THE DESCRIPTION AND ANTIBIOTIC PRODUCTION OF *STREPTOMYCES HYGROSCOPICUS* VAR. *GELDANUS*

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A new variety of *Streptomyces hygroscopicus* was isolated from a Kalamazoo soil. This isolate is described and identified as var. *geldanus*. When fermented in preferential media it produces geldanamycin, nigericin, nocardamine, and a libanamycin-like activity. Fermentation conditions, chromatographic separation, and antimicrobial spectra of the antibiotics are given.

Four types of antibiotics are produced in submerged culture by *S. hygroscopicus* var. *geldanus*[1] when the culture is fermented in different media. These antibiotics are geldanamycin, nigericin, nocardamine and a libanamycin-like activity. The taxonomy of the producing actinomycete is given together with the fermentation conditions, paper chromatographic separation of the antibiotics formed and their respective antimicrobial spectra.

Materials and Methods

Culture

An actinomycete isolated from a Kalamazoo soil was characterized as *Streptomyces hygroscopicus* var. *geldanus* var. *nova* (UC 5208).

Taxonomic studies were conducted by the methods described by DIETZ[3] and by SHIRLING and GOTTLIEB[4].

Inoculum

Seed flasks were inoculated with spores of this isolate which were stored in sterile soil and/or the gas phase of liquid nitrogen. The culture was grown in 500 ml Erlenmeyer flasks containing 100 ml of medium and was incubated for 72 hours at 28°C on a New Brunswick Model 52 shaker at 250 rpm. The seed medium consisted of 1% glucose monohydrate (cerelose), 0.25% yeast extract (Difco) and 1% Bacto-peptone (Difco) per liter. Fermentation flasks were routinely inoculated with 5% of the seed medium.

Fermentation

The media composition for the preferential production of the aforementioned antibiotics is summarized in Table I. Under our conditions the media used for the production of nigericin and nocardamine with *S. hygroscopicus* var. *geldanus* are specific. However, the medium which yields geldanamycin also produces small quantities of the libanamycin-like substance. The individual antibiotics were extracted from the culture filtrates and identified by Dr. D. H. PETERSON[2]. Azalomycin B which was extracted from a fermentation by PANDEY[2] was not detected under our conditions.

Fermentation samples taken from day one through day five were assayed on agar plates with susceptible microorganisms.

The four antibiotics were clearly separated by paper chromatography in a methyl isobutyl ketone solvent system. Table 2 indicates the respective assay organisms as well as the Rf's of the designated

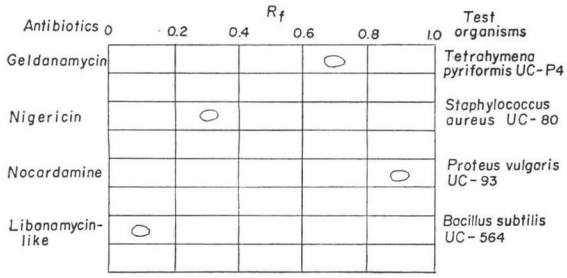
Table 1. The effect of ingredients on the preferential production of antibiotics by *S. hygroscopicus* var. *geldanus*.

Ingredients	Geldanamycin	Nigericin	Norcardamine	Libanamycin-like
Glucose monohydrate	40*		20	40
Dextrin		20		
Molasses (Brer Rabbit)	10	25		
Malt extract +			5	
Yeast extract +	2.5			1.0
Proteose peptone #3 +	2.5			10
Distiller's solubles **		10		
Fish meal (Viobin)		10		
Tryptone +	2.5			
Oatmeal (Gerber)	5			
Calcium carbonate			5	
Ammonium sulfate			3	

* Grams per liter.
Incubation at 28°C
Medium – 100 ml/500 ml Erlenmeyer flask
New Brunswick Model 52 shaker, 250 rpm.

\+ Difco
** Brown and Forman

Table 2. Antibiotics produced by *Streptomyces hygroscopicus* var. *geldanus*.



Solvent system: 0.075 N NH_4OH saturated with methyl isobutyl ketone, developed for 5 hours on Whatman #1.

antibiotics. The antimicrobial spectra of the identified antibiotics are given in Table 3.

Description of Culture

Streptomyces hygroscopicus var. *geldanus*, UC 5208, is compared with the type species *Streptomyces hygroscopicus*, (JENSEN) WAKSMAN CBS, UC 2317.

Color Characteristics. The aerial growth was white to gray-white or gray-cream to gray. Moist, black, hygroscopic patches appear on some media. Melanin-negative. Appearance on a Ektachrome is given in Table 4. Reference color characteristics are given in Table 5. The cultures may be placed in the White (W) and Gray (GY) color series of TRESNER and BACKUS[5].

Microscopic Characteristics. Sporophores occur in tight spirals, mostly in hygroscopic masses. Sporophores are spiral (S) in the sense of PRIDHAM *et al.*[6]. Spores are smooth with an irregular possibly warty surface by direct electron microscope examination. The spore surface is rugose (morel-like) when examined by the carbon replication and SEM methods of DIETZ and MATHEWS[7~11].

Cultural and Biochemical Characteristics. See Table 6.

Carbon Utilization: The ability of the culture to grow on carbon compounds was determined in the synthetic medium of PRIDHAM and GOTTLIEB[12], and their modified medium[4]. In PRIDHAM and GOTTLIEB's medium, both cultures grew well on D-xylose, L-arabinose, rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, lactose, cellobiose, raffinose, dextrin, soluble starch, glycerol, D-mannitol D-sorbitol, sodium acetate, sodium citrate and sodium succinate. Growth was moderate on sucrose and salicin; poor on inulin, and the control; negative on phenol, cresol and sodium salicylate. The cultures showed the following minor differences: The type culture grew moderately on dulcitol, inositol, sodium

Table 3. Antimicrobial spectra of antibiotics* produced by *Streptomyces hygroscopicus* var. *geldanus*.

Test organism	Culture number	Medium	Geldana-mycin	Nigericin	Nocarda-mine	Libana-mycin-like
Bacillus subtilis	UC-564	1	0	40 mm	0	28
Micrococcus luteus	UC-130	2	0	32	0	22
Staphylococcus aureus	UC-80	3	0	30	0	24
Escherichia coli	UC-51	3	0	0	0	0
Salmonella schottmuelleri	UC-126	3	0	0	0	0
Shigella gallinarum	UC-265	3	0	0	0	0
Klebsiella pneumoniae	UC-57	1	0	0	0	trace
Proteus vulgaris	UC-93	3	0	0	31	trace
Mycobacterium avium	UC-159	4	0	28	0	trace
Saccharomyces pastorianus	UC-1342	5	0	0	0	trace
Tetrahymena pyriformis	UC-P4	6	33	24	0	0
Crythidia fasciculata	UC-P23	6	22	0	0	0

* Solutions of 1 mg/ml; 8 mcl per disc (12.7 mm Schleicher & Schuell discs).
1 Streptomycin Assay Agar - Antibiotic Med. #5-BBL.
2 Seed Agar - Antibiotic Medium #1-BBL.
3 Nutrient agar - BBL.
4 Brain Heart Infusion agar - Difco
5 GRAY's agar - BBL.
6 Panmede agar - Ref. 1.

Table 4. Appearance of *S. hygroscopicus* cultures on Ektachrome[18]

Agar medium		*S. hygroscopicus* var. *geldanus* UC-5208	*S. hygroscopicus* UC-2317
BENNETT'S	S	Gray	Gray-white
	R	Yellow-tan	Yellow
CZAPEK's sucrose	S	Gray-white	Gray
	R	Yellow-gray	Gray
Maltose-tryptone	S	Pale gray-white	Trace gray-white
	R	Yellow	Yellow
Peptone-iron	S	—	—
	R	Yellow	Yellow
0.1% Tyrosine	S	Trace gray-white	Trace gray
	R	Red	Red
Casein starch	S	Gray white	Gray
	R	Yellow-gray	Yellow-gray

S=Surface. R=Reverse

oxalate, and sodium tartrate, whereas the new variety grew well. On sodium formate, the former grew poorly, the latter did not grow. In the modified medium, both cultures grew poorly on the basal medium without a carbon compound and well on the glucose control. Growth was also good on the basal medium with L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, and raffinose; somewhat less (moderate) on cellulose. The two cultures differed in growth on two compounds: on sucrose the type culture grew moderately and the new variety had doubtful growth – while on inositol the type culture had doubtful growth and the new variety good growth.

Temperature: Both cultures grew luxuriantly at temperatures of 18~28°C and moderately well at 37°C. The cultures do not grow at 45°C.

The new variety *S. hygroscopicus* var. *geldanus* produces geldanamycin, nigericin, nocardamine and a libanamycin-like activity.

Table 5. Reference color characteristics of *S. hygroscopicus* cultures

Agar medium		Color Harmony Manual, 3rd ed. 1948 (14)		NBS Circular 553, 1955 (15)	
		UC–5208 *S. hygroscopicus* var. *geldanus*	UC–2317 *S. hygroscopicus*	UC–5208 *S. hygroscopicus* var. *geldanus*	UC–2317 *S. hygroscopicus*
BENNETT'S	S	2fe(g) covert gray	1fe(g) griege, citron gray	94g light olive brown 112gm light olive gray	112m light olive gray 122g grayish yellow-green
	R	2gc(m) bamboo, chamois	2gc(g) bamboo, chamois	90gm grayish yellow	90gm grayish yellow
	P	3ec(g) bisque, light beige	2ec(g) biscuit, ecru, oatmeal, sand	79gm light grayish yellowish brown 90g grayish yellow	90gm grayish yellow
CZAPEK'S sucrose	S	3dc(g) natural	3fe(m) silver gray	—	63gm light brownish gray
	R	1dc(m) putty, griege	1fe(m) griege, citron, gray	121gm pale yellow green	112m light olive gray 122g grayish yellow green
	P	—	—	—	—
Maltose-tryptone	S	2ba(m) pearl, shell tint	2cb(m) ivory tint	92gm yellowish white	92m yellowish white 93m yellowish gray
	R	2gc(g) bamboo, chamois	2ec(g) biscuit, ecru, oatmeal, sand	90gm grayish yellow	90gm grayish yellow
	P	—	—	—	—
Yeast extract-malt extract (ISP–2)	S	2fe(m) covert gray	1dc(m) griege, citron, gray	94g light olive brown 112gm light olive gray	121gm pale yellow green
	R	2gc(g) bamboo, chamois	2gc(m) bamboo, chamois	90gm grayish yellow	90gm grayish yellow
	P	2ge(g) covert tan, griege	2gc(g) bamboo, chamois	90gm grayish yellow	90gm grayish yellow
Oatmeal (ISP–3)	S	3fe(g) silver gray	2fe(g) covert gray	63gm light brownish gray	94g light olive brown 112gm light olive gray
	R	2ec(g) biscuit, ecru, oatmeal, sand	2dc (g) natural, string	90gm grayish yellow	93m yellowish gray
	P	1·1/2 ec(g) putty	1dc(m) putty, griege	90gm grayish yellow 93m yellowish gray	121gm pale yellow green
Inorganic salts-starch (ISP–4)	S	2fe(m) covert gray	2fe(g) covert gray	94g light olive brown 112gm light olive gray	94g light olive brown 112gm light olive gray
	R	2ec(m) biscuit, ecru, oatmeal, sand	3fe(g) silver gray	90gm grayish yellow	63gm light brownish gray
	P	2cb(g) ivory tint	2dc(g) natural, string	92m yellowish white 93gm yellowish gray	93m yellowish gray
Glycerol-asparagine (ISP–5)	S	2fe(m) covert gray	b(m) oyster white	94g light olive brown 112gm light olive gray	263m white 264g light gray
	R	2ec(g) biscuit, ecru, oatmeal, sand	2cb(g) ivory tint	90gm grayish yellow	92m yellowish white 93gm yellowish gray
	P	—	—	—	—

S=Surface; R=Reverse; P=Pigment; (g)=glossy; (m)=matte.

Source. Soil.

Type culture. UC 2317 (CBS 482.48)

New variety. UC 5208 (NRRL 3602)

Table 6. Cultural and biochemical characteristics of *S. hygroscopicus* cultures

			S. hygroscopicus var. *geldanus*	*S. hygroscopicus*
Agar Media	Peptone-iron	S	gray white	v.s. trace white
		R	yellow tan	yellow tan
		P	melanin -	melanin -
	Calcium malate	S	trace white	trace white
		R	colorless	colorless
		P	no pigment	no pigment
		O	malate not solubilized	malate not solubilized
	Glucose - asparagine	S	gray white	gray white
		R	cream gray	cream
		P	pale pink pigment	no pigment
	Skim milk	S	white	gray pink white
		R	yellow	yellow pink tan
		P	yellow pigment	yellow pink pigment
		O	casein solubilized	casein solubilized around growth
	Tyrosine	S	gray cream	gray
		R	red tan	red tan
		P	red tan pigment	red tan pigment
		O	tyrosine solubilized	tyrosine solubilized
	Xanthine	S	cream gray	trace gray white
		R	pale yellow	pale yellow
		P	no pigment	no pigment
		O	xanthine solubilized around growth	xanthine not solubilized
	Yeast extract - Malt extract	S	gray white	cream gray white with moist black patches
		R	olive	yellow olive
		P	no pigment	no pigment
	Casein starch	S	gray	gray
		R	gray	gray
		P	no pigment	no pigment
		O	starch hydrolyzed	starch hydrolyzed
	Nutrient starch	S	white	white
		R	cream	cream
		P	pale yellow pigment	pale yellow pigment
		O	starch hydrolyzed	starch hydrolyzed
	SABOURAUD's dextrose	S	white with gray	white
		R	yellow tan orange	yellow tan orange
		P	no pigment	no pigment
	BENNETT's	S	heavy gray white	heavy gray white
		R	yellow	yellow
		P	no pigment	no pigment
	CZAPEK's sucrose	S	heavy gray	heavy gray
		R	gray	gray
		P	no pigment	no pigment
	Maltose tryptone	S	gray white	gray white
		R	yellow	yellow
		P	no pigment	no pigment
	Peptone - yeast extract - iron (ISP-6)	S	trace white	no aerial growth
		R	yellow	yellow
		P	pale yellow pigment	pale yellow pigment
	Tyrosine (ISP-7)	S	gray	gray

(to be continued)

Table 6. (continued)

			S. hygroscopicus var. *geldanus*	*S. hygroscopicus*
Agar Media	Tyrosine (ISP-7)	R	tan	tan
		P	tan pigment	pale pink becoming pink tan
Gelatin Media	Plain	S	—	—
		P	tan 1/4	
		O	liquefaction 1/3	liquefaction 1/3~3/4
	Nutrient	S	—	—
		P	tan 1/4	tan 1/4
		O	complete liquefaction	liquefaction 1/2
Broth Media	Synthetic nitrate	S	white aerial grown on surface pellicle	gray aerial growth on surface pellicle
		P	pale yellow	pale yellow
		O	compact to flocculent bottom growth	compact to flocculent bottom growth
			nitrate reduced to nitrite	nitrate not reduced to nitrite
	Nutrient nitrate	S	no aerial growth	no aerial growth
		P	none	none
		O	compact to flocculent bottom growth	compact to flocculent bottom growth
			nitrate not reduced to nitrite	nitrate not reduced to nitrite
	Litmus milk	S	gray-white aerial growth on blue-gray surface ring	tan surface ring
		O	peptonization complete pH 7.2	peptonization partial pH 6.6

S=Surface; R=Reverse; P=Pigment; O=Other characteristics.

Discussion

S. hygroscopicus var. *geldanus* is a new soil isolate of the genus *Streptomyces* which differs in certain characteristics from the type culture, *Streptomyces hygroscopicus*. The new variety is readily distinguished by its ability to produce geldanamycin, nigericin, nocardamine, and a libanamycin-like activity. Other distinguishing characteristics may be noted by perusal of the cultural characteristics given in the description. These characteristics are not of significant value to merit the creation of a new species. The new soil isolate is readily characterized *hygroscopicus* by the strong color pattern and microscopic characteristics of the type species.

It is proposed that the organism described here be designated *Streptomyces hygroscopicus* var. *geldanus* var. *nova* after the antibiotics first isolated from a fermentation of this culture.

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