	439.9	326.3	10.0	—	—
L-1-Methylhistidine	—	—	—	6.2	5.1	5.0	—	—	—
L-Arginine	958.9	948.6	958.1	717.2	247.7	10.0	—	—	—

[a] Each value is the average of duplicate determinations and is given in nanamoles/ml.
[b] Not detected.

half-cystine fell into this category for the NC isolate (TABLE II). The NC isolate utilized L-ornithine after 48 h of incubation. Both isolates demonstrated a consistent pattern of initial utilization of the media components L-threonine, L-phenylalanine, L-arginine, glycine, L-aspartic acid, L-valine, L-proline, L-methionine, L-histidine, L-serine, L-leucine, L-glutamic acid, L-alanine, L-α-amino-n-butyric acid, L-isoleucine, L-tyrosine and L-lysine during the period of rapid growth. Both isolates produced and then subsequently utilized cysteic acid, L-glutamine, and γ-aminobutyric acid during the incubation period. The NFLD isolate produced L-α-aminoadipic acid during the period of lysis, whereas the NC isolate produced this compound late in the rapid growth period and then subsequently utilized it. The NFLD isolate utilized DL-cystathionine after 6 h of incubation, whereas the NC isolate produced this substance after 24 h of incubation and then subsequently utilized it. The NC isolate produced L-1-methylhistidine after 12 h of incubation and then subsequently utilized it. In addition to the substances listed above, the analyzer was calibrated for DL-o-phosphoserine, glycerophosphoethanolamine, phosphoethanolamine, urea, methionine sulfoxide, hydroxy-L-proline, L-asparagine, sarcosine, L-citrulline, homocitrulline, glucosamine, galactosamine, β-alanine, DL-β-aminoisobutyric acid, DL + allo-δ-hydroxylysine, ethanolamine, L-3-methylhistidine, L-anserine, L-tryptophan, creatinine, carnosine, glutathione (reduced) and glutathione (oxidized) which were not detected in the initial media or after the growth of the isolates. The polyamine spermidine produced by the NFLD isolate was first detected after 12 h of incubation and by the NC isolate was first detected after 24 h of incubation. In the case of the NFLD isolate, spermidine was utilized after 36 h of incubation; whereas in the case of the NC isolate, spermidine was produced during the remainder of the period of incubation. The polyamines putrescine, cadaverine and spermine were neither present initially in the media nor produced during the period of incubation. During the lag period and the period of rapid growth, the NFLD isolate utilized 76% and the NC isolate utilized 92% of the glucose. Over the entire period of incubation, the NFLD isolate utilized 91% and the NC isolate 92% of the glucose.

DISCUSSION

The utilization of glucose and some ninhydrin-positive substances as carbon sources and of some ninhydrin-positive substances as nitrogen sources for mycelial growth by representatives of the Saprolegniaceae has

been previously reviewed in detail (see Nolan and Lewis, **1974**; Nolan, **1975, 1976**). Therefore, the discussion will be limited to the comparative aspects of the present study.

The slightly higher optimum temperature for mycelial growth for the NC isolate (29 C versus 26 C, see FIG. 1) and also the growth of the NC isolate at the maximum temperature tested (32 C) where none occurred for the NFLD isolate, would appear to reflect the adaptation of the NC isolate to growth in a temperate region. It should be noted that the isolates produced equivalent growth in the lower range of temperatures tested (10 C). In Newfoundland representatives of *S. australis* occur essentially throughout the yr with a maximum in July (water temperature ≅ 13 C) (Maestres and Nolan, **1978**). The optimum *p*H range for growth was broader and extended more into the acidic range for the NFLD isolate (*p*H 4.75 to 5.7 versus *p*H 5.3 to 5.7, see FIG. 2) with growth occurring for both isolates over the entire range tested. Newfoundland waters tend to be acidic because of the run-off from peat bogs. The river from which the NFLD isolate was obtained ranges from *p*H 5.5 to 6.6 (Maestres and Nolan, **1978**) and a nearby stream ranges from *p*H 5.5 to 6.0 (Nolan and Lewis, **1974**). Thus, the NFLD isolate appears to be adapted for growth in the more acidic environment.

An interesting outcome of the studies on the effects of casamino-acid concentration on mycelial growth is the tolerance of and requirement for much higher levels by the NC isolate (FIG. 4). Although this appears to be a simple reflection of a higher optimal level for nitrogen, it could be a "masked" requirement for a higher C/N ratio. The number of ninhydrin-positive substances capable of functioning as carbon sources for representatives of the Saprolegniaceae, however, is generally low (one to ten; see Nolan, **1975, 1976**).

The production of L-1-methylhistidine by the NC isolate is the second report for the fungi (see Nolan, **1976**). Both isolates produced ammonia during growth.

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