A NEW GENUS OF THE ACTINOMYCETALES: *KITASATOA* GEN. NOV.

AKIHIRO MATSUMAE, MARIKO OHTANI,
HIDEO TAKESHIMA and TOJU HATA

The Kitasato Institute, Tokyo, Japan

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In the course of isolating soil microorganisms four strains of actinomycetes were isolated. Based on the morphological studies these organisms were found to belong to a new genus in the family *Actinoplanaceae*. These organisms produced abundant aerial mycelia as *Streptomycetaceae*, and produced a club-shaped sporangium and a motile zoospore. With these characters it was designated as *Kitasatoa* gen. nov., belonging to the family *Actinoplanaceae*. Among four strains, *Kitasatoa purpurea* is the type genus. The three other strains are: *K. kauaiensis*, *K. nagasakiensis* and *K. diplospora*.

Actinomycetes are filamentous bacteria which are often considered intermediate between true bacteria and the fungi. According to the latest edition of BERGEY's Manual (1957)[1], the organisms which formed spores in sporangium were grouped under the family *Actinoplanaceae*. In this family, two genera have been described. The one is the genus Actinoplanes which formed motile spore, but not formed aerial mycelium, and the other is the genus *Streptosporangium* which formed aerial mycelium and non-motile spore. Thereafter other new genera, which formed motile spores and exhibited different types of sporangium and growth were reported[3~5]. Recently the authors isolated several strains of soil microorganisms and found that these organisms belonged to the family *Actinoplanaceae* but not to the *Streptomycetaceae*. Based on their characteristics, *Kitasatoa*, a new genus was established in the family *Actinoplanaceae*. The present paper deals with morphological and biological studies of *Kitasatoa*.

Materials and Methods

Isolation: Organisms were isolated from soil samples collected from many places. They were incubated on KRAINSKY's glucose asparagine agar or on PRIDHAM's starch agar for 5 days at 27°C.

Strain No. KA-279 (*K. purpurea*), No. KA-280 (*K. diplosporus*), and No. KA-281 (*K. kauaiensis*) were obtained from a soil of Kauai island in Hawaii, U.S.A. Strain No. KA-282 (*N. nagasakiensis*) was obtained from a cultivated field in Nagasaki Prefecture, Japan.

Culture medium: Spores of each of the strains were incubated at 27°C on agar plate or on agar block described by WAKSMAN[6].

Utilization of carbon source: Utilization of carbon sources was studied by the method described by PRIDHAM *et al.*[7]

Color characteristics: Color characteristics were compared to the Color Harmony Manual (U.S.A.)[8] and Guide to Color Standard (Japan)[9].

Microscopic and electron microscopic studies were done with microphotographs taken by a Nikon S-Ke Camera and with electron microphotographs taken by a Nihon Denshi JEM-5Y electron microscope.

Results

Description of *Kitasatoa* MATSUMAE and HATA genus nov.

The vegetative mycelium was 0.8~1.2 μ in width and well diverged. The septum on the mycelium was usually not confirmed, but in the older culture it was recognized by staining. Gram staining was positive, and acid-fast staining negative. The vegetative hyphae grew in or on the substrate of the agar media, and formed a colony as that of streptomyces. The vegetative mycelium grown in the fluid media or on the agar media produced short sporophores. At the extreme point of the sporophore a club-shaped sporangium was produced (Plates 1 & 2). Spores were arranged in a straight chain within the sporangium.

The sporangium was easily separated from the vegetative mycelium in water, and released zoospore. The zoospore swam rapidly in water.

By the electron-microscopic examination the spore showed spherical or ellipsoidal form (*K. purpurea* and *K. diplosporus*) as shown in Plate 3 and cylindrical form (*K. kauaiensis* and *K. nagasakiensis*) as shown in Plate 4. In most cases two spores adhered in pairs, and it appeared like a diplococcus or a chain of two spores (Plates 3 and 4). The spore had commonly a single flagellum at the one end as shown in Plates 3 and 4. Sometimes two spores adhered in parallel (Plate 5).

On the other hand, aerial mycelium grew abundantly from the vegetative mycelium on various kinds of media and diverged in all directions. Aerial hyphae were two types.

One was slightly thick (1.2~1.5 μ in width) and diverged a little.

The top of the hyphae was swollen, 2~2.5×5 μ in size, and formed a club-shaped sporangium. This sporangium produced a diplococcus-like sporangiospore which was released in the water.

This thick aerial hyphae produced also a round, vesicle body (Plate 8), mycelial net-like body (Plate 9), sclerotic granule (Plate 10) or crystals (Plates 11a and b).

The other type of hyphae was 1.0~1.2 μ in width and formed a long chain of cylindrical conidiospores as shown in Plates 12 and 13.

(1) Description of *Kitasatoa purpurea*, MATSUMAE *et* HATA, sp. nov. (No. KA-279)

Morphology:

Vegetative hyphae varied from 0.8 to 1.2 μ in width. These hyphae grew on the surface and in the substrate of the media, and formed mycelium. On the older culture, the hyphae were segmented, but not separated.

Gram-stain was positive, and acid-fast was negative. On the agar medium a club-shaped sporangium (2.5~3×1.0 μ in size) grew on the extreme point of a litte thicker hyphae as shown in Plates 1 and 2. Sporangiospore formed a diplococcus-like zoospore (2.0~2.8 μ×1.6~2.3 μ in size), having a polar single flagellum (Plate 3) and easily released from the sporangium. This zoospore swam rapidly in the water. The flagellum was 10~50 μ in length.

Table 1. Cultural findings of *Kitasatoa* on various media

		K. purpurea	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*
Glycerol Czapek's agar	G.	wet, elevated, dark brownish purple	poor, wet, pale yellowish brown	poor, wet, reddish brown	wet, elevated penetrated, grayish brown
	A.M.	none or scant, white	none or scant, white	none or scant, white	scant, white
	S.P.	dark brownish purple	light brown	reddish brown	pale brown
Glucose Czapek's agar	G.	poor, dewdrop-like, flat pale reddish brown	brown	poor, dewdrop-like, grayish red brown	small, flat, center elevated, grayish yellow brown
	A.M.	scant, white	white	none or scant, white	cottony, brownish white
	S.P.	brown	dull orange	reddish brown	grayish red brown
Sucrose Czapek's agar	G.	poor, dry, sand-like, grayish yellow brown	pale yellow	poor, penetrated colorless	poor, dry sand-like small, penetrated, brownish white
	A.M.	light pinkish gray	pinkish orange	poor, brownish white	dark brownish gray
	S.P.	none	none	none	none
Glucose Czapek's agar	G.	pale yellowish brown sedimentation	pale yellow	colorless sedimentation	brownish white sedimentation
	A.M.	none	none	none	none
	S.P.	none	none	none	none
Glucose asparagine agar	G.	small, center elevated, grayish brown	pale yellowish brown	large, wet, center, elevated, pale yellow	round center, elevated, yellowish brown
	A.M.	light gray	none	none or scant brownish gray	yellowish brown
	S.P.	light brown	grayish yellow brown	yellowish brown	grayish yellow brown
Ca-Malate agar	G.	large, elevated dark brown	large, flat, center elevated reddish brown	brown	center elevated brownish black
	A.M.	brownish white	none or scant, white	brownish white	yellowish white
	S.P.	dark brown	reddish brown	light brown	dark brown
Pridham's starch agar	G.	flat, pale yellowish brown	dark yellowish brown	moderate, center elevated yellow	center elevated
	A.M.	light brownish gray	brownish white	cottony, light brownish white	light brownish gray
	S.P.	none	light brownish gray	grayish yellow	yellowish brown
Bennett's agar	G.	brown	grayish yellow brown	light brownish gray	light brownish gray
	A.M.	brownish white	light brownish gray	cottony, brownish white	grayish white
	S.P.	dark brown	grayish yellow brown	brown	brownish black

(To be continued)

Table 1 (continued)

		K. purpurea	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*
Peptone agar	G.	flat, colorless	brownish white	light brownish gray	light brownish gray
	A.M.	brownish white	light brownish gray	cottony, brownish white	grayish white
	S.P.	pale yellowish brown	grayish yellow brown	pale brown	brownish black
Glucose nutrient agar	G.	grayish yellow brown	pale yellowish brown	large, round wrinkled, pale brown	large, round wrinkled, pinkish gray
	A.M.	brownish white	white	cottony, white	none
	S.P.	reddish brown	grayish red brown	brown	dark brown
Nutrient broth	G.	yellowish brown sedimentation	colorless sedimentation	colorless sedimentation mold like growth	pale yellow brown sedimentation
	S.P.	pale brown	yellowish brown	yellowish brown	pale brown
Oat meal agar	G.	poor, yellowish brown	yellowish gray	thin, flat, poor, pale olive	light brownish gray
	A.M.	light brownish gray	brownish white	cottony, white	grayish white
	S.P.	yellowish brown	grayish yellow brown	none or pale olive gray	brownish black
Potato plug	G.	wrinkled, dark brownish gray	grayish yellow brown	wrinkled, yellowish brown	light brownish gray
	A.M.	none or scant, white	white	none or scant, white	white
	S.P.	grayish red brown	grayish brown	grayish yellow brown	grayish yellow brown
Carrot plug	G.	grayish red brown	grayish yellow brown	pale brown	light brownish gray
	A.M.	white	none	none or scant, white	none
	S.P.	none	none	none	black
Milk	G.	sedimentation	sedimentation	ring and sedimentation yellowish brown	ring
	A.M.	none	none	poor, white	none
	S.P.	pale brown	yellowish brown	yellowish brown	brown
Gelatin	G.	pale brown	pale brown	pale brown	pale brown
	A.M.	none	none	none	none
	S.P.	brown	yellowish brown	brownish black	brown

G. : Growth, A.M. : Aerial mycelium, S.P. : Soluble pigment.

When dried, the zoospore was considered to change its form from flat to round, and to lose the flagellum and membrane-like structure surrounding the spore.

On the slide cell culture with BENNETT's agar, germ tubes (Plate 6) having no septum, grew from both ends of the zoospore and formed hyphae after 9~12 hours of incubation. Whorls or spirals were not developed from the aerial mycelium. The conidium (1.0~1.2 μ in size) developed on the aerial mycelium was cylindrical (1.5~1.8 μ in length by 0.8~1.0 μ in width) and separated one by one (Plate 13). The surface of the conidium was smooth and it had no spin structure and flagellum.

Table 2. Physiological characteristics of *Kitasatoa*

	K. purpurea	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*
Nitrate reduction	+	±	+	+
Starch hydrolysis	+	+	+	+
Milk coagulation	−	−	±	+
Milk peptonization	−	−	−	+
Hemolysis	−	+	±	−
Melanine production	+	+	+	+
Tyrosinase production	+	+	+	+
Serum liquefaction	−	+	−	−
Gelatine liquefaction	+	+	+	+
Temperature (optimum temperature)	10～37°C (20～30°C)	10～37°C (27～30°C)	10～37°C (25～32°C)	10～37°C (27～30°C)
pH	5.0～9.0	5.0～8.5	5.0～8.5	5.0～9.0
Production of antibiotics	Chloramphenicol	Chloramphenicol Anti-leukemic substance	Chloramphenicol Bottromycin Fradicin	Chloramphenicol

Table 3. Utilization of carbon sources

	K. purpurea	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*		*K. purpurea*	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*
Adonitol	−	−	−	−	Mannose	±	±	±	+
Aesclin	±	±	±	−	Melezitose	−	−	−	−
Arabinose	+	+	+	+	Melibiose	−	−	−	±
Cellobiose	+	+	+	+	Raffinose	−	−	−	−
Cellulose	−	−	−	−	Rhamnose	−	−	−	−
Dextrose	+	+	+	+	Saccharose	−	−	±	−
Dulcitol	±	−	−	−	Salicine	+	+	+	+
Galactose	±	±	±	±	Sorbitol	±	−	+	+
Glucose	+	+	+	+	Starch	+	+	+	+
Glycerol	+	+	+	+	Sucrose	−	−	±	−
Inulin	±	−	−	+	Xylose	±	±	±	±
Inositol	−	−	±	−	Na-Acetate	+	+	+	+
Lactose	−	−	+	+	Na-Citrate	−	−	−	−
Levulose	+	+	+	+	Na-Succinate	−	−	−	−
Maltose	+	+	+	+	Control	−	−	−	−
Mannitol	−	−	−	−					

Small vesicle bodies (30～50 μ in size) were observed on a little thicker, cottony aerial hyphae (1.2～1.5 μ in size). Surface of the vesicle body was smooth and round, and was covered with a thin membrane. When compressed, a small amount of fluid was flowed out from the vesicle body.

Mycelial body conglomerated with aerial hyphae (Plates 9a and b), mass of spore (Plate 7) and crystalline body (Plates 11a and b) on the mycelium and sclerotic granules (Plates 10a and b) in the substrate of the medium were found.

Cultural findings of the strain are shown in Tables 1～3.

Kitasatoa purpurea were differentiated from other species of *Kitasatoa* in several points as shown in Table 4.

Source: soil in Kauai Island.

Production of antibiotic: chloramphenicol.

This strain K-279 is the type culture of *Kitasatoa*.

Table 4. Characteristics of 4 species of *Kitasatoa*

		K. purpurea	*K. diplospora*	*K. kauaiensis*	*K. nagasakiensis*
Zoospore		globose single or in pairs 2.0~2.8×1.6~2.3 μ	ellipsoidal single or in pairs 2.5~3.0×1.5 μ	cylindrical single or in pairs 2.4~5.0×1.1~1.35 μ	rod single or in parallel 2.9×0.8 μ
Conidia		ellipsoidal, smooth 1.5~1.8×0.8~1.0 μ	cylindrical, smooth 1.5~1.3×0.7 μ	cylindrical, warty 1.2~1.5×0.8 μ	ellipsoidal, smooth 1.2~1.6×0.8~1.0 μ
Glycerol Czapek's agar	G.	dark brownish purple	pale yellowish brown	reddish brown	grayish brown
	A.M.	white	white	white	white
	S.P.	dark brownish purple	light brown	reddish brown	pale brown
Sucrose Czapek's agar	G.	grayish yellow brown	pale yellow	colorless	brownish white
	A.M.	light pinkish gray	pinkish orange	brownish white	dark brownish gray
	S.P.	none	none	none	none
Oat meal	G.	yellowish brown	yellowish gray	pale olive	light brownish gray
	A.M.	light brownish gray	brownish white	white	grayish white
	S.P.	yellowish brown	grayish yellow brown	pale olive gray	brownish black
Milk coagulation		−	−	±	+
Milk peptonization		−	−	−	+
Hemolysis		−	+	±	−
Serum liquefaction		−	+	−	−

G. : Growth, A.M. : Aerial mycelium, S.P. : Soluble pigment.

(2) Description of *Kitasatoa kauaiensis*, Matsumae, Ohtani *et* Hata, sp. nov. (No. KA-281)

Morphology:

Morphological findings of this strain were closely related to that of the type culture KA-279. Electron microscopic examination indicated that a cylindrical or ellipsoidal zoospore, 2.4~5.0×1.1~1.35 μ in size, was covered with a thin membrane and had a single flagellum (Plates 4a, b). When an agar block inoculated with the strain on the slide cell culture was violently shaken in the water, a sporangium with or without zoospore was observed. Aerial mycelium formed a chain of cylindrical or ellipsoidal conidia. This conidium was 1.5~1.2×0.8 μ in size. Its surface was slightly warty.

Cultural findings of the strain are shown in Tables 1~3.

Kitasatoa kauaiensis was differentiated from the other species of *Kitasatoa* in several points as shown in Table 4.

Source: the soil collected in Kauai Island.

Production of antibiotics: chloramphenicol, bottromycin A_2, B and fradicin.

(3) Description of *Kitasatoa diplospora*, Matsumae, Ohtani *et* Hata, sp. nov. (No. KA-280)

Morphology: Vegetative mycelium was a mass of hyphae, 1.0 μ in width. Gram and acid fast staining were indefinite. Its morphological characteristics was very similar to the type culture KA-279.

The conidium was cylindrical, 1.5~1.3×0.7 μ in size and its surface was smooth.

The sporangium of this strain contained a chain of two ellipsoidal zoospores having a single polar flagellum.

Besides of the sporangium, there were also observed vesicle body, sporangia like body and crystals.

Cultural characteristics: The cultural characteristics of this strain are shown in Tables 1~3.

Nitrite reduction was weak. Peptonization and coagulation of milk were negative. Liquefaction of serum was strong.

Source: soil collected in Kauai Island.

Production of antibiotics: chloramphenicol, antileukemic substance against mouse leukemia L-1210.

(4) Description of *Kitasatoa nagasakiensis*, MATSUMAE *et* HATA, nov. sp. (No. KA-282)

Morphological characteristics are closed related to the type culture KA-279.

Gram positive and acid fast negative. The conidia were ellipsoidal, 1.2~0.6 $\mu \times 0.8$~1.0 μ in size. Its surface was smooth. This strain formed a long bat-shaped sporangium.

The zoospore was rod or ellipsoidal in shape, $2.9 \times 0.8\ \mu$ in size, and sometimes adhered in parallel, having a single polar flagellum.

Cultural characteristics: Cultural findings of this strain are shown in Tables 1~3.

Coagulation and peptonization of milk were positive.

Source: soil collected in Nagasaki prefecture.

Production of antibiotics: chloramphenicol.

Discussion

The authors established a new genus *Kitasatoa* in the family *Actinoplanaceae*, because *Kitasatoa* differs from the other genera of *Actinoplanaceae* in the following generic characteristics: (1) all four strains belonging to *Kitasatoa* produce abundant aerial mycelium, (2) the vegetative mycelium grown on liquid or agar media produces a club shaped sporangium on the extreme point of the hyphae, (3) the aerial mycelium produces a long chain of conidia by segmentation of the hyphae in addition to the sporangium, (4) sporangiospores developed in the sporangium, (5) sporangiospore has a single flagellum at one pole and swims rapidly in the water (zoosporangiospore).

The genera of the family *Actinoplanaceae* (COUCH[2], 1950) are classified into A group having motile spores and B group having no motile spores.

According to the arrangement of sporangiospores, A group can be classified into four types including *Kitasatoa*.

Family *Actinoplanaceae*: COUCH, 1950

A. Motile spore.....(*Actinoplanoideae*)
1. Spores arranged in coiled chains within sporangium.
   a. Sporangium spheroid. Aerial mycelium lacking. .....*Actinoplanes* COUCH, 1950
   b. Sporangium spherical. Aerial mycelium present.....*Spirillospora* COUCH, 1963
2. Spores arranged in parallel chains within sporangium.
   a. Sporangium cylindrical or irregular. Aerial mycelium lacking. ..... *Ampulariella* COUCH, 1966
   b. Sporangium spherical or cylindrical. Aerial mycelium present. .....*Pilimelia* KANE, 1966
3. Spores arranged in straight chain within sporangium.
   a. Sporangium finger shaped .....*Dactinosporangium* THIEMANN, 1867
   b. Sporangium club-shaped. Aerial mycelium present. .....*Kitasatoa* gen. nov.
4. Sporangium formed intercalary in the mycelial hyphae.

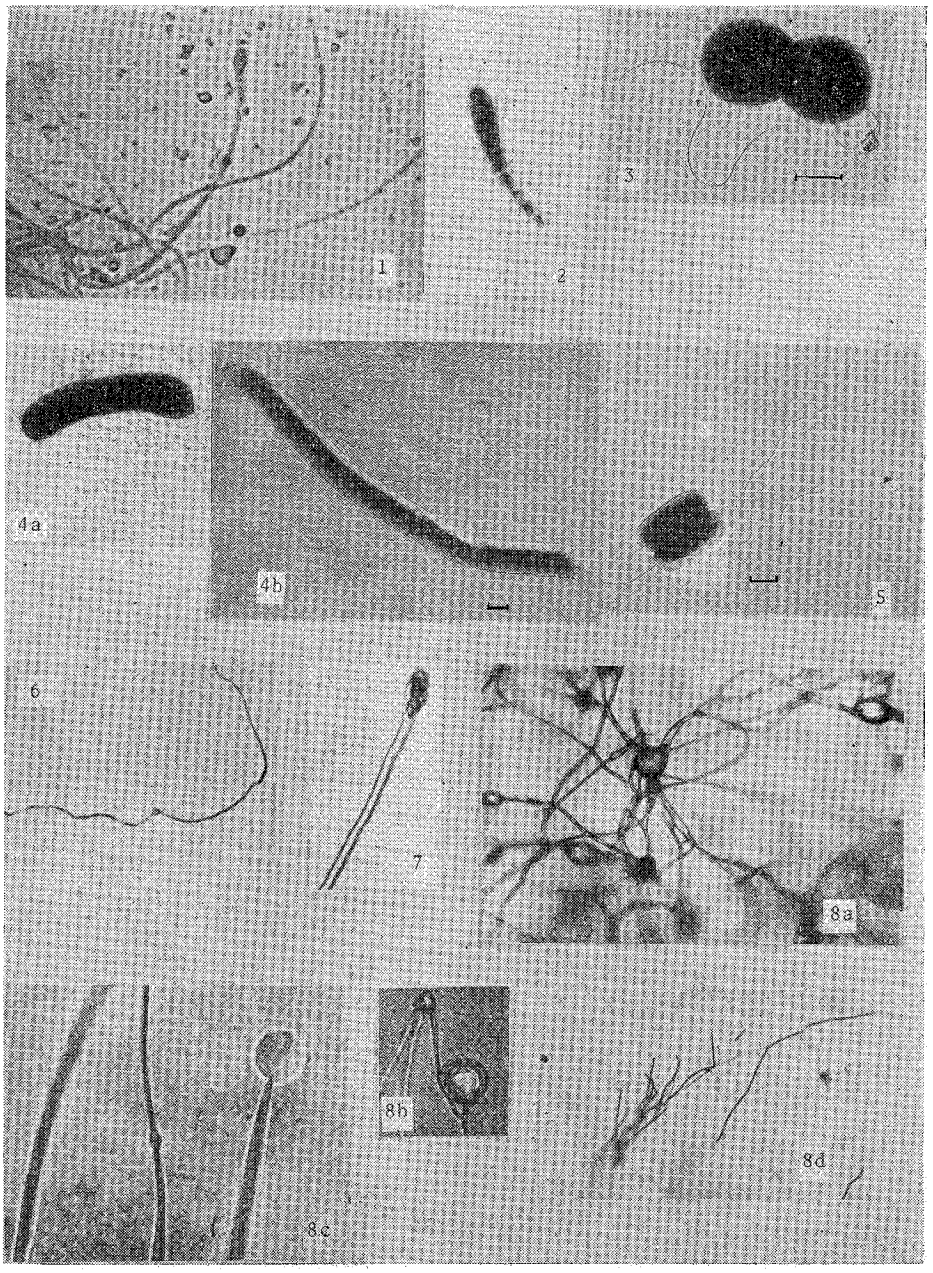

Plate 1. *K. purpurea* in BENNETT's solution, ×200×2/3
Plate 2. *K. purpurea*; a club-shaped sporangium in BENNETT's solution, ×800×2/3
Plate 3. *K. purpurea*; electron microphotograph of a diplococcus-like chain on synthetic agar, ×800×2/3
Plate 4-a. *K. kauaiensis*; electron microphotograph of a cylindrical-shaped zoospore, ×7,000×2/3
Plate 4-b. *K. kauaiensis*; electron microphotograph of sporangium with zoospore, ×25,000×2/3
Plate 5. *K. nagasakiensis*; electron microphotograph of a rod-shaped zoospore, ×5,000×2/3
Plate 6. *K. purpurea*; a germ tube on BENNETT's agar, ×1,200×2/3
Plate 7. *K. purpurea*; a mass of spores, WAKSMAN's solution, ×600×2/3
Plate 8-a, b, c, d. Scrotic vesicular body on PRIDHAM's starch agar

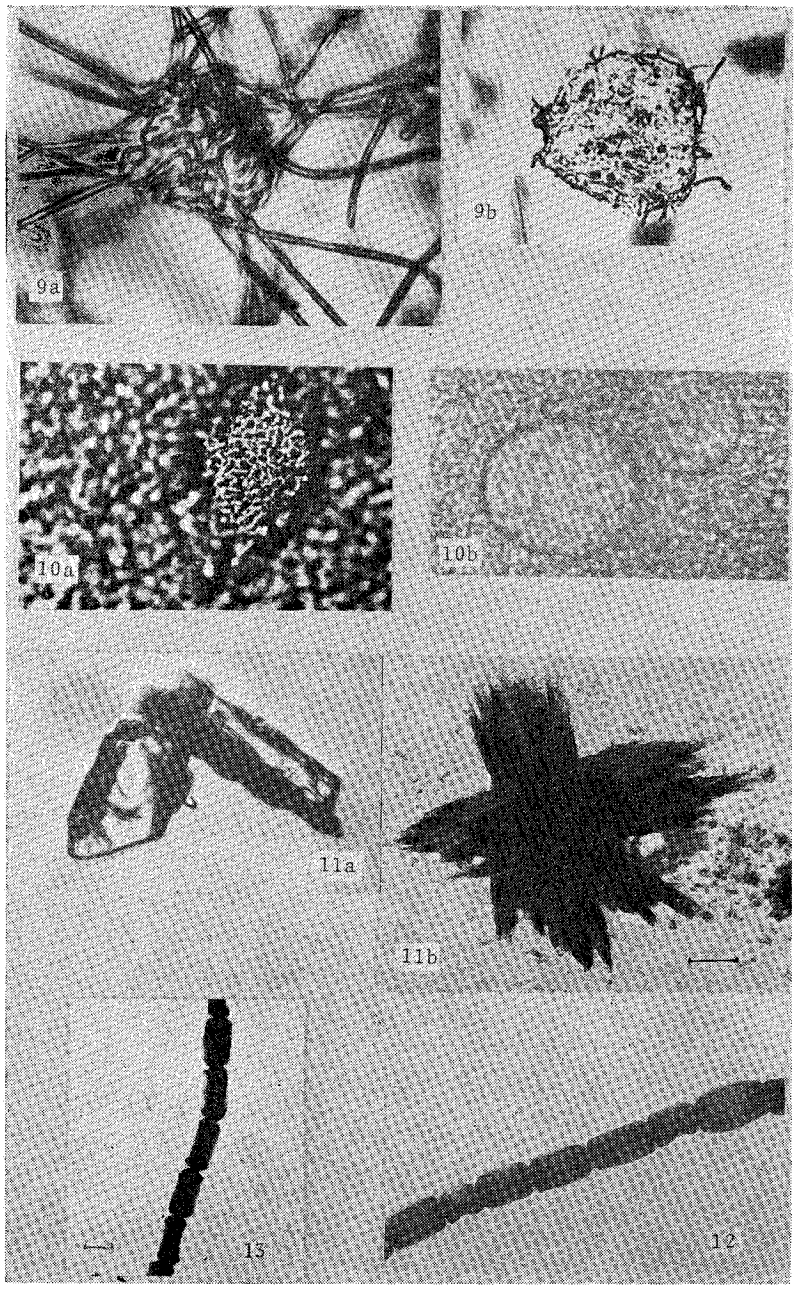

Plate 9-a. *K. purpurea*; a mycelial net-like body on PRIDHAM's starch agar, ×2,400×2/3
Plate 9-b. *K. purpurea*; a mycelial net-like body on bouillon, ×1,500×2/3
Plate 10-a. *K. purpurea*; sclerotic granule in BENNETT's agar
Plate 10-b. *K. purpurea*; sclerotic granule in BENNETT's agar, ×2,000×2/3
Plate 11-a. *K. kauaiensis*; crystalline body on WAKSMAN's solution
Plate 11-b. *K. nagasakiensis*; crystalline body on WAKSMAN's solution
Plate 12. *K. diplospora*; electron microphotograph of a chain of conidia, ×10,000×2/3
Plate 13. *K. purpurea*; electron microphotograph of a chain of conidia, ×5,000×2/3

B. Non-motile spore.....(*Streptosporangideae*)
1. Spores arranged in coiled chains within sporangium.
   a. Sporangium spheroid or short rods. Aerial mycelium present.
   .....*Streptosporangium* COUCH, 1955
   b. Sporangium irregularly shaped. Aerial mycelium usually lacking.
   .....*Amorphosporangium* COUCH, 1963
2. Spores arranged in parallel chains within sporangium.
3. Spores arranged in straight chain within sporangium.
   a. Sporangium cylindrical. Aerial mycelium present.
   ..... *Microellobosporia* CROSSETAL, 1963
   b. Sporangium fusiform. Aerial mycelium present.
   ..... *Elytrosporangium* FALCAO *et* MASSA, 1967
4. Sporangium formed intercalary in the mycelial hyphae.
   ..... *Intrasporangium* KALAKOUTSKII *et al.*, 1966

The genera, motile *Nocardia*, *Dermatophilus* and *Streptomyces*, must be differentiated from *Kitasatoa*. Motile *Nocardia* resembles *Kitasatoa* in having a single flagellum, but the development of the aerial mycelium of the former is very poor or not at all develop, while that of the latter is abundant. Vegetative mycelium of *Nocardia* produces the septum regularly and separates to short rods or coccoidal forms very easily, while in *Kitasatoa* vegetative mycelium produces a little and irregular septum only on the solid media. Segmentation of vegetative mycelium does not occur in the liquid media even after two months or more of incubation. *Dermatophilus* produces motile spore, but does not produce the sporangiospore. The genus *Streptomyces* is closely related to *Kitasatoa* in the growth characteristics but does not produce the septum on the aerial mycelium. Moreover, this genus forms a chain of spores on the top of the aerial mycelium, but does not form sporangium and the conidia produced do not move in the water.

Kitasatoa produces a soft vesicular body (a moist substance was contained in it and covered with a thin membrane) (Plate 8), a sclerotic granule like body which is produced in the substrate of the agar medium and crystalline body.

The cultural findings of the four species belonging to *Kitasatoa* are summarized in Table 4. There are differences in the cultural findings among these strains, therefore, they are named *K. purpurea*, (the type species) *K. kauaiensis*, *K. diplospora* and *K. ngasakiensis*, respectively.

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