

Studies on Hydrocarbon-utilizing Microorganisms

Part II. Screening of Hydrocarbon-utilizing Bacteria Producing Antibiotic Substances from Kerosene

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Forty-four bacterial isolates were subjected to screening test for production of antibiotic substance from kerosene. Twenty-two isolates were found to produce antibiotic substance: 19 isolates against *Streptomyces* and 3 isolates against bacteria. But no isolates produced any antibiotics against moulds and yeasts.

Introduction

It is well-known fact that hydrocarbon-utilizing microorganisms produce many valuable substances, such as vitamins, protein, organic acids, amino acids, coenzymes, enzymes and so on. But there have been few reports on production of antibiotics from kerosene. Only pyocyanine¹⁾, phenazine and the derivatives^{2) 3) 4) 5)} were the antibiotics produced from kerosene.

In the present paper, screening test for production of antibiotic substance from kerosene is described, using 44 bacterial cultures which were isolated and stocked in the authors' laboratory.

Materials and Methods

1) Bacterial cultures employed.

Forty-four cultures of bacteria employed in the present investigation were isolated in the authors' laboratory from oil-soaked soils by means of enrichment or plate culture method as described in the previous paper⁶⁾.

2) Microorganisms employed for antibiosis test.

Moulds: *Mucor racemosus* HUT 1193, *Rhizopus nigricans* HUT 1262, *Aspergillus niger* HUT 2119, *Cladosporium herbarum* OUT 4014, *Penicillium expansum* HUT 4021, *Alternaria tenuis* HUT 4021.

Yeasts: *Saccharomyces cerevisiae* HUT 7099, *Zygosaccharomyces salsa* HUT 7191, *Pichia farinosa* HUT 7309, *Candida utilis* HUT 7526.

Streptomycetes: *Streptomyces olivaceus* HUT 6022, *Streptomyces griseus* HUT 6037.

Bacteria; *Escherichia coli* IFO 3044, *Proteus vulgaris* IFO 3045, *Staphylococcus aureus* 209 P, *Bacillus subtilis* ATCC 6633.

3) Production and detection of antibiotic substance.

One loopful bacterial isolate was inoculated into 10 ml of cultural medium A previously sterilized in a L type test tube and incubated on Monod's type shaker for 7 days at 30°C. The culture broth was centrifuged at 12,000 r. p. m. for 30 min. The supernatant liquid was

adjusted to pH 7.0 with 0.1 N NaOH solution and used for detection of antibiotics.

Cultural Medium A for Hydrocarbon-utilizing Bacteria

Na ₂ HPO ₄ · 12H ₂ O	3.0 (g)
KH ₂ PO ₄	3.0
NaCl	0.5
MgSO ₄ · 7H ₂ O	0.5
NH ₄ NO ₃	5.0
tap water	1000 ml
pH	7.0
kerosene	100 ml

For detection of antibiotics, agar plate was prepared as follows. Fifteen ml of cultural medium B (for moulds and yeasts), C (for *Streptomyces*) or D (for bacteria) was poured into a petri dish (ϕ 90 mm) and solidified. A hole was made by a cork borer (ϕ 8 mm) at center of the agar plate. Microorganisms for antibiosis test were smeared by a hook-shaped nichrome wire to four directions from the hole in which 0.08 ml of the supernatant liquid obtained above was put, and incubated for two to four days at 28° C. When the growth of some test

organism was not observed around the hole, it was considered that antibiotic substance against the organism was produced in the culture broth of the hydrocarbon-utilizing bacterium.

Cultural Medium B for Moulds and Yeasts

malt extract	3.0 (g)
glucose	1.0
yeast extract	0.3
agar	2.0
tap water	100 ml
pH	5.5

Cultural Medium C for *Streptomyces*

peptone	0.2 (g)
meat extract	0.1
yeast extract	0.1
glucose	1.0
agar	2.0
tap water	100 ml
pH	7.0

Cultural Medium D for Bacteria

meat extract	1.0 (g)
peptone	1.0
agar	2.0
tap water	100 ml
pH	7.0

4) Fractionation of the supernatant liquid of culture broth.

Strain S15K2 which produced antibiotic substance against *Streptomyces griseus* was cultivated in 500 ml shaking flask containing 100 ml of medium A. The supernatant liquid adjusted to pH 7.0 was fractionated by means of the procedure described below and the fractions were tested for antibiosis. Before test, fractions I, II, IV and V were adjusted to pH 7.0 with 0.1 N NaOH solution or 0.1 N HCl solution and fractions III and VI were dissolved in Tris-HCl buffer solution (pH 7.2) after evaporation of ether.

Table. 2 Antibiotic Activity of Bacterial Isolates against Moulds, Yeasts, Bacteria and *Streptomyces*.

Bacterial Isolates	Test Microorganisms															
	<i>Mucor racemosus</i> HUT 1193	<i>Rhizopus nigricans</i> HUT 1262	<i>Aspergillus niger</i> HUT 2119	<i>Cladosporium nerbarum</i> OUT 4014	<i>Penicillium expansum</i> HUT 4021	<i>Alternaria tenuis</i> OUT 4621	<i>Saccharomyces cerevisiae</i> HUT 7099	<i>Zygosaccharomyces sulfus</i> HUT 7191	<i>Pichia farinosa</i> HUT 7309	<i>Candida utilis</i> HUT 7526	<i>Escherichia coli</i> IFO 3044	<i>Proteus vulgaris</i> IFO 3045	<i>Staphylococcus aureus</i> 209 P	<i>Bacillus subtilis</i> ATCC 6633	<i>Streptomyces olivaceus</i> HUT 6022	<i>Streptomyces griseus</i> HUT 6087
S18K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
S18K2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
S20K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S22K2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S24K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
S24K3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
S30K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S30K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S31K2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S34K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
S37K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S40K2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
S40K3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
S40K4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
S41K2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
S42K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S42K2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S43K1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S43K2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S43K3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table. 3 Antibiotic Activity against *S. griseus* of Six Fractions from the Cultural Broth of S15K2

Each Fraction	Antibiosis
fr-I	+
fr-II	-
fr-III	+
fr-IV	+
fr-V	-
fr-VI	+

Table. 4 Growth-inhibiting Effect of Various Acids on *S. griseus*

	Acids	(C. N*)	Concentration (%) of acids administered					
			0.01	0.1	0.5	1.0	2.0	3.0
Monobasic acids	Propionic	(3)	—	—	—	—	—	—
	n-Butyric	(4)	—	—	—	—	—	—
	n-Caproic	(6)	—	—	—	—	—	—
	n-Heptanoic	(7)	—	—	—	—	—	+
	n-Caprolic	(8)	—	—	—	±	+	+
	Pelargonic	(9)	—	—	±	+	+	+
	Capric	(10)	±	+	+	+	+	+
	Lauric	(12)	—	—	NT	NT	NT	NT
	Myristic	(14)	—	—	NT	NT	NT	NT
	Stearic	(16)	—	—	NT	NT	NT	NT
Dibasic acids	Malonic	(3)	—	—	—	—	—	—
	Succinic	(4)	—	—	—	—	—	—
	Glutaric	(5)	—	—	—	—	—	—
	Adipic	(6)	—	—	—	—	—	—
	Pimelic	(7)	—	—	—	—	—	—
	Suberic	(8)	—	—	—	—	—	—
	Azelaic	(9)	—	—	—	—	—	—
	1, 10-Decane-dicarboxylic	(12)	—	—	—	—	+	+

Symbols ; *: Carbon Numbers of Acid. — : Did not inhibit ± : Slightly inhibited
 + : Inhibited NT: Not tested

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