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Scopulariopsis brevicaulis: Effect of pH and Substrate on Growth

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Summary. Scopulariopsis brevicaulis Bainier NRRL 5867, isolated from ammonia-treated corn during preservation studies, was grown in shaken and still liquid cultures on Blakeslee's malt extract. The medium was adjusted to different pH values between 5.0 and 10.6 with sodium hydroxide and ammonium hydroxide. Maximum mycelium was produced at an initial pH of 9.0-10.0. Considerably more mycelium was produced in shaken flasks than in still cultures. When the initial pH was adjusted to 10.0 with ammonium hydroxide, 1350 mg mycelium/200 ml Blakeslee's malt extract was produced in contrast to 540 mg with sodium hydroxide. Approximately 28% of the total solids and 25% of the nitrogen in an ammoniated corn infusion broth were converted to mold mycelium high in essential amino acids and protein by both NRRL 5867 and NRRL 3273, another strain of S.brevicaulis. When S. brevicaulis was grown 7 days on a solid substrate of ammoniated corn, ammonia was converted to organic material, carbohydrate was utilized and the protein of the fermented corn increased in lysine and methionine. Approximately 9% of the weight of the corn was lost during the process.

Almost all research with Scopulariopsis brevicaulis Bainier has centered on the ability of the mold to convert arsenic in wallpaper or tapestry into poisonous gas (Thom & Raper, 1932). Although S. brevicaulis readily utilizes simple compounds of nitrogen, such as ammonia and nitrates, it grows quite commonly on substrates consisting largely of protein (Morton & Smith, 1963). Bainier (1907a,b) described S. brevicaulis from moldy cheese. Thom (1930) described how S. brevicaulis attacks Camembert cheese, overgrowing the Camembert mold, producing an ammoniacal odor and imparting an ammoniacal taste to the cheese.

Lorah et al. (1954) reported the same species growing on stored eggs. Thom (1930) indicated that S. brevicaulis grows on ham, both on the surface and deep into the tissues, imparting a moldy taste. Raper & Thom (1949) reported S. brevicaulis on wool, silk, soybeans, ground nuts and cellulosic materials. Scopulariopsis brevicaulis has also been implicated in the microbial flora of chicken litter (Halbrook et al., 1951; Schefferle, 1965; Lovett et al., 1971; Dennis and Gee, 1973). Allegedly, some strains of Scopulariopsis produce toxic metabolites (Christensen et al., 1968; Lovett, 1972; Martin et al., 1971) and also cause an infection of toenails(onychomycosis) in man (Morton & Smith, 1963).

Little biochemical work has been conducted with S. brevicaulis on foodstuffs. When insufficient carbohydrate is present, the mold apparently breaks down more protein than it uses for synthesis, liberates nitrogen as ammonia from the excess and utilizes carbon for energy production (Morton & Smith, 1963). Morton & Broadbent (1955) have shown that when the mold grows on media containing inorganic nitrogen compounds, extracellular nitrogen is produced in the form of peptides.

When we found S. brevicaulis growing on ammonia-treated corn during preservation studies (Bothast et al., in press), we explored the conditions for growth and the effect on substrate of this peculiar mold.

Experimental

Experiment 1. pH and Nitrogen

A typical isolate of S. brevicaulis NRRL 5867 from ammonia-treated corn was grown in duplicate shaken (200 rpm) and still flasks (1 liter) containing either 200 ml of Blakeslee's malt extract (BMX, 20 g malt extract; 20 g dextrose; 1 g peptone, per liter distilled H_2O) or 200 ml of Czapek's-Dox broth (CDB, 3 g $NaNO_3$; 1 g K_2HPO_4; 0.5 g $MgSO_4 \cdot 7H_2O$; 0.5 g KCl; 0.01 g $FeSO_4 \cdot 7H_2O$; 30 g sucrose, per liter distilled H_2O). These media were adjusted after sterilization to different pH values between 5 and 10.6 with 1N and 19N sodium hydroxide and with 1N and 15N ammonium hydroxide. The amount of alkali added was determined in preliminary titration studies. Aluminum foil was placed over

the mouth of each flask to block the escape of volatile ammonia. Each flask was inoculated with 1.0 ml suspension of spores prepared by washing conidia from a 7-day-old Czapek's agar slant with sterile distilled H_2O.

After incubation for 7 days at 28°C the mycelium produced in each flask was harvested by filtering on a fine mesh cloth and washing with 100 ml distilled H_2O. The mycelium from each flask was quantitatively transferred to a porcelain pan of known weight and dried for 16 hr at 100°C. Weights are reported as milligram of dry mycelium per 200 ml of medium or flask. Duplicate cultures agreed quite well in yield of mycelium, and the data reported in Tables 1 and 2 are the average

Table 1. Alkalinity (sodium hydroxide) enhances growth of *Scopulariopsis brevicaulis* in Blakeslee's malt extract (BMX)

Pre-fermentation pH	mg Dry mycelium /200 ml BMX	mg Mycelium /mg total N[a]	Post-fermentation pH
5.3	420	8.1	5.1
7.0	440	8.5	5.2
9.0	570	11.0	7.2
10.0	540	10.4	7.6
10.6	540	10.4	7.6

[a] BMX contains 52 mg N/200 ml

Table 2. Ammonium hydroxide enhances growth of *Scopulariopsis brevicaulis* in BMX

Pre-fermentation pH	mg Dry mycelium/ 200 ml BMX	mg N added to 200 ml BMX as NH_4OH	mg Mycelium/mg total N[a]	Post-fermentation pH
5.3	420	0	8.1	5.1
7.0	400	4.2	7.1	5.2
9.0	500	18.2	7.2	4.6
10.0	1350	105.0	8.6	4.4
10.6	10	693.0	0.01	9.9

[a] BMX contains 52 mg N/200 ml

of duplicates. Also, the post-fermentation pH of each spent medium was determined.

Experiment 2. Material Balance and Amino Acid Composition

An ammoniated corn-infusion broth (ACIB) was prepared by infusing in a four-layer bag of cheesecloth 200 g of whole corn (which had sufficient aqueous ammonia added to bring the ammonia content to 0.67%, dry basis) in 1000 ml of boiling H_2O for 30 min. The resulting broth had a pH of 8. ACIB (200 ml) was dispensed into flasks (1 liter) and sterilized. Duplicate flasks were inoculated as above with 1 ml spore suspensions of NRRL 5867 and NRRL 3273, another strain of S. brevicaulis. After incubation for 4 days at 28°C in shaken flasks (200 rpm), the mycelium was harvested as above and air dried at 25°C for 24 hr. Total solids were determined on the unspent and spent ACIB according to a modified AOAC (1970) procedure; total nitrogen was determined for both on an automatic analyzer (Uhl et al., 1971). Also total nitrogen (Uhl et al., 1971) and moisture (AOAC, 1970) were determined on the air-dried mycelium. Mycelia were combined (NRRL 5867 and 3273) and hydrolyzed by refluxing 24 hr in 6N HCl for amino acid analysis. A Beckman Spinco model 121 amino acid analyzer was used for the determination.

Experiment 3. Solid State Fermentation of Corn

Fermentations were carried out on two lots of corn--U.S. Grade No. 2, yellow dent corn (A) and a mold-damaged corn (B) that had been dried at high temperatures. Ten Fernbach flasks (2.8 liter) containing 500 g were set up for each lot. Approximately 0.8% (dry basis) ammonia (aqua) was added to each lot. Heat sterilization was not deemed necessary because of the fungicidal properties of ammonia (Bothast et al., 1973). The flasks were covered with gauze discs and aluminum foil and allowed to equilibrate for 24 hr. After the foil was removed, the treated corn was aired in a venting hood for an additional 24 hr. Distilled H_2O (150 ml/flask) was added to the treated corn and the mixture was allowed to stand for 24 hr. Five flasks from each lot were inoculated, as in experiment 1, with 1-ml spore

suspensions of NRRL 5867. Ten inoculated and 10 control flasks (five from each lot) then were incubated at 28°C and shaken manually, twice a day. Sterile water (25 ml) was added after 4 days to each inoculated flask.

After 7 days of fermentation, all flasks and contents were autoclaved for 15 min at 121°C. The contents were pooled according to treatment, spread out in a hood and allowed to equilibrate in the air for 24 hr. Fermentation weight losses then were calculated by comparing the weight of ammonia-treated corn (control) to S. brevicaulis fermented corn on a dry basis. The effect of ammonia and S. brevicaulis on total nitrogen, ammoniacal nitrogen, sucrose, reducing sugars and amino acids in each lot of corn was determined on ground samples. Total nitrogen was evaluated by the procedure of Uhl et al. (1971) and ammoniacal nitrogen according to Lancaster et al. (1974).

Sucrose and reducing sugars were extracted and analyzed by a modified AOAC (1970) procedure. Amino acids were determined as stated in experiment 2.

Results and Discussion

Experiment 1

Scopulariopsis brevicaulis grows best on an alkaline medium (Table 1). Almost all molds will not grow on a medium having a pH above 8.5, and the majority favor an acid pH (Frazier, 1958). The optimum pH appears to be about 9 for this mold, and its growth is not inhibited by a starting pH up to 10.6 in BMX or CDB. However, growth in CDB was only about onefifth that in BMX. Also, growth in shaken flasks was considerably greater than in still flasks; therefore, the data on CDB and still flasks are not included here. Scopulariopsis brevicaulis produced more mycelium/mg of nitrogen in the medium as alkalinity increased (Table 1), and post-fermentation pH remained high due to the concentration of unneutralized sodium.

These data are in contrast to those obtained when the initial alkalinity was controlled with ammonium hydroxide (Table 2). Up to pH 9, growth was similar to that with sodium hydroxide.

However at an initial pH of 10, ammonium hydroxide enhanced growth 2.5 times more than sodium hydroxide. The post-fermentation pH reflected removal and metabolism of ammonia. Overall, the efficiency of mycelium production per unit weight of nitrogen decreased. These results agree with those of Morton & Macmillan (1954) who observed incomplete assimilation of ammonia due to the fall in pH of the medium, induced by the initial uptake of ammonia. Also, ammonia dilution of the BMX protein may be involved in this less efficient use of total nitrogen. Nevertheless, growth was considerably greater if the pH was adjusted to 10 with ammonia. Since the post-fermentation pH dropped to the lowest level in this test, the average alkalinity was probably in the most favorable range in the tests with sodium hydroxide. The improved utilization of ammonia to produce mycelium is reflected in the improved ratio of mycelium to nitrogen. Scopulariopsis brevicaulis has the ability either to incorporate ammonia into mycelium, or extracellular peptides, or to produce ammonia from substrate protein depending on the availability of carbohydrate (Morton & Broadbent, 1955; Morton & Smith, 1963). Thus either nitrogen or carbon can limit growth.

The last entry in Table 2 illustrates the potency of large quantities of ammonia as a fungicide, in contrast to sodium hydroxide. Whether this fungicidal effect can be explained by lack of reaction of ammonia with substrate by chemical mechanisms to cause a reduction eventually in pH or by postulating a special inhibitory effect of soluble unionized ammonia is a moot question at this time. Evidently S. brevicaulis is not destroyed by media with alkalinity to pH 10.6, but it will not grow appreciably until the pH falls at least below 9.9.

Experiment 2

Two strains of S. brevicaulis had about the same effect on total solids and total nitrogen (Table 3) when grown on a natural substrate

Table 3. Growth of *Scopulariopsis brevicaulis* on ACIB[a]

Materials	mg/200 ml ACIB	
	Total solids	Total nitrogen
NRRL 5867		
Unspent medium	780	78
Spent medium	440	52
Utilized	340	26
Mycelium	232	21
Loss	108	5
NRRL 3273		
Unspent medium	780	78
Spent medium	460	54
Utilized	320	24
Mycelium	204	18
Loss	116	6

[a] ACIB (ammoniated corn-infusion broth) was prepared by infusing 200 g of ammonia-treated corn, tied in a four-layer bag of cheesecloth, in 1000 ml of boiling H_2O for 30 min

(ACIB). The energy cost of producing fungal mycelium is reflected by approximately 15% loss in total solids. Conversion of solids from a soluble form to mycelium produced 42% reduction in total solids and 32% reductions in total nitrogen. Approximately 28% of the total solids and 25% of the nitrogen were converted to mold mycelium.

Since an objective of certain foodstuff fermentations is to convert low-cost nitrogen (ammonia) to fungal protein, attention must be focused on nitrogen balance of the process. Prior experience and experiment 3 indicate little, if any, nitrogen loss when the mold grows on solid substrate. Although losses (6-8%) are reported in Table 3, we suspect that these may be in part artifacts of the analytical method. If losses did occur,

they could have been through evaporation of free ammonia from the substrate either present originally or produced by the mold from substrate protein. This latter phenomenon is one possibility that must be considered in using ammonia at high pH's in a fermentation medium. The total solid losses are not believed to be subject to the same degree of error as the nitrogen losses and are probably a good estimate of the use of carbohydrate to fuel fungal development in a fermentation of this type.

Almost all mold mycelia consist of reasonably high-quality protein (Rhodes et al., 1961). Our data (Table 4) indicate that protein constitutes approximately 40% of S. brevicaulis mycelium. Notably, lysine is higher than is found in cereal grains (Pike & Brown, 1967) with concomitant reduction in the proportion of glutamic acid and leucine.

Table 4. Amino acid composition of *Scopulariopsis brevicaulis* mycelium[a]

Amino acid	g/16 g Nitrogen
Lysine	5.4
Histidine	2.1
Ammonia	5.0
Arginine	4.4
Aspartic	7.3
Threonine	3.8
Serine	3.6
Glutamic	12.0
Proline	3.3
Glycine	4.8
Alanine	6.5
1/2-Cystine	0.7
Valine	4.4
Methionine	2.4
Isoleucine	2.9
Leucine	4.9
Tyrosine	2.4
Phenylalanine	2.7

[a] Composite mycelium of NRRL 5867 and 3273 on ACIB Yield 436 mg (7.11% N).

Experiment 3

Although solid-state fermentations are far from being technologically simple, ensiling agricultural commodities is an old practice. Scopulariopsis brevicaulis appears to be particularly adapted to growing on solid substrates, judging by its occurrence on such high-nitrogen substrates as meat, cheese, eggs, and chicken litter. Our most recent data (Table 5) show that the mold, when grown on ammonia-treated corn, efficiently converts ammonia to organic material with a loss in corn dry substance (9%) typical of an ensilage process. S. brevicaulis tends to use up both sucrose and reducing sugars (Table 5).

Table 5. Solid-state fermentation with Scopulariopsis brevicaulis plus ammonia changes constituents of corn

	Weight %, dry basis			
Sample identity[a]	Total N	Ammoniacal N	Sucrose	Reducing sugars
A (control)	1.51	0.016	1.93	0.35
A + NH_4OH	1.77	0.234	2.14	0.38
A + NH_4OH + 5867[b]	1.99	0.098	1.46	0.24
B (control)	1.62	0.012	2.43	0.27
B + NH_4OH	1.90	0.181	1.89	0.59
B + NH_4OH + 5867[c]	2.09	0.032	1.04	0.21

[a] A was a U.S. No. 2 yellow dent corn. B was a mold-damaged corn dried under extremely high temperatures

[b] Fermentation produced a 10.0% loss in dry weight

[c] Fermentation produced a 8.0% loss in dry weight

As expected from the work of Morton & Broadbent (1955) and the amino acid analysis of S. brevicaulis mycelium, protein quality is changed by the fermentation (Table 6). Amino acids are expressed in Table 6 as a percentage of those listed, rather than as g/16 g nitrogen. This percentage allows a more accurate comparison since it corrects all treatments to an ammonia-free basis. Lysine, methionine and proline increased in proportion to total amino acids while a decrease was noted in glutamic acid, leucine, alanine and phenylalanine. These changes are

Table 6. Scopulariopsis brevicaulis plus ammonia changes the amino acid composition of corn

Amino acid	Control	Weight % of those listed[a]	
		Corn + NH_4OH	Corn + NH_4OH + 5867[b]
Lysine	3.4	3.4	3.8
Histidine	3.6	3.4	3.1
Arginine	3.8	4.0	3.5
Aspartic	6.5	6.4	6.8
Threonine	3.4	3.4	3.6
Serine	4.6	4.8	4.8
Glutamic	17.9	18.3	16.3
Proline	12.9	12.0	14.4
Glycine	3.7	3.6	3.6
Alanine	7.4	7.6	6.7
1/2-Cystine	1.2	1.0	1.0
Valine	4.5	4.8	4.5
Methionine	2.6	2.8	5.7
Isoleucine	3.9	3.8	3.7
Leucine	12.1	12.2	10.2
Tyrosine	3.1	3.2	2.8
Phenylalanine	5.4	5.3	4.9

[a] Mean values of lots A and B

[b] Seven-day fermentation

consistent with a preliminary experiment, except for the increase in methionine which needs further study. In addition to the result that the final fermented product was higher in certain amino acids than untreated corn, the total amount of protein the corn contained also increased because of the loss in carbohydrate and mycelium synthesis.

Of fermentative process, one of the most important to man is the nitrogen cycle. That S. brevicaulis fits ecologically into this natural process at least in the ammonia-protein portion cannot be doubted. Since S. brevicaulis prefers alkaline and high-nitrogen substrates, it is an ideal organism for studying mold growth under less than ideal mycological conditions. What specific function it performs in the deterioration or synthesis of foods and fiber remains to be determined.

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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