CELLULOSE DIGESTING BACTERIA FROM LIVE TERMITE MOUND SOILS

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ABSTRACT

Six mesophilic aerobic bacteria, degrading cellulose were screened from live termite mound soils (*Odontotermes obesus*) located in semi-arid areas. The cultural and physiological characteristics of two purified forms (*Cellulomonas* species) were studied.

INTRODUCTION

MICROORGANISMS that degrade cellulose are abundant in nature. They include aerobic and anaerobic bacteria, fungi and actinomycetes. Besides, the ruminants organisms, the termites which subsist on a diet rich in cellulose have been found to harbour cellulose digesting microorganisms[1]. In termite mounds prevalent in semi-desert ecosystem in the Arravali ranges[2], decomposition rates of organic compounds are limited by available water, nitrogen and carbon[3]. Millipede species are detritivores and feed on soils, plant litter and other items found on soil surface.

Although several reports are available on the anaerobic cellulose-digesting bacteria, information on aerobic ones is very scanty. This communication reports the occurrence of two efficient aerobic bacteria from termite mound soils capable of solubilizing cellulose.

MATERIALS AND METHODS

Bacterial isolates from live termite mounds (*O. obesus*) were screened. Aliquots from soil serial dilution tubes, were plated on minimal medium fortified with 0.2% yeast extract, containing cycloheximide (50 μg/ml) as antifungal agent[4]. Purified isolates were obtained by 8 to 10 transfers on the fresh medium. The number of total viable bacteria was estimated for 4 replicates using minimal basal medium. Agar plates were incubated at 35 C for 7–28 days before counting the microbe colonies[5, 6].

Pure isolates were grown on a basal medium containing (g/l) CH_3COONa, 3.0; $Na_2SO_4 . 7H_2O$, 0.4; $MgSO_4 . 7H_2O$, 0.2; $MgCl_2 . 6H_2O$; 1.8, K_2HPO_4, 0.25; KH_2PO_4, 0.25; $CaCl_2 . 2H_2O$, 0.2; $FeSO_4$, 0.01; EDTA, 0.04; Yeast extract (Difco), 2.0 and 5 ml of trace element solutions[5]. Final pH was adjusted to 7.2. Multiple points inoculation was adopted to test utilization of glucose, cellobiose or cellulose (Carboxymethyl cellulose) at a concentration of 1%. Cellulose digestion was estimated by using the following procedures.

(i) *Hydrolysis of carboxymethyl cellulose:* Liquefaction of gel resulted in water-like viscosity[7]. Uninoculated controls were also included. Concentration of CMC used was 20 gm/l, where it forms a gel.

(ii) *Colorimetric method:* Isolates were grown on various native celluloses. The growth medium consisted of basal inorganic salts and one of the carbohydrates like cellulose pulver, cotton, toilet tissue paper, silk cotton etc. After a predetermined incubation in a rotary shaker (250 rpm) the incubation mixture was centrifuged to remove the residual insoluble substrate. The supernatant was treated with phenol-sulphuric acid to measure soluble sugars released as a result of cellulose activity. Glucose was used as a standard[8].

(iii) *Rate of cellulose degradation:* This was followed using the method of Hiltner and Dehority[9]. To the sediment fraction 5 ml of acid detergent fibre solution was added.

Total protein was estimated by Bradford[10] method. Pure strains were subjected to diagnostic tests following Bergey's manual. *Cellulomonas flavigens* obtained from ATCC (491), Virginia, U.S.A. served as reference species. Reduction of nitrate, methyl red and Voges-Proskauer reactions, hydrolysis of starch were tested as described by Holding *et al*[11] and routinely used in our laboratory[12]. For cleavage of carbohydrates, the method of Yamada and Komagata[13] was followed.

Chemicals were purchased from Sigma Chemicals, USA with following exceptions: yeast extract, peptone, from Difco, Michigan and CMC cellulose from BDH, England.

RESULTS AND DISCUSSION

The maximum bacterial number was detected during rainy season and the minimum in hot summer months. In all, sixteen aerobic strains were screened from termite mound soils and six were capable of solubilizing cellulose. The morphological and the salient physiological characteristics of two *Cellulomonas* isolates $DORP_1$ and WRS_1 are given in table 1. $DORP_1$ formed dark orange, small colonies whereas WRS_1 had white, large opaque colonies.

Cellulose was found to be a suitable substrate for growth. Carboxymethyl cellulose and glucose were the next preferred carbohydrates (table 2). Isolates showed little growth on inorganic nutrient medium substituted with 0.5 % yeast extract. No growth was recorded when the medium was prepared without yeast extract. Very little digestion of starch was detected, the regenerated cellulose pulvers were easily digested whereas cotton fibre and silk wood were poorly degraded (table 3).

A rapid rise in growth of isolate $DORP_1$ was seen upto 1 % CMC, further addition resulted in a sharp decline (figure 1). Isolate WRS_1, however, showed a linear growth upto 2 % CMC and beyond this level, a decline in growth was observed. Optimal pH for maximum activity was around 7.6 (data not given).

A correlation was recorded between cellulose digestion and bacterial growth. When there was an increase in bacterial population, paralleled with increase in protein value (index of growth rate), the pH of the incubation medium invariably turned alkaline. Isolate $DORP_1$ consumed most of the CMC in less than 52 hr of incubation and the pH changed from 7.8 to 8.7 (figure 2a). After 52 hr a sharp decline was seen in cell growth with no significant change in pH. The isolate WRS_1 attained the maximum growth after 58 hours and the pH turned alkaline, (figure 2b). Although the cause of alkalinity is yet to be established, this observation agrees with earlier reports[14, 15] from aerobic cellulose solubilizing bacteria *C. fulvus* and *Cellulomonas*, respectively. Preliminary HPLC analysis indicated the synthesis of aldehydes and ketones in the culture filtrate.

Increase in bacterial growth on media containing cellulose, CMC cellulose, or cellobiose indicated that active termite mound soil harbour bacteria which would produce C_1 (active upon crystalline cellulose) and C_x (active upon non-crystalline cellulose) cellulases and β-glucosidases or cellobiases[16]. Data pre-

Table 1 *Descriptive chart of cellulose utilizing organisms–$DORP_1$, WRS_1*

Characteristics	$DORP_1$	WRS_1	*Cellulomonas flavigens*
Morphological characteristics			
Form	Short rods, curved	Long roads, straight	Rods curved
Size	0.28 × 0.5–0.9 μ	0.7–0.8 × 1.2–1 4 μ	0.4–0 6 × 0.7–1.8 μ
Motility	Non-motile	Motile	Non-motile
Grams stain	Positive	Positive	Variable
Cultural characteristics			
Nitrient agar (Difco)	Grow feebly	Grow feebly	Smooth, glistering opaque, yellow
Yeast extract agar (Difco)	Dark orange, small, circular colonies, opaque, raised	White, large circular colonies, opaque, raised	—
Broth	Uniformly turbid	Uniformly turbid	Uniformly turbid
Optimal temp.	33–37°C	35–37 C	30–33 C
Biochemical characteristics			
Gelatin lique-faction	Slow	Fast	Slow
Methyl red test	Negative	Negative	--
Nitrate reduction	Reduced to NO_2	Reduced to NO_2	Reduced to NO_2
CM-Cellulose gel hydrolysis	Positive	Positive	Positive
Voges-Proskauer test	Negative	Negative	Negative

Table 2. Growth on carbohydrates (after 48 hr incubation)

Substrates	mg protein/ml culture broth DORP₁	WRS₁
Yeast extract (YE)	0.075	0.12
YE+CMC	0.265	0.22
+CMC (−YE)	0.056	ND
Glucose (−YE)	ND	ND
YE+Glucose	0.253	0.33
+Cellobiose (−YE)	ND	ND
YE+Cellobiose	0.283	0.395
+Starch (−YE)	ND	ND
YE+Starch	0.085	ND

CMC represents carboxymethyl cellulose, ND– not detectable. Incubation (48 hr) was completed on a rotary shaker (250 rpm) at 35 C.

Table 3 *Digestibility of various cellulosic substrates*

Cellulosic substrates	Cellulose made soluble (mg/ml)* equivalent mg glucose/ml broth DORP₁	WRS₁
Filter paper	0.210	0.265
Tissue paper	0.195	0.225
Cellulose pulver	0.315	0.660
Cotton fibre	0.165	0.110
Wood cotton	0.083	0.107

*Glucose was calculated following the method of Dubois *et al*[8].

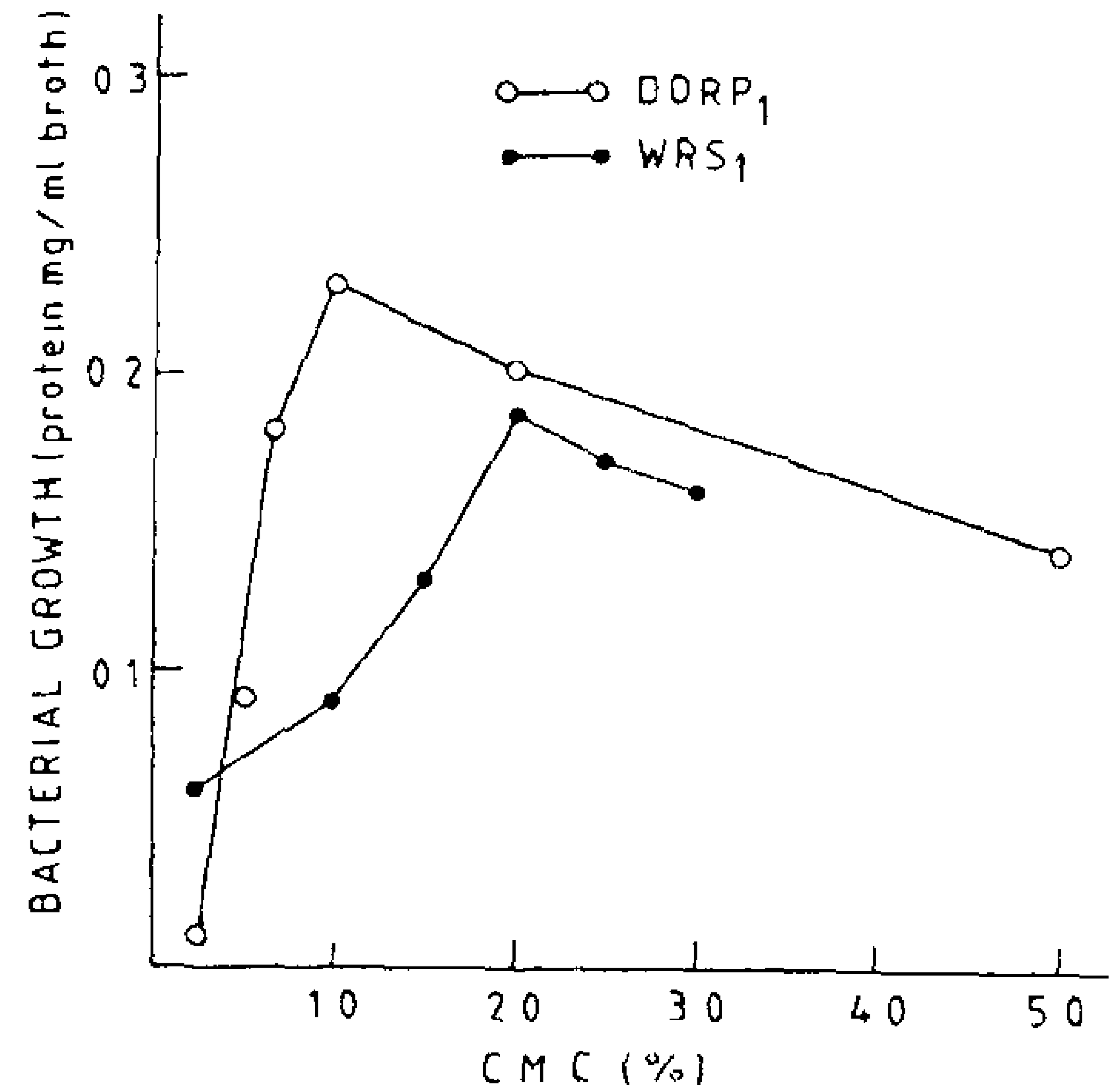

Figure 1. Effect of different carboxymethyl cellulose concentrations on bacterial growth O——O DORP, ●——● WRS_1 strain.

sented here indicates that much of the food consumed by these millipedes is already somewhat degraded by aerobic and anaerobic free-living microflora possessing necessary C_I and C_x cellulases. The association between millipede and termite mound soil bacteria

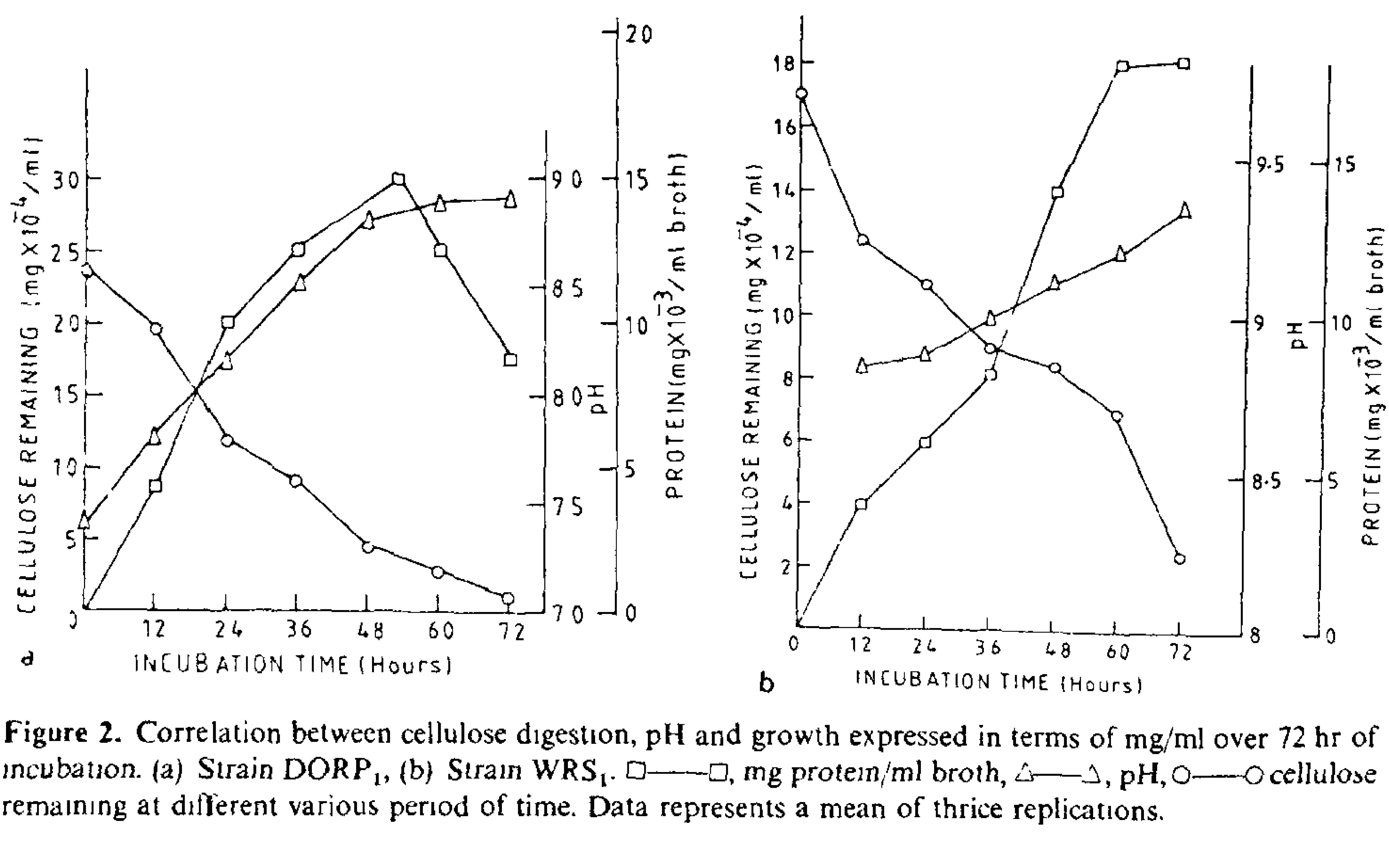

Figure 2. Correlation between cellulose digestion, pH and growth expressed in terms of mg/ml over 72 hr of incubation. (a) Strain $DORP_1$, (b) Strain WRS_1. □——□, mg protein/ml broth, △——△, pH, O——O cellulose remaining at different various period of time. Data represents a mean of thrice replications.

may be mutualistic. Free bacteria through the production of cellulolytic enzyme make available to millipedes otherwise unutilizable substrates, which would be of paramount importance to millipedes surviving in deserts, semi-arid zones where production of detritus is low. The millipedes in turn provide bacteria an environment with regulated moisture and temperature and supply bacteria with constant flow of substrates (cellulose, cellobiose and other cellulosic materials) to degrade. This type of association has important implications for nutrient cycling in semi-arid soils and would play a vital role in making semi-arid dry soils habitable for higher trophic levels.

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NEWS

COMPUTER PROGRAM FOR NUCLEAR CONTAINMENT

. ."The ultimate defense against the escape of radioactive material from today's nuclear power plant is the bunker-like building that surrounds the key components of the reactor. . . . Researchers at the Massachusetts Inst. of Technology's Energy Laboratory have developed a computer program that simulates flows and calculates local temperatures and pressures in the containment during both mild and severe accidents. By producing such detailed information, the program can help engineers design containment structures more accurately. In addition, the program can predict whether gases in the containment building will ignite when hydrogen is present, as it was during the accident at Three Mile Island."

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