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A simple method to study microbial coal solubilization using Pluronic F-127-amended media

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SUMMARY

Microbial coal solubilization and the extraction of solubilized coal products were carried out in media amended with polyol (Pluronic F-127), an agent which gels above 18 °C but reverts to a liquid state at low temperature (4 °C). The solubilized coal products, the unsolubilized coal particles and the mycelial mat were separated effectively by centrifugation at 4 °C. The amount of coal solubilization was 30–50% higher in polyol-amended media than in agar media regardless of the microorganism. On the other hand, the amount of coal solubilization in polyol-amended control media was less compared to agar-amended control media.

INTRODUCTION

The reversible gel, polyol (Pluronic F-127) is composed of polypropylene and ethylene oxide. At ambient temperature the polyol forms a semi-solid gel but on lowering the temperature to 4 °C it becomes a liquid [3]. This gelling agent has been used to culture *Coprinus congregatus* to harvest the mycelia and study sporulation [1], and for the selection of microbial antagonists [5]. Because of some desired qualities such as non-toxicity to microorganisms, chemical inertness, and reversible gelation and liquefaction, we were encouraged to pursue the feasibility of applying polyol as a substratum for microbial coal solubilization. Coal solubilization has been demonstrated by various microorganisms such as *Candida tropicalis* [4], *Polyporus versicolor* and *Poria monticola* [2], *Coriolus versicolor* [6], *Streptomyces setonii* [8] and a host of other organisms including *Trametes versicolor*, *Candida* sp. (ML-13), *Aspergillus* sp. and *Sporothrix* sp. [7]. Microorganisms have been cultured on solid agar surface sprinkled with coal particles. The liquid product formed from the surface of coal diffused into the agar, darkening the medium during the incubation period [2]. Because of problems in separating unsolubilized coal particles from solubilized products and mycelia, polyol medium was used to grow the organisms with coal particles at ambient temperature. At the end of the incubation period, the polyol media were reverted to the liquid state by transferring the plates to 4 °C. The mycelial mat, unsolubilized coal particles and the solubilized coal product were separated by centrifugation at 4 °C. We report in this paper the application of polyol media as potential substrata for microbial coal solubilization.

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MATERIALS AND METHODS

Chemicals and organisms. Polyol (Pluronic F-127) was obtained from BASF, Parsippary, NJ. Other chemicals and media were purchased from Fisher Scientific, Pittsburgh, PA. North Dakota Wyodak bituminous powdered coal and the organisms, *Streptomyces setonii*, *Candida* sp. (ML-13) and *Trametes versicolor* were obtained from Dr. C.D. Scott, ORNL, Oak Ridge, TN. Cultures were maintained in Sabouraud maltose broth (SMB) or Sabouraud maltose agar (SMA) media and incubated at 30 °C.

Preparation of polyol (Pluronic F-127) medium. The polyol medium was prepared by dissolving 250 g of polyol in 1000 ml of distilled water and allowing it to solubilize at 4 °C overnight. Czapek Dox (CD) (3.5%) and Sabouraud maltose (SM) (5.0%) were reconstituted in four concentrations: 18, 20, 22.5 and 25% of polyol. No gel formation was observed when the polyol concentration was less than 18%. Media were sterilized at 121 °C for 15 min at 15 p.s.i. and allowed to cool at 25 °C. They were then shifted to the cold room where the media liquefied. Liquefied polyol-amended media were dispensed aseptically into sterile plates, 20 ml per plate. The plates

were kept at ambient temperature (25 °C) to solidify [3]. Bacto agar (2%) was used in Czapek Dox and Sabouraud maltose media as the solidifying agent in control plates.

Coal solubilization. Plates were inoculated with test organisms by introducing aseptically inoculum discs (7 mm in diameter, one disc/plate) containing *Candida* sp. ML-13, *I. versicolor* or *S. setonii* at the center of the plates containing polyol or agar-amended media and incubated at 30 °C. After the seventh day of growth, 300 mg of sterilized coal were sprinkled over the mycelial surface aseptically and plates were incubated for another 7 days [7]. Growth was determined by measuring the dry weight of the mycelia obtained from 10 plates. Values reported represent the mean of triplicate experiments.

Extraction of solubilized coal. At the end of the 14th day of growth, experimental plates containing polyol were cooled to 4 °C to liquefy the media. The contents of the liquid medium were transferred into a sterilized centrifuge tube kept in ice and centrifuged at 4 °C for 15 min at 12000 × *g*. The supernatant fluid and pellet were separated aseptically. The supernatant fluid contained the solubilized coal product and the pellet contained mycelium and unsolubilized coal. Coal particles were removed from the mycelium by gentle vortexing and mild stirring. The separated coal particles were collected on preweighed and predried filter paper. The residual coal weight was used for determining the percentage of coal solubilization. Solubilized coal particles present in the broth were separated through filtration on Whatman No. 1 filter paper using a Buchner funnel and the residual coal weight was determined. Coal particles and solubilized products from agar plates were collected by adding sterile water and analyzed spectrophotometrically. The supernatant fluid obtained from the plates was scanned from 900 to 190 nm at 4 °C, using the supernatant fluid without coal as blank.

RESULTS AND DISCUSSION

Candida ML13, *Streptomyces setonii* and *Trametes versicolor* were successfully cultured in different growth media amended with polyol as the solidifying agent. Mycelial growth was greater (55, 20 and 24% in *Candida*, *S. setonii* and *T. versicolor*, respectively, data not shown) in polyol Sabouraud media than in the agar media. Though there was a difference in the growth of organisms among media, polyol concentrations did not affect the growth of the organisms significantly (Fig. 1). This is in agreement with earlier findings [1,3,5]. Data obtained from the higher concentrations only are shown in Fig. 1. Therefore, polyol can be used for the growth of microorganisms without significant inhibition or toxic effects on the organisms.

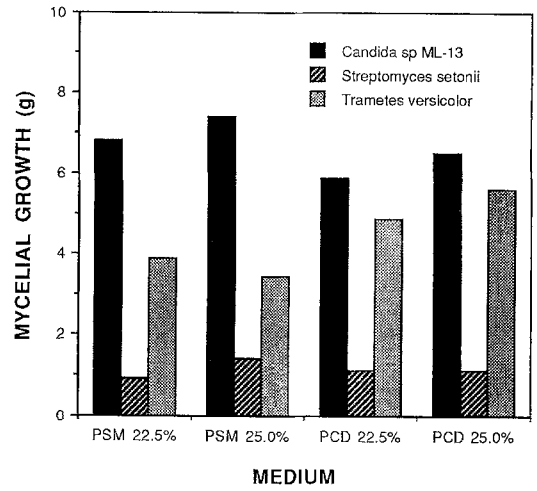

Fig. 1. Effect of polyol concentration on growth of various organisms. PSM, Polyol Sabouraud maltose medium; PCD, Polyol Czapek Dox.

Coal solubilization by microorganisms has been well documented [2,7,8]. However, the solubilized coal product diffused extensively into the agar substratum which may impede extraction and recovery of the solubilized products. We have eliminated this problem to a great extent by using polyol as a solidifying agent using three organisms known for their coal solubilization potential. The amount of coal solubilization in polyol-amended media compared with agar-amended media is shown in Fig. 2.

Coal solubilization by the three organisms was higher in polyol media than in agar media. Overall, polyol-amended media facilitated a higher degree of coal solubili-

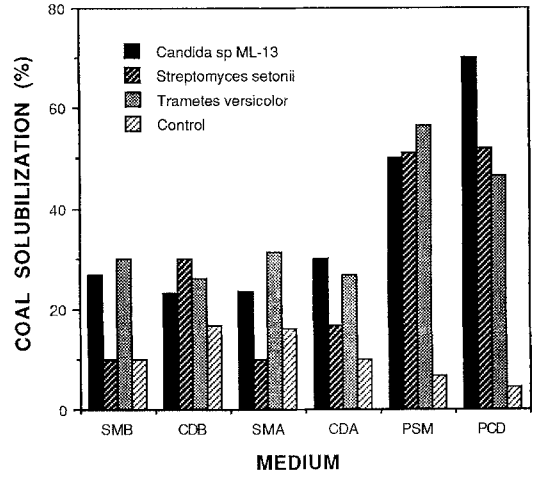

Fig. 2. Effect of media on coal solubilization. SM, Sabouraud maltose broth; CD, Czapek Dox broth; PSM, Polyol Sabouraud maltose medium; PCD, Polyol Czapek Dox medium. In control plates agar was substituted for polyol.

zation (40–70%) compared to agar containing media (10–30%). The organisms varied in their capacity to solubilize coal irrespective of the type of substratum used. Media composition also played an important role in the amount of growth and coal solubilization. In both broth and agar media, considerable non-microbial coal degradation was observed which ranged from 10–16%. However, such non-microbial coal solubilization was lower (3–5%) in the polyol supplemented medium. Non-microbial or non-specific degradation of coal has been reported earlier [8]. Alkaline buffers alone solubilized coal from 23–80%.

The pH of the media was measured before and after the experimental period. Changes in pH varied depending upon the medium and the presence or absence of coal. The pH increased from pH 5.6 to neutral in SMB and SMA, whereas in SM supplemented with polyol there was little or no change in pH regardless of the polyol concentration. In CDA there was a decline from pH 7.6 to 6.7 in medium with coal, and in medium with the organism alone pH increased to 8.3. In medium with coal and the organism, pH was in the neutral range. These observations were similar for all three organisms under the same culture conditions. The coal degradation products (non-microbial mediated coal degradation and microbial coal solubilization) were scanned from 900–190 nm. Despite the changes in pH, only one major absorption peak was associated with each organism with absorption maxima at: CDB and coal control 504 nm, polyol medium and coal control 576 nm, *Candida* 364 nm, *S. setonii* 380 nm and *T. versicolor* 308 nm.

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