CHEMICALLY DEFINED MEDIA FOR SYNNEMATIN PRODUCTION[1]

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Cephalosporium salmosynnematum was found by Gottshall *et al.* (1951) to produce the antibiotic synnematin. Synnematin was later separated into two components, synnematin A and synnematin B (Olson *et al.*, 1953). Cephalosporin N, one of the antibiotics produced by a species of the genus Cephalosporium, was found to be identical to synnematin B (Abraham *et al.*, 1955). Cephalosporin N was shown to be (D-4-amino-4-carboxy-*n*-butyl-) penicillin (Newton and Abraham, 1954). Synnematin B was found to be an effective therapeutic agent against typhoid (Benavides *et al.*, 1955).

The presence of the perfect stage in the life cycle of Cephalosporia together with other morphological similarities have indicated that *C. salmosynnematum* belongs to the genus *Emericellopsis* in the family Aspergillaceae (Grosklags and Swift, 1957). It was found that several members of the genus *Emericellopsis* produce antibiotics similar to synnematin.

Chemically defined media suitable for the growth of *C. salmosynnematum* have been reported (Pisano *et al.*, 1954). Olson and Jennings (1954) found a medium containing corn meal, soybean meal, ammonium sulfate, and calcium carbonate suitable for synnematin production with *C. salmosynnematum*. A chemically defined medium for the production of synnematin has not yet been reported. Natural media, because of their extreme chemical complexity, are not well adapted to studies of chemical changes during fermentation. The work reported here was undertaken to develop a chemically defined medium for synnematin production with *C. salmosynnematum* and *Emericellopsis terricola* var. *glabra*.

MATERIALS AND METHODS

Fermentation techniques. Cephalosporium salmosynnematum strain 3590A (obtained from B. H. Olson, Division of Laboratories, Michigan Department of Health, Lansing, Michigan) and *Emericellopsis terricola* var. *glabra* (obtained from the Department of Botany, University of Wisconsin) were used in these experiments. The cultures were maintained on soil in the manner described by Backus and Stauffer (1955). *C. salmosynnematum* spores were grown on Czapek-Dox agar medium and *E. terricola* spores were grown on honey-peptone-agar medium. The Czapek-Dox agar medium contained (per L): sucrose, 30 g; $NaNO_3$, 3.0 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7\ H_2O$, 0.01 g; and agar, 20 g. The honey-peptone-agar medium contained (per L): honey, 60 g; peptone (Difco), 10 g; and agar, 15 g. Ten ml of sterile water were dispensed into each 6 ounce spore bottle to prepare the spore suspension.

With *C. salmosynnematum*, 5 ml of the spore suspension were used to inoculate 50 ml of the first stage inoculum medium in 500 ml Erlenmeyer flasks. The first stage inoculum medium contained (per L): glucose, 40 g; L-glutamic acid, 10 g; and salts mixture (composition in table 1), 100 ml. The inoculum flasks were incubated on a Gump rotary shaker for 48 hr and 5 ml of the vegetative growth obtained were used to seed 50 ml of the second stage inoculum medium. The second stage inoculum medium contained (per L): glucose, 40 g; ammonium sulfate, 8 g; calcium carbonate, 10 g; and salts (composition in table 1), 100 ml. Five ml of the vegetative growth obtained after the flasks had been shaken for 24 hr were used to seed 100 ml of the fermentation medium.

With *E. terricola* var. *glabra*, 5 ml of the spore suspension were used to inoculate 50 ml of inoculum medium. The inoculum medium contained (per L): glucose, 40 g; ammonium sulfate, 8 g; calcium carbonate, 10 g; biotin 100 μg; and salts (composition in table 1), 100 ml. Five ml of the vegetative growth obtained after these flasks had been incubated for 48 hr were used to seed 100 ml of the fermentation medium.

The inoculum was always grown on 50 ml medium in 500 ml Erlenmeyer flasks. All fermen-

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tations, except when indicated otherwise, were run in 500 ml Erlenmeyer flasks containing 100 ml of medium. The flasks were placed on a Gump rotary shaker which operated at 250 rpm and described a 2¼ in circle. The temperature of incubation was 30 C in all cases. All fermentations were run in duplicate and the results reported here are the averages of two flasks.

All synthetic media contained 10 ml of salt mixture per 100 ml of medium. The composition of the salt mixture is given in table 1.

After sterilization, the pH of all media was adjusted with sulfuric acid to approximately pH 6.

Samples for synnematin assay and chemical analyses were taken under aseptic conditions and handled in the manner described by Gailey *et al.* (1946).

Analytical procedures. Synnematin was assayed by the Oxford cup method with *Staphylococcus aureus* as the test organism and synnematin B (obtained from B. H. Olson, Division of Laboratories, Michigan Department of Health, Lansing, Michigan) was used as the standard.

The pH was determined with a glass electrode immediately after removal of the sample. All sugars were determined by the method of Shaffer and Somogyi (1933). Sucrose was hydrolyzed with 1 N sulfuric acid at 100 C for 10 min. Maltose was hydrolyzed with 1 N sulfuric acid at 100 C for 40 min. Lactose was hydolyzed with 1.5 N sulfuric acid in an autoclave at 120 C for 15 min. Starch was hydrolyzed with 2 N sulfuric acid in an autoclave at 120 C for 25 min. Standard curves were prepared for every carbohydrate except starch, for which the glucose standard curve was used. Ammonia nitrogen was determined by the method described by Gailey *et al.* (1946). Soluble Kjeldahl nitrogen was determined by the method of Johnson (1941). The mycelial nitrogen was determined by subtracting the soluble nitrogen present at the time of sampling from the soluble nitrogen present at the time of inoculation.

Materials. Ground whole corn meal obtained from the University of Wisconsin farms was used. Soybean meal was obtained as soybean oil meal flakes from the Archer-Daniels-Midland Co. (Decatur, Illinois). The soybean meal extract was prepared by mixing 100 g of soybean meal with 1500 ml of boiling water for 30 min. The mash was then filtered through two thicknesses of cheesecloth. This procedure gave approximately 1000 ml of the extract.

TABLE 1

*Composition of salt mixture**

	g
KH_2PO_4	10.9
$MgSO_4 \cdot 7H_2O$	8.12
Na_2SO_4	4.45
$CaCl_2 \cdot 2\ H_2O$	2.42
$Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$	0.28
$CuSO_4 \cdot 5\ H_2O$	0.033
$MnSO_4 \cdot H_2O$	0.02

* Water was added to make 1000 ml.

The mixture of 19 different amino acids was was sterilized by filtration through a Seitz filter. For use, enough of this solution was added to give 33 mg of each amino acid per 100 ml of medium.

The mixture of B vitamins was sterilized by filtration through a Seitz filter. For use, an aliquot of this solution was added to give the following amounts of each vitamin per 100 ml of medium: thiamin hydrochloride, 0.075 mg; calcium pantothenate, 0.15 mg; nicotinamide, 0.15 mg; *p*-aminobenzoic acid, 0.025 mg; riboflavin, 0.1 mg; folic acid, 0.025 mg; biotin, 0.025 mg; pyridoxine hydrochloride, 0.05 mg; and choline, 0.15 mg.

DL-α-Aminoadipic acid was dissolved as the sodium salt and the pH adjusted to 6. It was sterilized by filtration through a Seitz filter.

RESULTS

Conditions for the growth of C. salmosynnematum. Glucose, maltose, sucrose, and starch were utilized rapidly and good growth was obtained. Xylose and lactose were utilized at a slow rate and poor growth was obtained. Since good growth was obtained with glucose, it was used as the main carbon source throughout these studies.

Pisano *et al.* (1954) reported that inorganic nitrogenous compounds were incapable of replacing organic nitrogenous compounds as the sole source of nitrogen in a medium. In our laboratory the growth obtained on inorganic nitrogenous compounds in media of the proper composition was found to compare favorably with that obtained on single organic nitrogenous compounds. The results are given in table 2. The utilization of ammonia during the growth phase in the glucose-ammonium sulfate-salts medium caused a rapid drop in pH. It was found that pH values lower than 4 were unsuitable for

TABLE 2

Effect of the composition of media on growth of Cephalosporium salmosynnematum

Nitrogen Source		$CaCO_3$	pH		Mycelial N
			0 hr	48 hr	
	%	%			μg/ml
$(NH_4)_2SO_4$	0.4	0.0	6.0	4.0	64
$(NH_4)_2SO_4$	0.4	0.5	6.1	6.85	750
$(NH_4)H_2PO_4$	0.4	0.0	6.0	4.0	76
$(NH_4)H_2PO_4$	0.4	0.5	6.1	6.75	800
NH_4-Citrate (dibasic)	1.0	0.0	5.8	5.7	600
$NaNO_3$	0.4	0.8	5.9	7.6	good growth†
L-Glutamic acid*	0.5	0.0	6.2	6.4	600

All media contained 4 g of glucose and 10 ml of salt mixture per 100 ml of medium.

* Data from Pisano *et al.* (1954).

† Analysis for nitrate-N not performed.

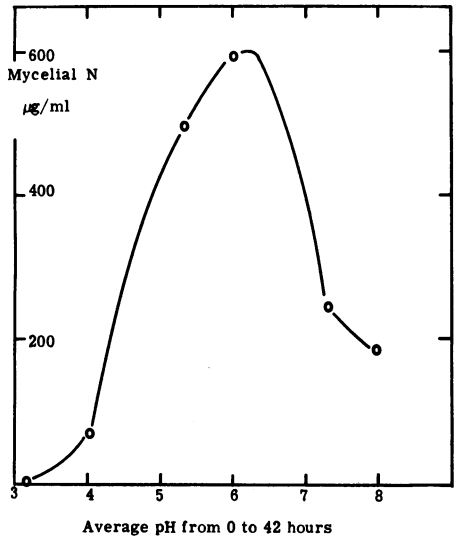

Figure 1. Effect of pH on the growth of *Cephalosporium salmosynnematum*. The medium contained glucose, 4 g; dibasic ammonium citrate 1.6 g; and salts, 10 ml per 100 ml of medium. The pH values of the media were adjusted at 8 hr intervals to the proper pH level. Analyses were made after 42 hr.

growth. When calcium carbonate was added to the same medium the pH was maintained in a a region suitable for growth, approximately pH 6. Therefore good growth was obtained on media containing calcium carbonate.

The optimum pH for growth was studied in media containing glucose, diabasic ammonium citrate, and salts. The media were adjusted initially to different pH levels, and the growth obtained in 42 hr was determined. The pH of the media was adjusted manually at 8 hr intervals. The drifts in pH were small and were taken into account in the preparation of the data. The results are shown in figure 1. The optimum pH for the growth of the mold was found to be approximately 6. Chemical analysis at the end of 42 hr showed that the growth was not limited by the exhaustion of sugar or nitrogen.

Synthetic media for synnematin production with C. salmosynnematum. It has been found for penicillin (Jarvis and Johnson, 1947) and several other antibiotics (Biffi *et al.*, 1954; Corum *et al.*, 1954) that the fermentation can be divided into a rapid growth phase and an antibiotic producing phase. During the latter phase the mycelium should be maintained under adequate aeration, at a pH level suitable for antibiotic production, and under conditions which support a very slow rate of growth. Similar observations were made with *C. salmosynnematum.* The slow rate of growth necessary during the synnematin producing phase was obtained by slowly feeding glucose at the rate of 0.4 g per 100 ml at 12 hr intervals.

When synnematin fermentations were carried out in a medium containing corn meal, soybean meal, calcium carbonate, and ammonium sulfate, it was found that the pH during the synnematin production phase was between 7 and 7.8. The effect of pH on synnematin yield was then studied in a synthetic medium containing glucose, ammonium sulfate, calcium carbonate, and salts. The initial pH values of all fermentations were approximately 6. At the end of 40 hr, when the growth phase was essentially completed, the media were adjusted with sodium hydroxide to

different pH values. The fermentations were maintained at the required pH levels by manual adjustment with acid or alkali. No significant differences in yields were observed when the pH during the synnematin producing phase ranged from 6.6 to 7.6. The yields obtained averaged 33 units per ml.

From the foregoing considerations it was apparent that for good synnematin production the growth phase should progress at a pH of about 6 and the synnematin producing phase at the pH range of 6.6 to 7.6. However, media devised to give such pH control did not give good synnematin yields. DL-α-Aminoadipic acid was tested as a precursor for synnematin. Lysine has been shown (Rothstein and Miller, 1954) to be converted by rats to glutaric acid via α-aminoadipic acid and α-ketoadipic acid. Therefore lysine was tested for its effect as possible precursor of the α-aminoadipic acid side chain of synnematin. The effect of the addition of a mixture of B vitamins and amino acids to the basal medium on the synnematin yield was also studied. The results are given in table 3. No significant increase in yield was obtained with any of these substances.

TABLE 3

Media for synnematin production with Cephalosporium salmosynnematum

The basal medium contained glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; and salts, 10 ml per 100 ml of medium. Glucose (0.4 g) was added to all fermentations at 12 hr intervals starting from 48 hr. DL-α-Aminoadipic acid and DL-lysine were added at a level of 0.1 g at 24 hr intervals starting from 48 hr. The composition of the mixture of amino acids and B vitamins included in the medium is given in the section on Methods.

Supplement	pH Plateau*	Synnematin
		units per ml
None (basal medium)	7.4	36
DL-α-Aminoadipic acid	7.7	37
DL-Lysine	7.4	32
Amino acids plus B vitamins	7.4	35

* The pH plateau is defined as the pH during the synnematin producing phase.

Synnematin production with E. terricola. The production of synnematin with *E. terricola* was studied with several natural materials. The yields are compared with those obtained with *C. salmosynnematum* in table 4. It was found that much better yields were obtained with *E. terricola* than with *C. salmosynnematum*. Further studies on synthetic media were made with *E. terricola*

Growth factor requirement of E. terricola. It was found that a basal medium containing glucose, ammonium sulfate, calcium carbonate, and salts did not support the growth of the mold. The effect of adding a mixture of amino acids and a mixture of B vitamins to the above medium was studied. The medium containing the mixture of amino acids was unable to support the growth of the mold. However, good growth was obtained on media containing either the B vitamins or a mixture of the B vitamins and amino acids. The B vitamins were then added individually to the basal medium and it was found that the medium containing biotin would support the growth of the

TABLE 4

Synnematin production on natural materials

Culture	Medium	Vol of Medium	Synnematin
		ml	*units per ml*
Cephalosporium salmosynnematum	Corn meal-soybean meal*	100	160
Emericellopsis terricola var. *glabra*	Corn meal-soybean meal*	100	300
Cephalosporium salmosynnematum	Soybean meal extract†	100	30
Emericellopsis terricola var. *glabra*	Soybean meal extract†	100	400
Emericellopsis terricola var. *glabra*	Soybean meal extract‡	50	900

* The corn meal-soybean meal medium contained the following per 100 ml of medium: corn meal, 4 g; soybean meal, 4 g; ammonium sulfate, 0.1 g; and calcium carbonate, 1 g.

† The medium contained (per 100 ml) glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; salts, 10 ml; and soybean meal extract, 40 ml. The method of preparation of the soybean extract is given under Methods.

‡ The medium had the same composition as the above medium. Cottonseed oil was added at a level of 0.2 ml at 12 hr intervals starting from 24 hr.

TABLE 5

Effect of different additives on growth of Emericellopsis terricola

Additive	Mycelial N
	μg/ml
None (basal medium*)	15
Amino acids†	25
B-Vitamins†	1123
Amino acids plus B vitamins	1200
Biotin	1100
B Vitamins except biotin	160

* The basal medium contained glucose, 2 g; ammonium sulfate, 0.4 g; calcium carbonate, 0.5 g; and salts, 5 ml in a total volume of 50 ml.

† The composition of amino acids and B vitamins is given under Methods.

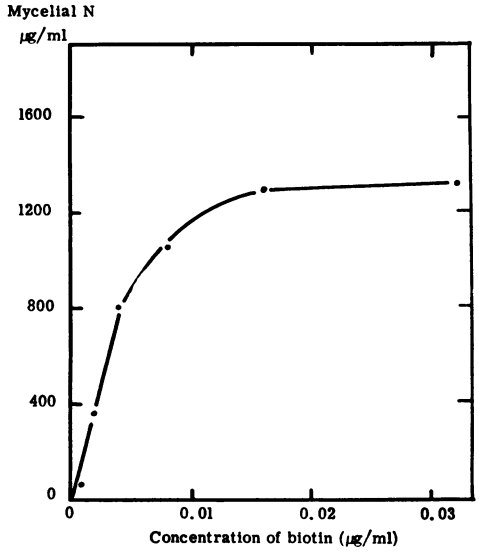

Figure 2. The effect of the concentration of biotin on the growth of *Emericellopsis terricola*. The medium contained glucose, 2 g; ammonium sulfate, 0.4 g; calcium cargonate, 0.5 g; and salts 10 ml; in a total volume of 50 ml.

mold. The results of these fermentations are given in table 5.

The level of biotin needed for the growth of the mold was investigated by adding it at different levels to the above basal medium and determining the growth obtained at the end of the growth phase. The results are given in figure 2. As may be seen from the figure, 0.02 μg of biotin per ml of medium was sufficient to support the maximum growth (1300 μg of mycelial nitrogen per ml) of the mold. In all subsequent fermentations, unless otherwise mentioned, 0.1 μg of biotin was included per ml of medium.

TABLE 6

Carbohydrate utilization by Emericellopsis terricola

Carbohydrate	Carbohydrate Utilized in 24 hr	Mycelial N Formed in 24 hr
	mg/ml	μg/ml
Glucose	27.0	1128
Sucrose	22.0	902
Maltose	24.0	850
Xylose	1.6	30
Galactose	4.0	142
Lactose	1.0	55

All media contained carbohydrate, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; biotin, 10 μg; and salts, 10 ml in a total volume of 100 ml.

Carbon requirement of E. terricola. The carbohydrate utilization by the mold was studied in a medium containing ammonium sulfate, calcium carbonate, salts, biotin, and the carbohydrate. The results are given in table 6. Glucose, maltose, and sucrose were utilized at a rapid rate and good growth was obtained. Xylose, galactose, and lactose were used at a very slow rate and poor growth was obtained. Since good growth was obtained with glucose, all further studies were made with glucose as the main carbon source.

Nitrogen requirement of E. terricola. It was found that the mold was capable of utilizing various forms of nitrogen compounds. In media of the proper composition good growth was obtained on inorganic nitrogenous compounds. The results are given in table 7. Since good growth was obtained with ammonium sulfate, it was used, unless otherwise mentioned, in all subsequent experiments.

Optimum pH for growth of E. terricola. The optimum pH for growth was studied in a medium containing glucose, dibasic ammonium citrate, biotin, and salts. The pH was adjusted manually at 8 hr intervals and was averaged over the period of the experiment. The results are shown in figure 3. The pH range of 4.75 to 6.5 was found to be optimum for the growth of the mold. Unless otherwise indicated, the initial pH of the medium

TABLE 7

Effect of composition of medium on growth of Emericellopsis terricola

All media contained glucose, 4 g; calcium carbonate, 1 g; salts, 10 ml; and biotin, 10 μg, in a total volume of 100 ml. Salts and biotin were not added to the corn steep liquor medium.

Nitrogen Source		Mycelial N
	g/L	*μg/ml*
Ammonium sulfate	8.0	1400
Ammonium chloride	7.0	1230
Ammonium lactate	10.75	1300
Ammonium citrate (dibasic)	10.0	1200
Corn steep liquor	30.0 (dry wt)	1500

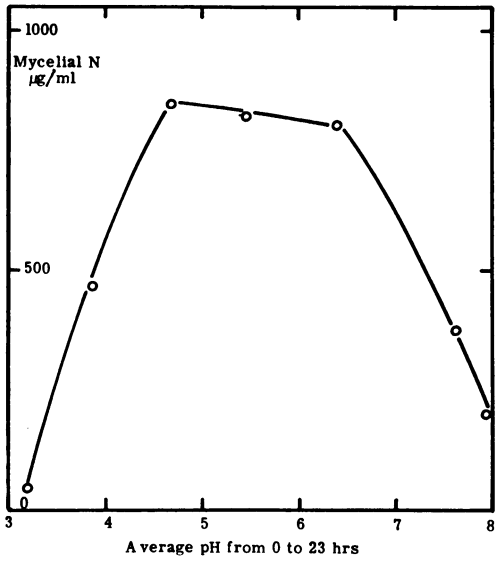

Figure 3. Effect of pH on the growth of *Emericellopsis terricola*. The medium contained glucose, 4 g; ammonium citrate (dibasic), 1 g; biotin, 10 μg; and salts, 10 ml; in a total volume of 100 ml. The pH values of the medium were adjusted to the proper levels at 8 hr intervals. Analyses were made after 23 hr.

was always adjusted, under aseptic conditions, to 6 after sterilization.

Synthetic medium for synnematin production with E. terricola. It was found that the maximum rate of synnematin production was obtained under fermentation conditions which supported a very slow rate of growth during the synnematin production phase. This result was obtained from several fermentations and is shown in figure 4. The slow rate of growth during the synnematin producing phase was obtained by slowly feeding glucose at the rate of 0.4 g per 100 ml at 12 hr intervals. When galactose, xylose, or lactose (sugars which are very slowly utilized by the mold) were used in attempts to maintain a slow rate of growth during the synnematin production phase, autolysis took place with resulting rise in pH and low synnematin yields.

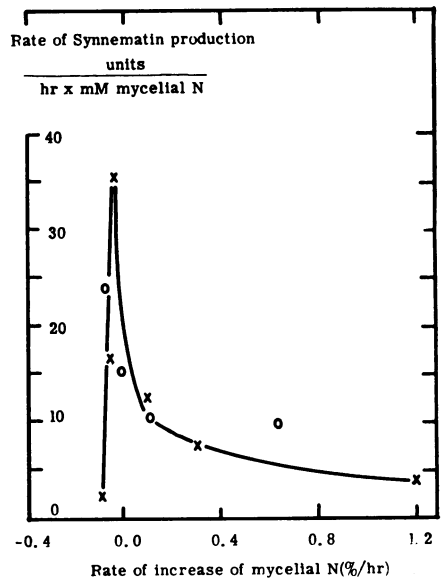

Figure 4. Effect of the growth rate on the rate of synnematin production. Negative growth rates indicate autolysis. ×, Fermentations conducted on a medium containing (per 100 ml) glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; salts, 10 ml; and B vitamins. ○, Fermentations conducted on a medium, same as above, to which a mixture of amino acids was added.

A synthetic medium was devised in which a rapid growth phase and separate synnematin producing phase were obtained. This medium contained glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; biotin, 10 μg; and salts, 10 ml in a total volume of 100 ml. The chemical changes on this medium are shown in figure 5. The rapid utilization of glucose and ammonia nitrogen during the first 42 hr caused a rapid increase in mycelial nitrogen. Glucose was slowly fed to the fermentation, starting from 42

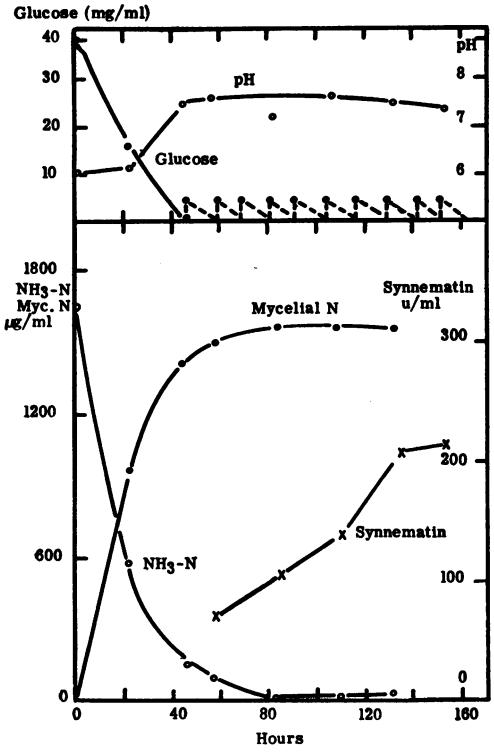

Figure 5. Chemical changes in a synnematin fermentation with *Emericellopsis terricola*. The medium contained glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; biotin, 10 µg; and salts, 10 ml; in a total volume of 100 ml. Glucose (0.4 g) was added at 12 hr intervals starting from 48 hr.

TABLE 8

Synnematin production on various media with Emericellopsis terricola

All media contained (per 100 ml) glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; biotin, 10 µg; and salts, 10 ml. Glucose was added to all fermentations at a level of 0.4 g at 12 hr intervals starting from 48 hr. The pH plateau is defined as the pH during the synnematin production phase.

Additive	Mycelial N	pH Plateau	Synnematin	Time to Max. Yield
	µg/ml		*units per ml*	*hr*
None (basal medium)	1400	7.0	303	130
B Vitamins	1560	7.2	225	150
B Vitamins plus amino acids	1500	7.3	210	150
DL-α-Aminoadipic acid		7.4	240	140

hr, which served to maintain the pH during the synnematin producing phase between 7 and 7.5.

The effect of the addition of different growth factors and DL-α-aminoadipic acid, to the above medium on the synnematin yield was studied. The results are given in table 8. No increase in yield was observed on the addition of amino acids, vitamins, or DL-α-aminoadipic acid.

Effect of pH on synnematin production with E. terricola. The basal medium found suitable for synnematin production was used for these experiments. The pH of the medium was adjusted manually to different levels at the end of 40 hr when the growth was essentially completed. The pH was maintained at different levels by manual adjustment at 12 hr intervals. The results are shown in figure 6. It may be seen from the figure that a pH range of 6 to 6.5 is optimum for synnematin production.

However, when a fermentation was maintained under conditions of rapid growth at pH 6 for 96 hr, no synnematin production was obtained. It has not been possible to obtain conditions under which rapid growth and synnematin production proceed simultaneously.

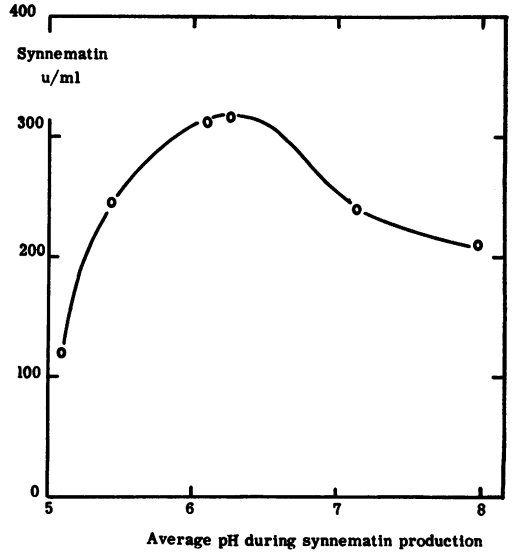

Figure 6. Effect of pH on synnematin yields. The medium contained glucose, 4 g; ammonium sulfate, 0.8 g; calcium carbonate, 1 g; biotin, 10 µg; and salts, 10 ml in a total volume of 100 ml. The pH values were adjusted manually at 12 hr intervals. Glucose (0.4 g) added at 12 hr intervals starting from 42 hr. In all cases the maximum yield was obtained at 144 hr.

DISCUSSION

Soltero and Johnson (1954) found that penicillin fermentations to which glucose was continuously fed gave higher yields than those to which glucose was fed intermittently. At feed rates higher than optimum, a rapid drop in pH, heavy growth, and low penicillin yields were obtained. Low feed rates led to eventual autolysis. In the synnematin fermentations reported here glucose was fed intermittently and at only one rate, to the fermentations. The effect of continuously feeding glucose at different feed rates, at the pH optimum for synnematin production (pH 6 to 6.5) is under investigation.

In penicillin fermentations, Owen and Johnson (1955) found a temperature of 30 C to be optimum for the mycelium producing phase, and about 20 C to be best for the penicillin producing phase. Fermentations with *E. terricola* var. *glabra* which were incubated at 25, 30, and 37 C for the entire period of the fermentation gave 170, 230, and 30 units per ml, respectively. Therefore the fermentations reported here were conducted at 30 C. However, further investigations are needed to define the optimum temperature for each phase of the fermentation.

Mutants of *E. terricola* var. *glabra* prepared at the Department of Botany of the University of Wisconsin are also under investigation.

It has been shown (Tardrew and Johnson, 1958) for *Penicillium chrysogenum* that the high yield mutants show a greater utilization of inorganic sulfate, and a greater excretion of organic sulfur than the low yield strains. The sulfur metabolism of synnematin producing molds is discussed in another paper (Bhuyan, Mohberg, and Johnson, 1958).

SUMMARY

The optimum conditions for growth and production of synnematin on chemically defined media by *Cephalosporium salmosynnematum* and *Emericellopsis terricola* var. *glabra* were investigated.

A pH of about 6 was found optimum for the growth of *C. salmosynnematum* and a pH range of 4.8 to 6.5 was found optimum for the growth of *E. terricola* var. *glabra*. Good growth was obtained on media containing glucose, sucrose, or maltose and inorganic or organic nitrogen sources. Biotin was found to be necessary for the growth of *E. terricola* var. *glabra*.

The maximum rate of synnematin production was obtained under conditions which supported a very slow growth rate. A medium containing glucose, ammonium sulfate, calcium carbonate, salts, (and biotin in the case of *E. terricola* var. *glabra*) was devised which supported a rapid growth rate during the growth phase and a slow growth rate during the synnematin production phase. The slow growth rate during the synnematin producing phase was obtained by feeding glucose intermittently to the fermentation after the end of the growth phase. The synnematin yields obtained with *C. salmosynnematum* (36 units per ml) were lower than those obtained with *E. terricola* var. *glabra* (300 units per ml). No improvement in synnematin yields was obtained when DL-α-aminoadipic acid, other amino acids, or vitamins were added to the medium.

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