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Factors Affecting Coal Solubilization by the Bacterium *Streptomyces setonii* 75Vi2 and by Alkaline Buffers

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ABSTRACT

Streptomyces setonii 75Vi2 produces an extracellular coal-solubilizing component(s) in the absence of coal. The heat stability, relatively low molecular weight, and insensitivity to proteases of the substance(s) responsible for coal solubilization indicate that the process is nonenzymatic. This report describes factors affecting the production and activity of this substance(s) and the similarity in its action to alkaline buffer solutions in solubilizing coal.

Index Entries: Coal solubilization; bacteria; *Streptomyces*; and alkali.

INTRODUCTION

Several recent papers have described the solubilization of low-ranked coals by fungi (*1–3*). Fungal coal solubilization appears, at this time, to be limited to the use of intact mycelia grown on the surface of liquid or solid media. This limitation was believed to be a potential impediment to the future development of a microbial process to solubilize coals.

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A bacterial system was considered as an alternative. Strandberg and Lewis (*4*) discovered that the bacterium *Streptomyces setonii* 75Vi2 could solubilize coals in submerged culture and that coal solubilization was caused by an extracellular component(s) produced by the bacterium in the absence of coal. The heat stability (30–40% loss in activity after 1 h at 121°C), apparent molecular weight range (1000–10,000 daltons), and insensitivity to proteases of the coal-solubilizing (CS) component(s) indicated that it was not an enzyme. In this report further details on factors affecting the production and activity of the CS component(s) and the similarity in action of weak bases in solubilizing certain coals are presented.

MATERIALS AND METHODS

The organism used in this study was *S. setonii* 75Vi2 (ATCC 39116). It was obtained from D. L. Crawford, University of Idaho, Moscow, ID. The methods used to culture the organism and determine CS activity have been reported previously (*4*). Briefly, in the experiments presented here, cell-free CS activity was determined by incubating known weights of nitric-acid-pretreated coals (*5*) for 2 d (30°C, 50 rpm) in filter sterilized (0.45 μm) culture broths from *S. setonii* 75Vi2 which had been grown on various carbon and nitrogen sources. Approximately 0.2 g coal (dry wt) was added to 10–20 mL of culture broth. However, as reported earlier (*4*) the ratio of coal to culture broth volume was not an important consideration. The details of the media formulations are presented in the appropriate tables. The extent of coal solubilization was assessed by determining the dry weight of residual, unsolubilized coal, recovered by centrifugation or filtration. Adjustments to the pH of the culture broths were made with either NaOH or HCl. The solubilization of coals by alkaline buffer solutions was determined in a similar manner.

RESULTS AND DISCUSSION

As reported previously (*4*), several characteristics of the extracellular CS component(s) produced by *S. setonii* indicated that it was not an enzyme. It was also observed that coal solubilization only occurred with culture broths that had undergone a substantial increase in pH (final pH 8 to 9) during the growth of the organism. In this regard, Pometto and Crawford (*6*) recently reported that the oxidative depolymerization and solubilization of lignin by *S. viridosporous* was maximal in the pH 8.4–8.8 range.

A definite correlation between the growth of *S. setonii*, an increase in pH during growth, and the appearance of CS activity is shown in Table 1. Furthermore, as shown in Table 2, coal solubilization is maximal above pH 8.

Table 1
The Effect of Culture Age on the Medium pH and the Production of Extracellular Coal-Solubilizing Activity by *Streptomyces Setonii* 75Vi2

Culture age[a] (d)	pH	Cells[b] mg/mL dry wt	Coal (mg)[b,c] Initial	Coal (mg)[b,c] Final	Coal solubilized (%)
0	5.6	0.05	236	233	2
3	7.5	2.1	233	227	2
4	7.8	2.3	212	184	13
5	8.0	2.4	260	209	20
6	8.4	3.1	218	174	20
7	8.4	2.4	279	241	14
10	8.9	1.9	215	173	19

[a]*Streptomyces setonii* 75Vi2 was grown in Sabouraud Maltose broth (30°C, 80 rpm) After the indicated days of growth the cells were removed by filtration (.45μm, Millipore) and the cell-free broth tested for coal solubilization.

[b]Dry weights were determined after oven drying at 95–100°C, 18 h.

[c]A nitric acid-treated (2 d, 8 *M* HNO_3) Wyodak subbituminous coal was used (*see* ref. 5). The extent of solubilization was determined after 2 d at 30°C.

Table 2
The Effect of pH on Coal Solubilization by Cell-Free Broths of *Streptomyces Setonii* 75Vi2

pH[a]	Coal solubilized[b] (%)
7	9, 1
7.5	22, 19
8.2[c]	21, 28
8.5	31, 36
9.5	44, 45

[a]The pH of cell-free culture broths (Sabouraud Maltose broth, 7 d, 30°C) was adjusted with either HCl or NaOH.

[b]Values shown are replicates. See Table 1 for conditions.

[c]The final pH of the culture broth after 7 d growth of *Streptomyces setonii* 75Vi2 was 8.2.

Pometto and Crawford (*6*) suggested that the pH change they observed was due to the conversion of nitrogen-containing components in the growth medium to alkaline substances. As shown in Table 3, this is probably what is occurring with *S. setonii*. Several, but not all, complex nitrogen sources supported growth and the production of CS activity. Several individual amino acids were also effective. In all instances, when

Table 3
The Utility of Various Carbon and Nitrogen Sources for the Production of Extracellular Coal-Solubilizing Activity by *Streptomyces Setonii* 75Vi2

Carbon/nitrogen for growth[a]	pH of culture		Coal solubilized[b,c] (%)
	Initial	Final 7 d	
Neopeptone[d] 1% plus maltose 4%	5.8	8.9	69, 64
Neopeptone[d] 1% plus glucose 1%	7.4	7.9	0,0
Neopeptone[d] 1%	7.4	8.5	38, 40
Yeast extract[d] 1%	7.0	8.8	62, 60
Amber-crude[e] 2%	5.1	8.8	48, 47
Amber-EHC[e] 1%	5.8	6.0	0
Whey[f] 2%	5.9	8.2	32, 36
Casamino acids[d] 3%	5.9	9.0	68, 72
Glutamic acid .5%	7.0	9.3	78, 79
Valine .5%	7.3	8.0	8, 10
Asparagine .5%	7.3	8.0	50, 54

[a]The medium components were dissolved at the concentrations indicated in demineralized, distilled water and sterilized at 121°C, 20 min. A mineral supplementation consisting of KC1, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.001%; and K_2HPO_4, .05% was included in the media containing only glutamic acid, valine, or asparagine.

[b]Values shown are replicates. *See* Table 1.

[c]The extent of solubilization in sterile controls of each medium was <5%.

[d]Difco Laboratories, Detroit, MI.

[e]Amber Laboratories, Juneau, WI.

[f]Whole powdered cheese whey (partially demineralized), Flav-O-Rich, Inc., Knoxville, TN.

CS activity was produced, there was a corresponding increase in pH. However, an increase in pH did not necessarily indicate the presence of CS activity. A carbohydrate was not required, and, notably, the presence of glucose suppressed the production of CS activity. This suppression was observed with all of the nitrogen sources listed in Table 3 (data not shown). The reason for the suppression of CS activity is unknown. Perhaps glucose metabolism suppresses the metabolic transformation of the nitrogenous substrate to the CS component(s). Carbon catabolite regulation of secondary metabolite formation has been observed in *Streptomyces* (*7*). Further experiments are necessary in this regard.

Our initial attempts at separating and isolating the CS component(s) from culture broths of *S. setonii* grown on complex nitrogen sources (i.e., Sabouraud Maltose broth) by gel filtration chromatography were unsuccessful. These attempts were hampered by the large and varied number of compounds present in the culture broth originating from the complex nitrogen source (i.e., neopeptone) and also containing products of microbial metabolism. However, the use of individual amino acids to culture the organisms should greatly facilitate future attempts to isolate the active CS component.

During the attempts to isolate the active CS component(s) using gel permeation chromatography, the addition of sodium dodecyl sulfate (SDS) was examined as a possible means of enhancing the separation of components in the broth. When, as a control, the effect of SDS was tested in whole culture broths, an enhancement in coal solubilization was observed (Table 4). The reason for this effect is unknown.

Also, during the isolation and separation attempts, several alkaline buffers were employed, since it was known that activity was maximal at alkaline pHs. As shown in Table 5, these alkaline buffer solutions alone were very effective in solubilizing coals. Furthermore, the extent of solubilization was dependent upon the buffer concentration (Table 5), and the coal-to-buffer-volume ratio (Table 6). These results suggest that the number of available base equivalents determined the extent of solubilization.

The general characteristics of the CS activity (i.e., pH requirements, heat stability, molecular weight), the requirement of an organic nitrogen source for its production, and its similarity in action to weak bases sug-

Table 4
The Enhancement of Cell-Free Coal Solubilization by Sodium Dodecylsulfate

Conditions[a]	Coal solubilized[b] (%)
Cell-free culture broth	50, 47
Cell-free culture broth plus 0.1% SDS	59, 63
SDS	6, ND[c]

[a]The cell-free culture broth was from a 7-d culture of *S. setonii* 75Vi2 grown on Sabouraud maltose medium.
[b]Values shown are replicates. See Table 1.
[c]ND–not determined due to loss of sample.

Table 5
The Solubilization of Coal in Alkaline Buffers

Buffer	Molarity	Initial pH	Coal solubilized[a] (%)
TRIS[b]	0.05	8.5	73, 69
TRIS	0.15	8.5	79, 73
Glycylglycine	0.05	8.5	70, 74
Glycylglycine	0.15	8.5	79, 80
Potassium phosphate	0.05	7.8	59, 62
Potassium phosphate	0.15	7.9	69, 73
Potassium phosphate	0.05	7.0	23, 37

[a]Values shown are replicates. *See* Table 1.
[b]Tris(hydroxymethyl)aminomethane

Table 6
The Effect of the Coal to Buffer Volume Ratio on the Extent of Solubilization of Pretreated Wyodak Subbituminous Coal

g coal/20 mL buffer[a]	Coal solubilized[b] (%)
.2	64, 65
.4	61, 63
.6	60, 60
.8	56, 56
1.0	49, 49

[a].15M Tris(hydroxymethyl)aminomethane, pH 8.5
[b]Values shown are replicates. *See* Table 1

gest that the active component(s) may be basic polypeptides or polyamines. This idea is further supported by the findings of Pometto and Crawford (*6*), noted previously.

Of several coals examined, only one, a naturally oxidized North Dakota lignite (American Colloid, Skokie, IL), was readily solubilized by fungi (*3,5*) or bacteria (*4*). As described previously by Strandberg and Lewis (*5*), pretreatment of recalcitrant coals with nitric acid significantly enhances their susceptibility to microbial solubilization. As shown in Table 7, solubilization by dilute, alkaline buffers also requires a pretreatment step.

Table 7
The Enhancement of Solubilization by the Pretreatment of Coals with Nitric Acid

	Coal solubilized %[b]			
	Buffer			
	0.15 *M* Tris pH 8.5		0.15 *M* Potassium phosphate (pH 8.5)	
Coal[a]	Untreated	Treated[c]	Untreated	Treated[c]
Mississippi	0	43, 43	0	37, 38
Texas	0	45, 45	0	46, 46
Vermont	0	34, 28	0	ND[d]
Wyodak subbituminous	0	59, 59	0	33, 35

[a]The sources of the coals and their properties are described in refs 3 and 6.
[b]Values shown are replicates. See Table 1.
[c]Pretreatment consisted of soaking the coals in 8 *M* HNO_3 for 2 d at room temperature. The treated coals were washed extensively with distilled water and oven dried (95–100°C, 18 h) after treatment. Also see ref. 5.
[d]ND–not determined due to loss of sample.

A characterization and comparison of coal-solubilization products produced by both CS activity and alkaline buffers will be required to determine if their modes of action are similar.

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REFERENCES

1. Cohen, M. S., and Gabrielle, P. D. (1982), *Appl. Environ. Microbiol.* **44:** 23–37.
2. Ward, H. B. (1985), *Appl. Microbiol.* **6:** 236–238.
3. Scott, C. D., Strandberg, G. W., and Lewis, S. N. (1986), Biotechnol. Prog. **2:** 131–139.
4. Strandberg, G. W., and Lewis, S. N. (1987), *J. Industrial Microbiol.* **1:** 371–375.
5. Strandberg, G. W., and Lewis, S. N. (1986), *Biotechnol. Bioeng. Symp. Ser.* **17:** 153–158.
6. Pometto, A. L., and Crawford, D. L. (1986), *Appl. Environ. Microbiol.* **52:** 246–250.
7. Martin, J. F., and DeMain, A. E., (1980), *Microbial. Rev.* **44:** 230–251.