Short Communication

Tungsten Requirement of an
Extremely Thermophilic,
Cellulolytic Anaerobe
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Recently, extremely thermophilic or caldophilic anaerobes have been extensively isolated from geothermal habitats, potentially providing us with new microbial species^{1~5}. In the present study, we tried to isolate cellulolytic, anaerobic, extreme thermophiles from hot springs located in the Nozawa district (Nagano, Japan), using the following basal medium (per liter): KH_2PO_4 , 1.5 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 4.2 g; NH_4Cl , 0.6 g; Na_2CO_3 , 4 g; MgCl_2 , 0.38 g; L-cysteine $\cdot \text{HCl} \cdot \text{H}_2\text{O}$, 0.5 g; $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.5 g; vitamin mixture,⁶ 10 ml; trace mineral solution (Wolfe's modified mineral elixir),⁶ 50 ml; volatile fatty acids,⁷ 1 ml; inositol, 1 mg; resazurin, 1 mg; yeast extract (Difco), 1 g; Bactotryptone (Difco), 1 g; cellulose (KC floc W-300 from Sanyo Kokusaku Pulp, which was ball-milled in a 3% suspension), 6 g; and agar (if necessary), 35 g. The medium was anaerobically prepared with O_2 -free N_2 gas and the pH was about 8 after autoclaving (110°C, 15 min).

Media composed of relatively many nutrients and especially containing a trace mineral solution (containing Al, Mo, Se, etc.) have often been used for culturing extremely thermophilic anaerobes such as *Methanobacterium thermoautotrophicum*, *Thermoanaerobium brokii* and *Thermoanaerobacter ethanolicus*.^{6,8} In the present study, however, successful enrichment required addition of the original hot spring water to the medium. Ultimately, an extremely thermophilic, cellulolytic, non-

spore-forming anaerobe (Gram-negative rods of $0.6 \sim 0.9 \times 1.8 \sim 4.0 \mu\text{m}$ having an optimum growth temperature of 75°C) was isolated from positive enrichment cultures (80°C) by means of roll-tube cultures (65°C),⁹ in which the medium was supplemented with 400 ml of the hot spring water per liter. The isolate was provisionally designated as strain NA10. The identification and characterization of this strain will be reported and discussed in detail elsewhere. Strain NA10 actively decomposed cellulose as shown in Fig. 1, but it still required the original hot spring water as a source of essential nutrient(s) after the purification.

It is desirable that the natural nutrient(s) can be replaced with chemically defined compound(s) for investigation of the physiological characteristics of the strain. Thus, the effects of seven elements (W, Si, V, F, Br, Cr, Sn), which were divided into three groups for convenience, on cellulose decomposition of the strain were examined as shown in Table I (upper column). In the medium containing Na_2WO_4 , Na_2SiO_3 and VCl_3 , cellulose was successfully decomposed through eight subcultures. As a result of the detailed test on these chemicals, moreover, it was found that Na_2WO_4 was an effective nutrient (lower column in Table I). It is known that tungsten participates in acetic acid formation by *Clostridium thermoaceticum*,^{10,11} and that enzymes of some anaerobes contain this metal.^{12,13} Also in strain NA10, consequently,

FIG. 1. Photograph Showing Cellulose Decomposition by Strain NA10.

A, before cultivation; B, after cultivation at 75°C for 18 hr.

TABLE I. TRACE ELEMENT REQUIREMENT OF STRAIN NA10

The cultivations were carried out at 75°C with shaking (90 rpm), in test tubes (12 mm ϕ $\times$ 150 mm) containing 5 ml of the medium. About 0.5 ml of a growth culture was inoculated into new medium for the next subculture. The concentrations of the nutrients added to the medium were 30 μ g/liter except for Na₂WO₄·2H₂O (0.4 mg/liter) and the hot spring water (400 ml/liter).

Nutrient added	1st	2nd	3rd	4th	5th	6th	7th	8th
Na ₂ WO ₄ ·2H ₂ O, Na ₂ SiO ₃ , VCl ₃	+	+	+	+	+	+	+	+
	(42)	(23)	(23)	(25)	(28)	(17)	(20)	(21)
NaF, NaBr	+	+	+	+	—	—		
	(42)	(47)	(46)	(68)	(72)			
Cr ₂ (SO ₄) ₃ ·xH ₂ O, SnCl ₂ ·2H ₂ O	—	—						
	(220)							
All	+	+	+	+	+	+	+	+
	(32)	(64)	(32)	(24)	(37)	(59)	(42)	(30)
Hot spring water	+	+	+	+	+	+	+	+
	(42)	(23)	(23)	(20)	(19)	(22)	(20)	(21)
None	+	+	+	—	—			
	(40)	(48)	(81)	(96)				
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Na ₂ WO ₄ ·2H ₂ O	+	+	+	+	+	+	+	+
	(16)	(16)	(18)	(24)	(15)	(17)	(19)	(18)
Na ₂ SiO ₃	+	+	+	+	+	—	—	
	(16)	(36)	(24)	(36)	(51)	(72)		
VCl ₃	+	+	+	+	+	—	—	
	(16)	(16)	(18)	(36)	(76)	(96)		
Hot spring water	+	+	+	+	+	+	+	+
	(24)	(20)	(18)	(22)	(20)	(19)	(22)	(20)

+, cellulose was decomposed; —, cellulose was not decomposed. Figures in parentheses show the times (hr) when the cultures were transferred to the next subcultures.

tungsten may serve as a prosthetic group of enzyme(s) involved in acetic acid synthesis, because the strain formed the acid from cellulose as a main product.

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