PRODUCTION OF QUINOLINE-2-METHANOL AND QUINOLINE-2-METHANOL ACETATE BY A NEW SPECIES OF *KITASATOA, KITASATOA GRISEOPHAEUS*

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Two metabolites have been isolated from the fermentation broth of a new species of *Kitasatoa, Kitasatoa griseophaeus*. These alkaloids have been identified as quinoline-2-methanol and quinoline-2-methanol acetate. The former exhibits hypoglycemic activity in the rat.

In the previous articles[1], we described a method for screening for alkaloids produced by microorganisms and reported the new alkaloids pyrindicin[1], NA-337 A[2] and TM-64[3]. In the course of the screening, we found in addition two alkaloids produced by the soil isolate PO-1227. Strain PO-1227 was classified as a new species of *Kitasatoa, Kitasatoa griseophaeus*. Both alkaloids were shown to be quinoline alkaloids and were identified as quinoline-2-methanol[4] and quinoline-2-methanol acetate[5].

It has been reported that quinoline alkaloids are produced by *Pseudomonas* sp. and fungi[6]. However, there has been no report describing production of this group of alkaloids by actinomycetes. Consequently, this was the first observation of the production of quinoline-2-methanol and its acetate derivative by a microorganism.

The present paper deals with the taxonomy of the producing strain, as well as the production, isolation, identification and pharmacological activities of these alkaloids.

Taxonomy

1. Morphological Characteristics

Strain PO-1227 was cultured on glycerol-asparagine agar and oatmeal agar for 14 days at 27°C and observed microscopically. Its morphological characteristics were as follows: The aerial mycelium was 0.8~1.2 μ in diameter, showing straight and irregular branching (Fig. 1A). It formed no spirals or whorls, but had many spore chains resembling *Streptomyces* species (Fig. 1B). It also had stick-like sporangia (Fig. 2A) with motile spores (zoospores). The spores were 1.0~1.2 $\mu \times 0.6$~0.7 μ in size, oval with smooth surfaces. The formation of zoospores was very scanty and these zoospores were less motile in comparison with zoospores of known *Kitasatoa* species. Fig. 2B illustrates an electronmicrograph of the zoospores, 0.2 $\mu \times 1.4$ μ in size, with several flagella. In contrast zoospores of known *Kitasatoa* species have a single polar flagellum.

2. Cultural Characteristics

The culture was observed after incubation at 28°C for two weeks, except where otherwise noted. Color names and hue numbers indicated are those of the Color Harmony Manual (4th edition) published by Container Cooperation of America. The cultural characteristics of strain PO-1227 are summarized in Table 1.

Fig. 1. Sporophores (A) and spores (B) of strain PO-1227. (A: Inorganic salts-starch agar, 14 days at 27°C, ×1,000; B: Glycerol-asparagine agar, 14 days at 27°C ×14,400)

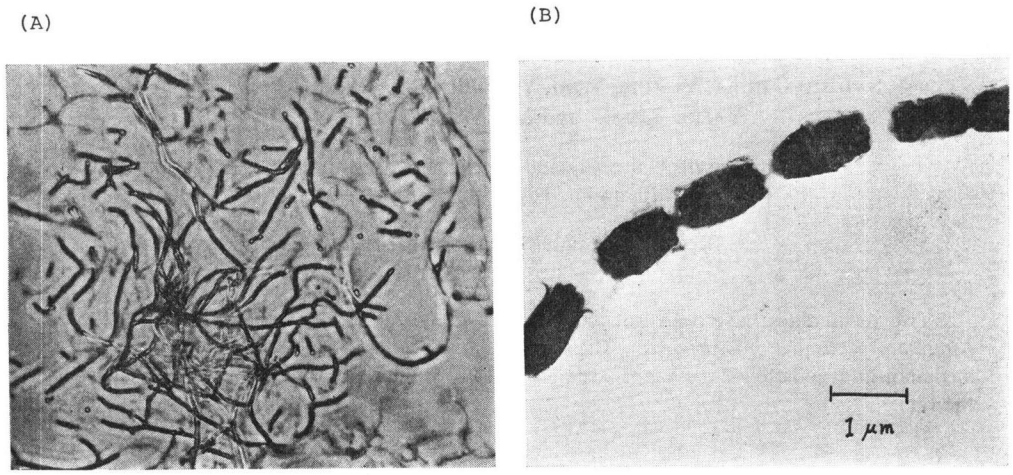


Fig. 2. Sporangia (A) and zoospore (B) of strain PO-1227 (Water agar 10 days at 27°C, A: ×1,000, B: ×35,000)

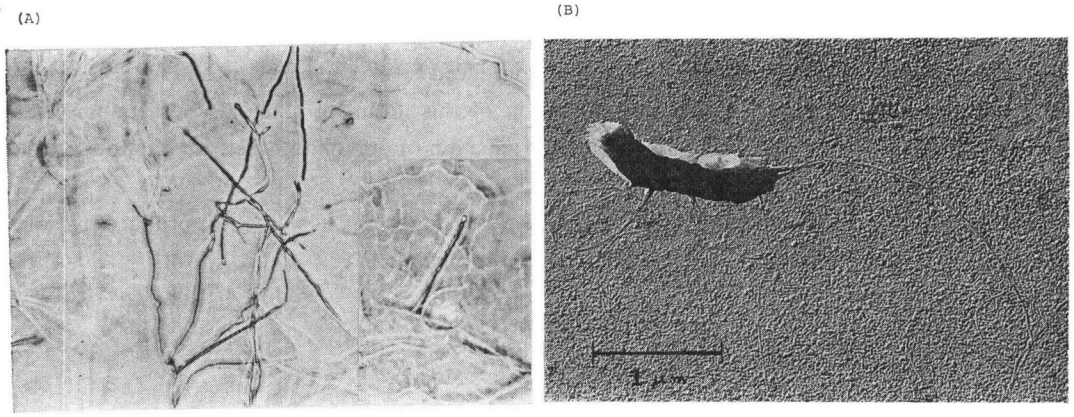


Table 1. Cultural characteristics of strain PO-1227.

	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	colorless	poor, 2dc (Natural)	—
Glucose-nitrate agar	2ic (Light Gold)	—	3ng (Yellow Maple)
Glycerol-calcium malate agar	21e (Mustard)	powder, 2ea (Light Wheat)	—
Glucose-asparagine agar	2gc (Bamboo)	cottony, white	2ne (Mustard Gold)
Glycerol-asparagine agar	colorless	poor, white	—
Inorganic salts-starch agar	colorless	poor, 2cb (Ivory Tint)	3ie (Camel)
Tyrosine agar	2ic (Light Gold)	poor, white	2ng (Yellow Maple)
Nutrient agar	2ea (Light Wheat)	—	—
Peptone-yeast iron agar	2ca (Light Ivory)	—	—
Yeast extract-malt extract agar	2ea (Light Wheat)	poor, 2dc (Natural)	3ng (Yellow Maple)
Oat meal agar	2ea (Light Wheat)	poor, white	2pg (Mustard Gold)

Table 2. Physiological characteristics of strain PO-1227.

	Response		Response
Melanin formation	−	Carbon utilization	
Tyrosinase reaction	−	Arabinose	+
Nitrate reduction	+	Xylose	+
Liquefaction of gelatin	+	Glucose	+
Coagulation of milk	−	Fructose	+
Peptonization of milk	+	Sucrose	+
Hydrolysis of starch	+	Inositol	−
Temperature range for growth	15~40°C	Rhamnose	−
		Raffinose	−
		Mannitol	−

Table 3. Comparison of strain PO-1227 with known *Kitasatoa* species.

		PO-1227	*K. nagasakiensis* KA-281	*K. diplospora* KA-280
Zoospore		rod single or in pairs, 1.4×0.2 μ	rod single or in pairs 2.4~5.0×1.1~1.35 μ	ellipsoidal single or in pairs, 2.5~3.0×1.5 μ
Conidia		oval, smooth, 1.0~1.2×0.6~0.7 μ	ellipsoidal, smooth, 1.2~1.6×0.8~1.0 μ	cylindrical, smooth, 1.3~1.5×0.7 μ
Glycerol-asparagine agar	G AM SP	colorless white 3ic (Light Amber)	3ng (Light Brown) white→ 5ca (Shell Pink) 4ng (Light Brown)	11/2gc (Dusty Yellow) white 3ie (Camel)
Inorganic salts-starch agar	G AM SP	colorless 2ig (Slate Tan) 3ne (Topaz)	colorless white→ 5ec (Dusty Peach) 2gc (Bamboo)	colorless 5ca (Shell Pink) 2gc (Bamboo)
Oat meal agar	G AM SP	colorless white 2pg (Mustard Gold)	colorless 5dc (Pussywillow Gray) 3ie (Camel)	colorless grayish white 31e (Cinnamon)

3. Physiological Characteristics

Utilization of carbon sources by strain PO-1227 was investigated according to the method of PRIDHAM and GOTTLIEB[7]. Excellent growth was observed when arabinose, xylose, glucose, fructose and sucrose were used. The physiological characteristics of strain PO-1227 are summarized in Table 2.

Using the procedures described by BECKER *et al.*,[8] the cell wall components of strain PO-1227 were analyzed with the following results: positive for LL type of diaminopimelic acid and ambiguous for the mesoisomer. Glycine was detected, but arabinose and galactose were not present.

From the above results, it was concluded that strain PO-1227 is a *Kitasatoa* species[9]. Strain PO-1227 was then compared with type cultures of the four known *Kitasatoa* species: *K. purpurea, K. diplospora, K. kauaiensis* and *K. nagasakiensis*. Morphological, cultural and physiological characteristics of strain PO-1227 resembled those of *K. nagasakiensis* and *K. diplospora*. However, the former is non-chromogenic while the latter two are chromogenic. Taxonomic comparison of strain PO-1227 with these species is summarized in Table 3.

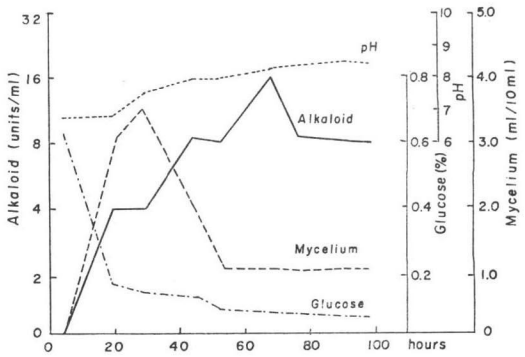
Based on the differences noted, strain PO-1227 is assigned to a new species of *Kitasatoa* and is designated as *Kitasatoa griseophaeus*. Strain PO-1227 has been deposited with the Fermentation

Research Institute, Agency of Industrial Science and Technology with the accession number FERM-P No. 3077.

Production

Strain PO-1227 was maintained on modified WAKSMAN's agar or as freeze-dried stock. The stock culture was inoculated into 100 ml of medium in a SAKAGUCHI flask and incubated at 27°C. A 48-hour culture was transferred into 20 liters of medium in a 30-liter jar fermentor and the fermentation was carried out for 3 days under the following conditions: temperature, 27°C; aeration, 10 liters/min; agitation, 250 r.p.m.; and pressure, 0.5 kg/cm^2. The composition of the seed medium was 2% glucose, 0.5% peptone, 0.5% meat extract, 0.3% dried yeast, 0.3% $CaCO_3$ (pH 7 before sterilization) and that of the production medium was 1% glucose, 2% starch, 0.5% yeast extract, 0.5% peptone and 0.4% $CaCO_3$ (pH 7 before sterilization). Adekanol LG-109 (Asahi Electro-Chemical Co., Ltd.,) was used as antifoam agent. The titer of alkaloid in units was determined as the maximum dilution number of a DRAGENDORFF positive solution[1]. A typical time course of PO-1227 production in a 30-liter jar fermentor is shown in Fig. 3. The concentration of the alkaloids produced reached a maximum 72~80 hours after inoculation and then gradually decreased.

Fig. 3. Time course of alkaloid PO-1227 production by *Kitasatoa griseophaeus*.



Isolation

Two alkaloids were detected on silica gel TLC upon eluting with $CHCl_3$-CH_3OH (10: 1, v/v); these were designated as PO-1227 A (Rf 0.7) and B (Rf 0.4). Attempts to selectively produce A and B was unsuccessful with the former being the minor product in many cases. In the following cases, PO-1227 A and B were accumulated in almost equal amounts in the fermentation broth. Culture broth (20 liters) of *Kitasatoa griseophaeus* obtained by incubation in a 30-liter jar fermentor was used as a starting material. The presence of alkaloids was determined by the DRAGENDORFF reaction. After the broth supernatant was adjusted to pH 10 with aqueous ammonia, the alkaloids were extracted with 4 liters *n*-butyl acetate and then transferred into 1.5 liters 0.1 N hydrochloric acid. The water layer was adjusted to pH 10 with aqueous ammonia and extracted twice with 1 liter chloroform. The solvent layer was again transferred into 600 ml 0.1 N hydrochloric acid. The water layer was adjusted to pH 10 with aqueous ammonia and extracted three times with 300 ml ether. The ether solution was dried over anhydrous sodium sulfate, concentrated to a small volume, and then chromatographed on silica gel (15 g) eluting with a solvent mixture of benzene and acetone. Alkaloids PO-1227 A and B were successively eluted from the column and were separated from each other by column chromatography on silica gel. Each alkaloid fraction was concentrated to a small volume, to which a saturated solution of picric acid in ether was added until no more precipitates formed. Thus PO-1227 A picrate (220 mg) and PO-1227 B picrate (240 mg) were obtained. Each picrate was suspended in a mixture of 100 ml chloro-

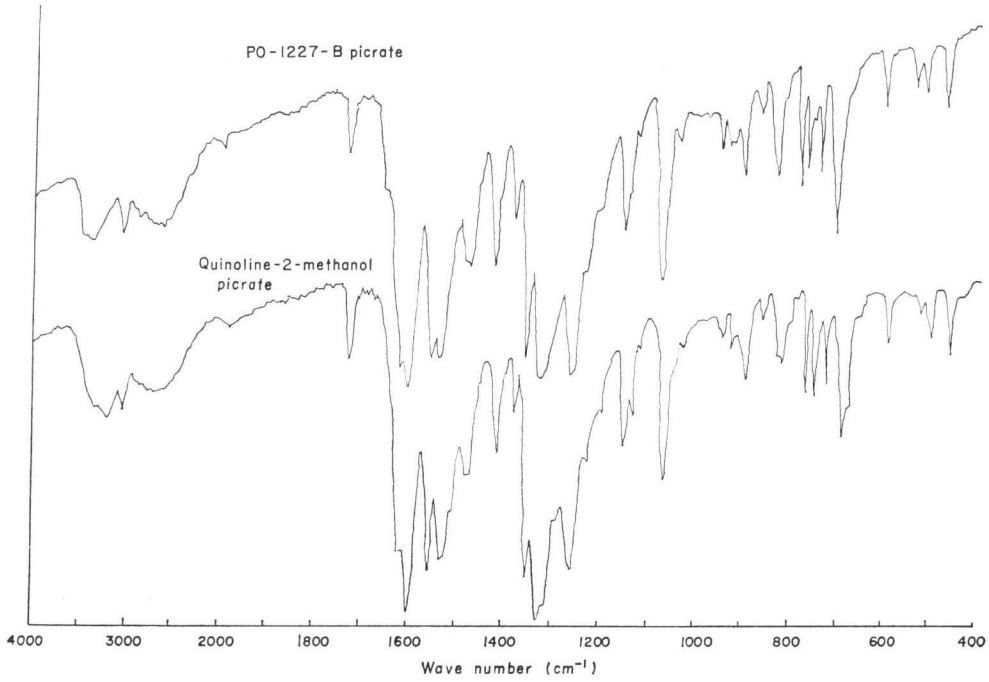
form and 20% aqueous potassium hydroxide (10~20 ml), and the mixture was vigorously shaken. The solvent layer was washed with water, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to dryness. Each dried material was recrystallized from *n*-hexane to yield 120 mg PO-1227 A and 140 mg PO-1227 B.

PO-1227 B melted at 67~68°C and its optical rotation showed $[\alpha]_D^{20}$ 0(*c* 1.0, methanol). Ultraviolet spectrum showed characteristic bands at λ_{max}^{MeOH}, nm(ε): 230 (47,700), 233 (44,500), 277 (4,000), 284 (3,800), 290 (3,700), 296 (3,300), 302 (4,000), 308 (2,500), 317 (5,000). Infrared absorption spectrum in KBr pellet exhibited characteristic bands at 3,200, 1,600, 1,500, 1,420, 1,310, 1,080, 820, 780 and 750 cm^{-1}. The molecular formula $C_{10}H_9NO$ for PO-1227 B was determined on the basis of elemental analysis and its mass spectrum. The elemental analysis of PO-1227 B picrate gave the following values: C 49.47, H 3.41, N 13.70 (%). The calculated values for $C_{10}H_9NO \cdot C_6H_3N_3O_7$ are C 49.52, H 3.43, and N 13.84. The mass spectrum of PO-1227 B showed *m/e* 159.0690 (M^+). The characteristic cracking pattern of PO-1227 B suggested the presence of quinoline or isoquinoline skeleton. These spectroscopic properties (IR, PMR and Mass) suggested alkaloid PO-1227 B to be either quinoline-2-methanol[4] or isoquinoline-1-methanol[10]. The melting point of both compounds in the literature were 66~67°C and 65°C, respectively. Consequently, alkaloid PO-1227 B melted at 67~68°C was presumed to be quinoline-2-methanol.

Identification

The physico-chemical properties of PO-1227 B indicated that it is most likely to be quinoline-2-methanol and its identity was confirmed by comparing it with an authentic sample prepared by independent synthetic methods[11,12].

Fig. 4. IR spectral comparison of alkaloid PO-1227-B with quinoline-2-methanol picrate.



A mixture containing 1 g of quinaldine, 3 ml of acetic acid and 1 ml of a 30% aqueous hydrogen peroxide was heated at 50°C for 10 hours. After cooling in ice, 80% aqueous potassium hydroxide was added to the mixture and the solution shaken to afford precipitate. The resulting precipitate was recrystallized from water to yield 350 mg quinaldine oxide; m.p. 77~78°C. A mixture of 1 ml acetic anhydride and 30 mg of the quinaldine oxide were refluxed for 1 hour, to which ether and 10% aqueous sodium carbonate were then added. The resulting ether layer was concentrated *in vacuo* to give 20 mg quinoline-2-methanol acetate. Twenty mg of the quinoline-2-methanol acetate was suspended in 1 ml of 20% aqueous potassium hydroxide and the solution shaken for 1 hour. It was then extracted with ether. The ether layer provided 10 mg quinoline-2-methanol.

Since the spectroscopic properties of the quinoline-2-methanol and its picrate were in complete agreement with those of alkaloid PO-1227 B and its picrate, respectively, as shown in Fig. 4, it was reasonably concluded that PO-1227 B is identical with quinoline-2-methanol.

The PMR (CCl_4) and IR (KBr) spectra of the quinoline-2-methanol acetate prepared by the above method were also compared with those of alkaloid PO-1227 A. As expected, these were completely identical. This observation as well as mixed melting point determination of the picrates unambiguously confirmed that PO-1227 A is identical with quinoline-2-methanol acetate.

Pharmacological Activity

When PO-1227 B was tested in a variety of pharmacological system, it was found to have hypoglycemic activity in the rat as shown in Table 4. The quinoline-2-methanol hydrochloride prepared by the following procedure and tolbutamide as control drug were used for this test. PO-1227 B picrate was made alkaline with 20% aqueous potassium hydroxide and treated with benzene. The benzene layer was dried over anhydrous Na_2SO_4, to which hydrogen chloride was introduced to give quinoline-2-methanol hydrochloride. Three Wistar male rats weighing approximately 110 g were administrated the compounds orally. Two hours before and after the administration of each drug, 0.1 ml of blood was taken from the lateral caudal vein and the glucose concentration in each blood sample was assayed by the modified HOFFMAN method[13].

Table 4. Effect of quinoline-2-methanol on hypoglycemic activity in rat.

Compound	Dose (mg/kg)	Exp. No.	Decrease of glucose level (%)
Quinoline-2-methanol HCl	100	1	23.1
		2	25.8
Tolbutamide	50	1	67.0
		2	49.5
Saline (control)		1	6.5
		2	11.5

Discussion

In subsequent screening for alkaloids from microorganisms, two actinomycetes strains, AM-2431 and AM-2435 were also found to produce quinoline-2-methanol. Strain AM-2431 was classified as *Streptomyces* species. The later organism was identified as *Kitasatoa* species, but differing from *Kitasatoa griseophaeus* strain PO-1227. It has been known that various quinoline alkaloids are present in nature and that a variety of organisms produce kynurenate and related compounds that are structurally similar to quinoline-2-methanol. Quinoline alkaloids are known to be produced by bacteria and fungi. For example, complex of seven related 4-oxyquinolines and one 4-quinolone has been elaborated by *Pseudomonas aeruginosa*. These are commonly called "pyo" compounds or pseudanes. It has also been reported that viridicatum, viridicatol and 3-O-methyl viridicatin are produced by

Penicillium species. It is thus concluded that our findings are new in that both quinoline-2-methanol and its acetate are naturally occurring compounds and actinomycetes produce quinoline alkaloids.

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