The Mold-isolates from the Oil-soaked Materials in the Petroleum Refinery

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In order to gain such microorganisms as might serve for producing microbial cell substances from petroleum and chemical transformation of many kinds of compounds, 33 oil-soaked materials collected in the petroleum refinery were employed[1,2].

In this note, the isolation of molds from the oil-soaked materials and their determination are described.

Isolation of molds was carried out in the same way that had been already reported by one of us[1,2]. Hydrocarbons employed were light naphtha (d=0.68, 34°-155°C, paraffines 89.2 v%), kerosene (Wako Pure Chemical Industries, Ltd.), liquid paraffine (Wako Pure Chemical Industries, Ltd.), and middle oil (d=0.84, 36°-330°C, benzene, toluene, and xylene 40 w%). Sample materials consist of 33 oil-soaked materials collected in Chiba Refinery, Maruzen Oil, Co., Ltd., 4 crude oil samples, and 2 soil samples of perfume plant fields (Table 1).

Determination was carried out mainly according to de Vries' methods[3], with two monospore cultures of each isolate. The test media for cultural characteristics were as follows: glucose agar[3], Czapek's agar with glucose[3], potato glucose agar[4], and malt extract agar[4]. The assimilation of hydrocarbons was determined in the mineral salts solution[1] containing ammonium nitrate, 2 g/L and hydrocarbon (light naphtha, kerosene, or liquid paraffine), 0.02 g/L, while ammonium sulfate and yeast extract were remo ved, after incubation for 1 month at 25°C. A strain which showed much more growth after two serial subcultures than that of control in the mineral salts solution, from which hydrocarbon was removed, was recorded as distinctly positive.

As shown in Table 1, molds were isolated from 17 of 39 sample materials. The isolates by each substrate were as follows: 19 strains, when light naphtha was employed as a substrate; 6 strains, when kerosene employed; 35 strains, when liquid paraffine employed. They totalled 60 strains. The isolates by each pH were as follows: 17 strains with light naphtha, 6 strains with kerosene, and 34 strains with liquid paraffine at pH 4.5; 2 strains with light naphtha, none with kerosene, and 1 strain with liquid paraffine at pH 7.2. These isolates were distinguished into 10 cultural groups with their colony appearances on the mineral salts agar slants containing hydrocarbons. The representatives of those groups were marked with Mo-1, Mo-2, Mo-3, Mo 4, Mo-5, Mo-6, Mo-7, Mo-8, Mo-9, and Mo-10, respectively, and served for identification.

These representatives were identified with the following species. The strain Mo-1 was considered to belong to *Aspergillus glaucus* group in respect of its morphological and cul-

Table 1. Isolates and their origins

Origins	Material no.	Mold isolates-their numbers					
		pH 4.5			pH 7.2		
		light naphtha	kerosene	liquid paraffine	light naphtha	kerosene	liquid paraffine
(aqueous materials)							
drain water in a ditch into the waste-treating plant	6	—	Y	Cc-1, T-1	—	—	—
	7	T-1	Y	—	—	—	—
waste water in the oil separator, in operation	8	T-6, An-4	Y	T-2, Y	—	—	—
	12	—	—	Cc-1, Cc*-1 Pi-1, Pd-3	—	—	—
	13	—	—	Cc-1	—	—	—
	14	—	Y	T-2	—	Y	—
	15	—	Y	—	—	Y	—
waste water in the oil separator, off operation	18	—	Y	Y	—	—	Y
	26	—	Y	T-5	—	—	Cc*-1
bottom water in the storage tank of crude oil	21	T-4	—	T-2	—	Y	—
	22	—	—	Cc*-1	—	—	—
	23	T-1	T-1	T-2	—	—	—
cooling water in the petro-chemical plant	34	T-1	—	Cc—1	—	—	—
(soil materials)							
soil about the topping unit	1	—	Y	T-1, Y	Cc-2	—	—
	5	—	Ca-3	—	—	—	—
soil about the waste-treating plant	16	—	Y, Ao-2	Y	—	—	—
(others)							
crude oil in a drain ditch	2	—	Y	Cc-1, Pd-6, Y	—	—	Y
	27	—	—	Pi-1,	—	—	—
crude oil (Kuwait)	39	—	—	Pi-1,	—	—	—

* An: *Aspergillus niger*. Ao: *Asp. oleovorans*. Cc: *Cladosporium cladosporioides*. Ca: *Cl. avcllaneum*. Pi: *Penicillium implicatum*. Pd: *Pen. decumbens*. T: *Trichoderma lignorum*. Cc*: *Cl. cladosporioides* Mo-6. Y: Hydrocarbonutilizing yeasts were isolated[1].

tural properties, but this strain was identified with no species in Thom and Raper's system[5]. *Asp. proliferans* is close to this strain, but different from this strain with the size of conidia. This strain should rather be designated as a new species in *Asp. glaucus* group than as a variety of *Asp. proliferans*, because of the absence of perithecium. Accordingly, this strain was given a new species name, *Asp. oleovorans*, because of its ability of hydrocarbon utilization and active lipolysis with tributyrin (Fig. 1). The strains Mo-2 and Mo-8 were identified with *Penicillium implicatum* and *Pen. decumbens* respectively, and the strains Mo-3 and Mo-4 were identified with *Trichoderma lignorum*[6,7] (Fig. 2, 3, and 4). The strain Mo-5 should be identified with *Cladosporium avellaneum* because of the rapid growth, grayish yellow brown conidial areas, production of brown diffusible pigment and incapacity to liquefy gelatin, and was considered as *Cl. avellaneum* form *avellaneum*, differing from *Cl. avellaneum* form *viride* in color of conidial areas, and shape and size of conidia (Fig. 5). The strains Mo-7, Mo-9 and Mo-6 were identified with *Cl. cladosporioides*, distinctly differing from *Cl. herbarum* in the smaller, usually 1-celled, smooth conidia, and the greater number of conidia per conidial head (Fig. 6). The strain Mo-6 was more or less different from the strains Mo-7 and Mo-9 in respect of appearances of colonies on Czapek's agar, size of conidia and lipolysis, but it was identified with the same species, as differences were negligible. The strain Mo-10 should be identified with *Asp. niger* because of rough brown-black conidia, double sterigmata, and large and globose conidial heads (Fig. 7).

Trying assimilation test with light naphtha, kerosene, and liquid paraffine respectively, it was ascertained that only one strain, *Asp. oleovorans* Mo-1, could utilize hydrocarbon distinctly, though only kerosene was employed as a sole source of carbon.

DESCRIPTION

Aspergillus oleovorans nov. sp.

strain: Mo-1.

o. le. o'vo. rans M. L. adj. oil-devouring, *oleum* L. oil, many kinds of oils, including mineral oils, *voro* L. v. to devour (Fig. 1).

Perithecia absent. Conidial heads abundant, radiate, grayish olive. Conidiophores, produced on foot cells, walls of which are smooth. Vesicles globose, 12.5 to 15 μ in diameter. Phialides in one series, 5 μ long. Conidia, pale green, globose, 2 to 3 μ, the surface of which is somewhat rough. Vegetative mycelium, colorless. Cleistothecia and sclerotia, not found.

Colonies on Czapek's agar spreading slowly, reaching a diameter of 65 to 68 mm in 20 days at room temperature, 23° to 28°C, with growth largely submerged, then partially with matted floccose aerial mycelium, white, with conidial areas grayish olive. Reverse pale yellowish brown to yellowish brown. Colonies on malt extract agar and potato glucose agar, moderately growing, velvety, grayish yellow green.

Amylolytic, proteolytic and lipolysis: positive with tributyrin. Produce brown pigment from gallic acid only at pH 6.0.

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SUMMARY

Sixty strains of molds were isolated from 33 oil-soaked materials collected in the petroleum refinery, 4 crude oil samples, and 2 soil samples of perfume plant fields, employing light naphtha, kerosene, or liquid paraffine as a sole source of carbon. They were distinguished into 10 cultural groups with their colony appearances. The representatives of those groups were identified as follows: *Aspergillus niger*, 1 strain; *Asp. oleovorans*, 1 strain; *Cladosporium cladosporioides*, 3 strains; *Cl. avellaneum*, 1 strain; *Penicillium implicatum*, 1 strain; *Pen. decumbens*, 1 strain; *Trichoderma lignorum*, 2 strains. Only one strain, *Asp. oleovorans* was considered to be able to utilize hydrocarbon as a sole source of carbon.

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Fig. 1 a Conidiophores of *Asp. oleovorans* Mo-1 on Czapek's solution.
1 b Branched conidiophore and radiated conidial head of the strain Mo-1
1 c Globose vesicle, phialides in one series and smooth conidiophore wall of the strain Mo-1.
1 d Giant colony of the strain Mo-1.
Fig. 2 Branched conidiophores of *Pen. implicatum* Mo-2.
Fig. 3 Branched conidiophores of *Pen. decumbens* Mo-8.
Fig. 4 Conidiophores of *Trich. lignorum* Mo-4.
Fig. 5 Conidiophore of *Cl. avellaneum* Mo-5.
Fig. 6 Conidiophore of *Cl. cladosporioides* Mo-7.
Fig. 7 a Globose conidia and their verrucose surface of *Asp. niger* Mo-10.
7 b Double sterigmata of the strain Mo-10.

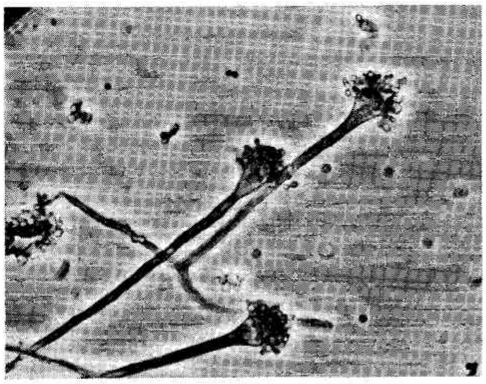

Fig. 1 a

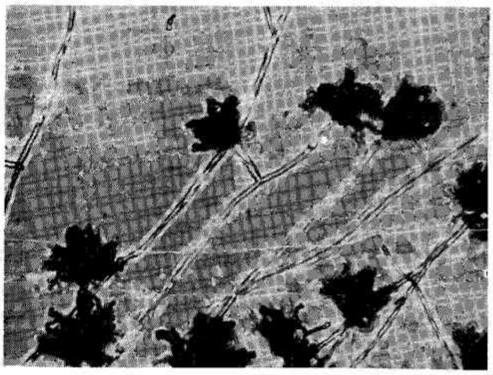

Fig. 1 b

Fig. c

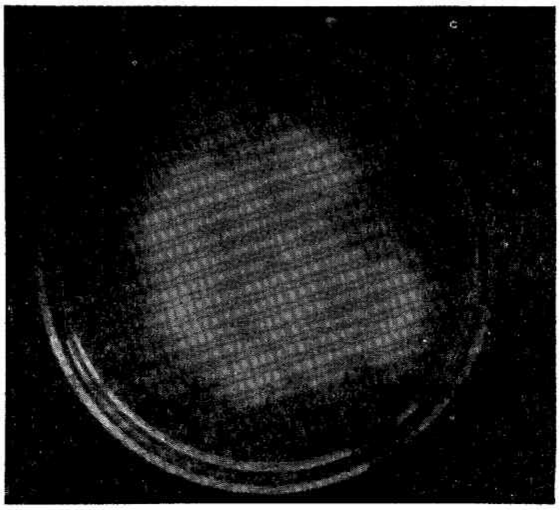

Fig. 1 d

Fig. 2

Fig. 3

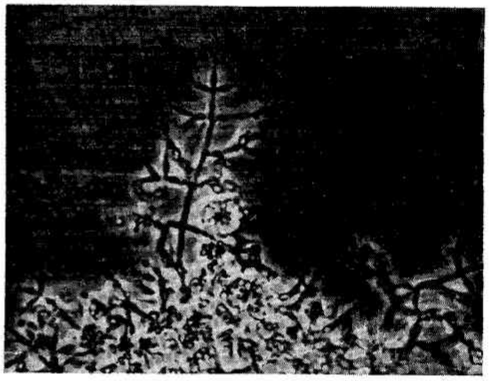

Fig. 4

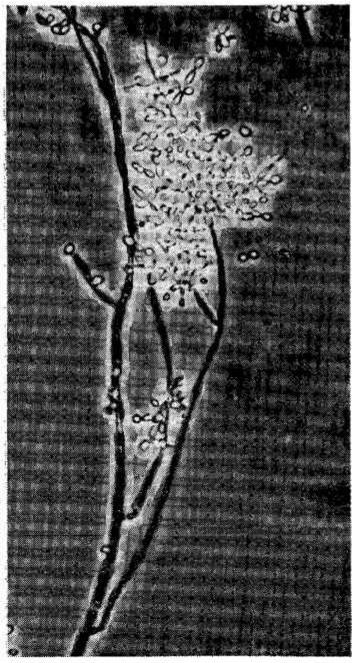

Fig. 5

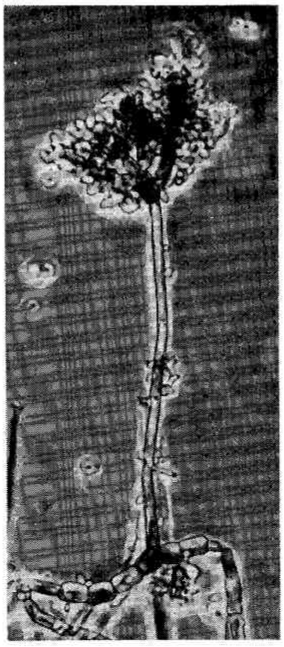

Fig. 6

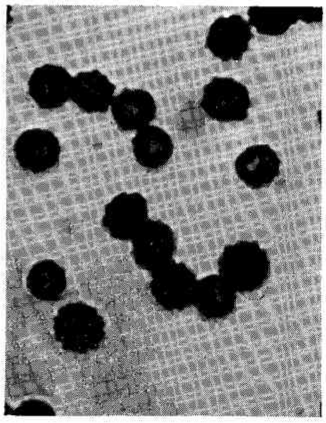

Fig. 7 a

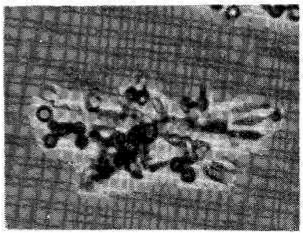

Fig. 7 b