A NEW *STREPTOVERTICILLIUM* SPECIES—*S. QUILONENSIS*

K. SAMBAMURTHY and P. ELLAIAH

Fermentation Technology Laboratory, Department of Pharmaceutical Sciences,
Andhra University, Waltair, India

(Received for publication February 17, 1975)

An extensive screening of soil samples from Kerala State, India, gave a new streptomycete, *Streptoverticillium quilonensis*. Its taxonomy is described in detail.

While conducting a systematic screening of the natural substrates from Kerala State, a new streptomycete was isolated from a brownish black, free-flowing soil sample collected at Quilon. The species is of interest as it is a verticillate type and the number of *Streptoverticillium* species reported in the literature are limited. The species produces an intracellular antibiotic, Antibiotic K 13. It is a heptaene and studies so far conducted revealed that the antibiotic is possibly new. Fermentation conditions, isolation of the antibiotic and its properties will be communicated separately. The present communication deals with the taxonomic studies on the isolate.

Morphological Characteristics

The species grows well on almost all the media as either irregular or round, flat to convex colonies either with an entire or fibrous edge. The aerial mycelium develops moderately to good on most of the synthetic media and poorly on organic media. The sporophores occur as straight, short verticillate forms (Plate 1). Predominantly secondary and occasionally primary verticils are observed on many media with sporophores 6.5 to 11 and rarely 22 μ (secondary verticils) in length. The inter-nodal distance is about 70 μ. Spore surface is smooth (Plate 2). The aerial mycelium is fine cottony to fluffy and is white to pale greenish yellow or rarely pale rose in colour. Substrate mycelium is light yellow to yellowish brown on synthetic media and brown with or without green tinge to deep brown on organic media. The species is chromogenic producing deep brown diffusible pigment on organic media. It

Plate 1. Aerial mycelium of *Streptoverticillium quilonensis* (×650) on yeast extract-malt extract agar ISP

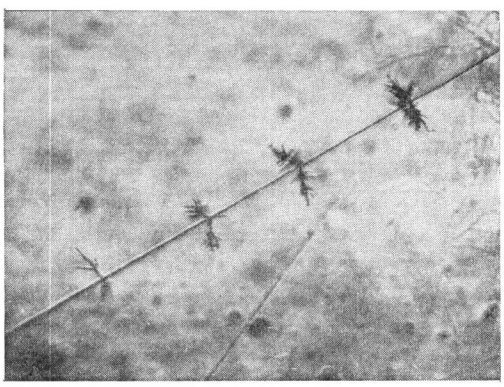
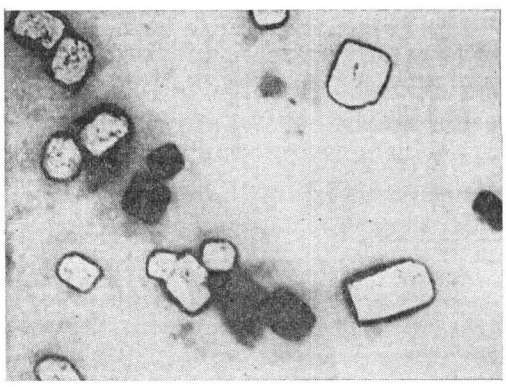


Plate 2. Electron-micrograph of the spores of *Streptoverticillium quilonensis* (×44,000) on yeast extract-malt extract agar ISP



also produces pale yellowish brown to light brown diffusible pigments on a few synthetic media.

Cultural and Biological Characteristics

The culture is melanin, H_2S and tyrosinase positive. It completely coagulates milk with partial peptonization giving a strong alkaline reaction. It exhibits a good catalase activity, good diastatic activity, hydrolyses casein, exhibits a rapid haemolytic activity and rapidly liquefies gelatin. The following reactions are negative: cellulolytic activity, reduction of nitrate, inversion of sucrose, production of acid from glucose, formation of indole from tryptone and liquefaction of coagulated serum. The culture grows well at pH 6.0~8.0 and does not grow at pH 4.0 and 10.5 (glycerol-peptone broth). It exhibits good growth between 25° and 37°C and shows no growth at 5° and 40°C (BENNETT's agar). Its growth is moderate at 4% sodium chloride but does not grow at 7% sodium chloride concentration (yeast extract - malt extract agar supplemented with various concentrations of sodium chloride). The detailed cultural and physiological characteristics and carbon source utilization are presented in Tables 1, 2 and 3. The colour numbers mentioned in Table 1 refer to the colour plates of the Dictionary of Color by MAERZ and PAUL[1].

Discussion

A detailed perusal of the literature[3~8] and the information available in the various journals on *Streptomyces* and *Streptoverticillium* species showed that our isolate was somewhat related to *Streptoverticillium cinnamoneum*[5], *Streptomyces eurocidicus*[5], *S. albireticuli*[5], *S. hachijoensis*[5], *S. netropsis*[5], *S. reticuli*[5], *S. olivoverticillatus*[5,7] and *S. olivoreticuli*[5,7,9].

S. cinnamoneum and *S. quilonensis*: Although the reference organism produces straight sporophores with verticil formation, it differs widely from our isolate when other characteristics are considered. The reference strain produces white to cinnamon to gray aerial mycelium, gives cream to light greenish yellow to dull yellowish orange to brownish growth on synthetic media and light yellow colours on organic media and does not produce soluble pigments. It is melanin and H_2S negative, utilizes xylose and produces a polypeptide antibiotic.

S. eurocidicus and *S. quilonensis*: The reference organism produces straight sporophores as atypical verticils, is chromogenic, is melanin and starch hydrolysis positive and does not reduce nitrate. But it differs in producing scant white to white with yellow tinge aerial mycelium, in giving yellowish brown substrate mycelium without frequent tinges of green, does not liquefy gelatin, shows a doubtful tyrosinase reaction and produces a pentaene. In the literature information is made available on the aspects mentioned above.

S. hachijoensis and *S. quilonensis*: The reference culture, although produces straight secondary verticils with absence of soluble pigment on synthetic media and agrees in gelatin liquefaction, nitrate reduction, blood haemolysis, coagulation and peptonization of milk, and gives the heptaene antibiotic trichomycin, it differs significantly from our isolate in being non-chromogenic and melanin negative, in the complete absence of green tinges in aerial mycelium and substrate mycelium colours and in the absence of growth on cellulose (rest of the carbohydrate utilization pattern has not been mentioned). Antibiotic K13 is different from trichomycin. Further, the differences magnify when characteristics on individual media are considered.

S. netropsis and *S. quilonensis*: *S. netropsis* agrees with our organism in the following respects: It is melanin positive, is a verticil and gives positive starch hydrolysis and negative nitrate reduction reactions. However, the two organisms differ in many respects. While the reference organism gives predominantly white aerial mycelium except sucrose-nitrate agar where it is pale vinaceous fawn, *S. quilonensis* produces dull white to rose-white to pale greenish yellow to green aerial mycelium on the media considered for the reference organism. As for the substrate mycelium, while the range of colours

Table 1. Cultural characteristics

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment
1. Sucrose-CZAPEK's agar	poor; 3 mm colonies with fine wrinkled surface	poor; dull white	colourless to pale yellow	none
2. Glucose-CZAPEK's agar	good; 6 mm flat colonies	good; yellow to greenish yellow (17-D-1 to 17-D-2)	bright yellow (17-I-1) to yellow-brown	light brown around big colonies
3. Glycerol-CZAPEK's agar	good; thick 2 mm colonies	good; yellow with trace green (19-F-1)	yellow with green tinge (18-F-2) to light brownish yellow (11-G-3)	pale yellow
4. Glucose-asparagine agar	good; 5 mm colonies with raised centres	moderate; dull white to pale greenish yellow	yellow with trace orange to yellowish brown (10-G-2 to 13-H-7)	none
5. Glycerol-asparagine agar ISP	moderate; 1~5 mm flat colonies	poor; dull white to pale greenish yellow (19-B-1)	colourless to brownish yellow (11-H-3) to yellowish brown (12-H-7)	pale yellowish brown
6. SHINOBU synthetic tyrosine agar ISP	moderate; 4 mm colonies with colourless guttation drops	good; white and pale yellow with green tinge	deep blackish brown	brown (deep blackish brown in broth)
7. Inorganic salts starch agar WAKSMAN	excellent; 2~5 mm convex colonies with colourless guttation drops	excellent; white to rose-white (9-A-2)	colourless to dull oyster white	none
8. Inorganic salts starch agar ISP	-do-	-do-	oyster white to oyster white with green tinge to light brownish yellow (11-D-2)	none
9. GAUSE mineral salts medium I	very good; 2~4 mm colonies with colourless guttation drops	abundant; white to light rose (10-A-2)	colourless to yellow with trace orange (10-D-2)	none
10. Starch agar plate (10 days)	very good	good; white	colourless to yellowish brown with green tinge	brown
11. Potato dextrose agar	excellent; dome-shaped colonies	abundant; dull white to pale yellow with green tinge	light brownish yellow (11-F-2) to light yellowish brown (12-H-6)	pale yellowish brown
12. CARVAJAL's oatmeal agar	good; 2~8 mm colonies with colourless guttation drops	good; dull white to light yellow with trace green (19-D-1)	light dull brown with trace green (13-H-6) to dark brown (16-C-11)	pale brown
13. Oatmeal agar ISP	very good; occasional 20 mm colonies with colourless guttation drops	excellent; white to rose white	brownish yellow (12-F-3) to dark brown (16-C-12)	deep brown
14. Tomato paste-oatmeal agar	good; 5 mm irregular colonies	good; white to white with rose tinge	—	brown
15. Egg albumin agar	moderate; discrete 10~15 mm colonies	poor; dull white	colourless	none
16. Potato plug	excellent; leathery and much wrinkled growth with colourless guttation drops	good; dull white to light yellow with green tinge (17-C-2)	brown with olive tinge	pale brown around growth and no decomposition of plug
17. Carrot plug	excellent; deeply folded, a few fissures on surface	good; pale dull yellow with trace green (19-B-1)	yellow with trace green (11-I-1) to dirty green with black tinge	none; no decomposition of plug
18. Plain agar	poor; 12 mm colonies	scant; dull white	colourless	none

(to be continued)

Table 1. (continued)

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment
19. Yeast extract-glucose agar	excellent	excellent; white	brownish yellow (11-J-4) to brown (14-J-8)	deep brown
20. Glycerol-peptone agar	excellent; 5 mm colonies with wrinkled surface	scant; white	deep brown with green tinge (15-L-8)	deep brown
21. a) Maltose-tryptone agar	good; 3 mm colonies with a few light yellow guttation drops	abundant; white to pale yellow (9-B-1)	straw (10-F-2) with a number of deep brown with trace green (15-E-7) to greenish black patches	dark brown
b) broth	excellent	moderate; dull white	deep to dark green with trace brown (15-J-4 to 16-J-6)	dark blackish brown
22. Nutrient agar	moderate; 3~8 mm flat colonies	scant; white	colourless to dull brown with green tinge (14-H-6)	brown
23. Glucose-nutrient agar	6~12 mm irregular colonies with much folded surface and with rough, hard and leathery texture	moderate; pale yellow (10-B-1)	deep to dark brown (15-L-10 to 16-H-12)	brown
24. Nutrient-salt agar	moderate; surface wrinkled	moderate; dull white to pale yellow with green tinge	deep greenish brown (15-H-5) to dark brown with green tinge (16-J-9)	light brown
25. Yeast extract-malt extract agar ISP	excellent; colourless guttation drops	excellent; dull white to pale yellow with green tinge	light brownish yellow (11-F-2) to yellowish brown (13-I-7)	brown
26. BENNETT's agar	good; 4~10 mm deeply penetrating colonies with a leathery, tough texture; colourless guttation drops	good; pale yellow (17-B-1) to pale yellow with green tinge (17-B-2)	brown with green tinge (14-I-6) to deep brown with trace green (15-H-7)	light brown
27. EMERSON's agar	good; 4~12 mm irregular colonies with much folded surface; colourless guttation drops	moderate; white to yellow with green tinge (17-B-2)	deep green with brown tinge (15-L-6) to dark brown with green tinge (16-J-9)	deep brown
28. Nutrient gelatin	good; pellicle	poor; light green (19-B-2)	light yellow to deep brown (15-A-10)	light reddish brown to deep brown
29. Bromocresol purple milk at 37°C (3 weeks)	excellent	none	colourless to amber white to brown	—
30. Skim milk at 37°C (3 weeks)	excellent	none	colourless to amber white to brown with a few violet patches	deep pinkish brown
31. Skim milk agar (10 days)	good	poor; white	light dull green (13-D-1)	light brown in hydrolysed zone
32. Blood agar at 37°C	moderate	scant; white	deep brown	deep brown
33. LOEFFLER's coagulated serum at 37°C	good; much wrinkled, thick, 10 mm colonies	none	creamy to greenish yellow	deep blackish brown around growth
34. Organic nitrate broth	moderate	poor; pale yellow	colourless	deep brown

Table 2. Physiological reactions

Reaction	Medium used	Response	Result
1. Melanin reaction	WAKSMAN's medium	brownish black SP in 24 hours.	positive
2. Melanin formation	Tryptone-yeast extract broth ISP	deep brown SP in 36 hours.	positive
3. H_2S production	Peptone-yeast extract-iron agar ISP	bluish black SP in 12 hours.	positive
4. Tyrosinase reaction	SHINOBU synthetic tyrosine agar ISP and broth	brown SP on agar and deep blackish brown SP in broth	positive
5. Proteolytic activity			
a) Gelatin liquefaction	Plain gelatin	1/4 liquefaction in 2 weeks	positive
	Nutrient gelatin	1/4 liquefaction in 2 weeks	positive
	Gelatin agar plate (10 days)	growth zone/hydrolysed zone = 7 mm/20 mm	positive
b) Coagulation	Bromocresol purple milk and skim milk	complete in one week	positive
Peptonization		3/4 in 3 weeks	positive (partial)
Reaction			strongly alkaline
c) Casein hydrolysis	Skim milk agar (10 days)	growth zone/hydrolysed zone = 5 mm/40 mm	strongly positive
d) Blood haemolysis	Blood agar at 37°C	growth zone/haemolysed zone = 4 mm/12 mm	positive
e) Serum liquefaction	LOEFFLER's coagulated serum at 37°C	—	negative
6. Nitrate reduction	Sucrose-nitrate, glucose-nitrate and organic-nitrate broths		negative
	Glycerol-nitrate broth		weakly positive
7. Amylolytic activity	Starch agar plate (10 days)	growth zone/diastatic zone = 12 mm/42 mm	positive
	Inorganic salts starch agar (WAKSMAN and ISP) and GAUSE mineral salts medium I		positive diastatic action
8. Cellulolytic activity	Cellulose disc + CZAPEK's salts solution, cellulose powder + SHIRLING and GOTTLIEB salts solution agar		negative
9. Acid from glucose	Glucose fermentation broth		negative
10. Indole production from tryptone	Tryptone-yeast extract broth		negative
11. Sucrose inversion	Sucrose solution (GAUSE)		negative
12. Catalase formation	BENNETT's agar and broth		positive

Table 3. Utilization of carbon compounds in PRIDHAM and GOTTLIEB's medium[2]

Carbon source	Utilization	Carbon source	Utilization	Carbon source	Utilization
Arabinose	−	Inositol	###	Sorbitol	+
Cellulose	+	Lactose	±	Salicin	±
Digitonin	−	Maltose	##	Sorbose	−
Dulcitol	−	Mannitol	###	Sodium succinate	+
Fructose	###	Mannose	###	Sodium acetate	±
Glucose	###	Paraffin	−	Sodium citrate	±
Galactose	###	Raffinose	±	Sucrose	−
Glycerol	###	Rhamnose	±	Xylose	±
Inulin	##	Starch	###		

###: excellent growth; ##: good growth; ##: moderate growth; +: poor growth; ±: growth similar to or only slightly better than on basal medium; −: growth less than the growth on basal medium.

Table 4. Differences between *S. quilonensis* and *S. olivoreticuli*

	S. quilonensis	*S. olivoreticuli*
1. Sporophores	mostly secondary verticils	mostly primary verticils
2. Glucose-CZAPEK's agar	AM: good; light yellow to yellow with trace green	poor; pale gray with trace yellow
3. Glucose-asparagine agar	R: yellow with trace orange to yellowish brown	light brown with trace green to deep olive brown
4. Inorganic salts-starch agar (WAKSMAN)	R: colourless to dull oyster white	brown with trace green
	AM: white to rose-white	white
5. Inorganic salts-starch agar ISP	R: oyster white to Italian straw	cream to deep grayish brown
	AM: light rose	dull white
6. GAUSE-mineral salts medium I	R: colourless to yellow with trace orange	deep olive brown
	AM: light rose	dull white to pale gray with yellow tinge
7. a) BENNETT's agar	R: brown with green tinge	blackish brown to black
	SP: deep brown	pale brown
b) Broth	AM: greenish yellow	none
8. a) EMERSON's agar	G: deeply wrinkled surface	smooth surface
b) Broth	SP: deep blackish brown	deep brown
9. Nutrient broth	R: colourless to yellow to brown	colourless
10. Glucose-nutrient agar	G: deeply wrinkled surface	smooth surface
11. a) Maltose-tryptone agar	R: colourless to straw with a number of deep brown with trace green to greenish black patches	deep brown
b) Broth	R: deep dull green with trace brown to dark green with trace brown	colourless
12. Oatmeal agar ISP	AM: white to pale rose	white
13. Tomato paste-oatmeal agar	AM: white to white with rose tinge	white to pale olive gray
14. Potato plug	SM: deep brown with olive tinge	deep brown to blackish brown
	SP: pale brown around growth	deep blackish brown throughout
15. Carrot plug	SM: yellow with trace green to dirty green with black tinge	amber white to brown
16. Gelatin agar plate (10 days)	R: light dull greenish yellow	colourless to pale yellowish brown
17. a) Tyrosine agar ISP	R: deep blackish brown	deep brown
	AM: good, pale yellow with green tinge	none
	SP: brown	none
b) Broth	R: colourless to deep brown	colourless
	AM: good	none
	SP: deep blackish brown	none
18. Nutrient gelatin	AM: light green	pale yellow with gray tinge
19. Skim milk	SP: deep pinkish brown	brown in the peptonized zone
20. Skim milk agar (10 days)	R: light grayish green	yellowish brown
21. Tryptone-yeast extract broth ISP	R: deep blackish green	colourless
	SP: deep blackish brown	pale brown
Biochemical reactions:		
Tyrosinase	positive	negative
Melanoids	strongly positive	weak

(to be continued)

Table 4. (Continued)

	S. quilonensis	*S. olivoreticuli*
Milk coagulation	complete in 1 week	complete in 3 weeks
Milk peptonization	complete in 4 weeks	little in 4 weeks
pH (Reaction)	strongly alkaline	no change
Nitrate reduction (glycerol-nitrate broth)	positive	negative
Starch hydrolysis	moderate	strong
Gelatin liquefaction	weak	moderate
Casein hydrolysis	strong	weak
Carbohydrate utilization:		
Glucose	++	trace
Inositol	+++	trace
Mannitol	+++	—
Fructose	++	trace
Cellulose	+	—
Inulin	++	—
Lactose	±	+
Antimicrobial activity	active on fungi, yeasts and on a few bacteria	active on *Mycobacteria*; very slight activity on other bacteria; no activity on fungi and yeasts
Antimicrobial product	Heptaene	Polypeptide (viomycin)

agrees, differences exist when individual media are compared. Further, the reference organism produces a brown soluble pigment on glucose-asparagine agar, does not liquefy gelatin, does not peptonize milk, gives a variable H_2S reaction and produces a basic antibiotic.

S. reticuli and *S. quilonensis*: The two organisms agree in being melanin positive and chromogenic, in producing verticils with straight or spiral sporophores, in the absence of soluble pigment formation on synthetic media, in the liquefaction of gelatin, in the coagulation and peptonization of milk, in hydrolysis of starch and in being H_2S positive. But *S. reticuli* differs from our isolate in producing ash-gray aerial mycelium on sucrose-nitrate agar and potato, in showing gray combination in substrate mycelium colours on sucrose-nitrate agar, nutrient agar, gelatin, potato and starch agar, in producing a black soluble pigment on potato, in exhibiting positive nitrate reduction and invertase reactions and in producing neomycin. ETTLINGER notes that the reference organism does not produce spiral verticils and is melanin negative.

S. albireticuli and *S. quilonensis*: The two organisms agree in being verticils, in being melanin and H_2S positive, in chromogenicity, positive gelatin liquefaction, starch hydrolysis and milk peptonization reactions. However, the reference culture differs in giving spiral sporophores, in producing predominantly white aerial mycelium, in substrate mycelium colours, in producing a black soluble pigment on gelatin and in exhibiting a positive nitrate reduction reaction, in the absence of growth on cellulose and in producing a pentaene.

In view of quite a few significant differences between our isolate and the above reference organisms as discussed above, it has to be considered that *S. quilonensis* is neither identical nor a variant of these.

As it has been found that *S. quilonensis* is more closer to *S. olivoreticuli* and *S. olivoverticillatus*, these two cultures were obtained from Centraalbureau Voor Schimmelcultures, Nederlands and simultaneous comparative studies were made in an extensive way. The data revealed that our isolate was closer to *S. olivoreticuli* in its aerial mycelial, substrate mycelial and soluble pigment colour characteristics than *S. olivoverticillatus* in a broad way and when differences on individual media were not taken into consideration but it was difficult to make a choice between the two reference organisms when

biochemical reactions alone were considered. However, when the characteristics of *S. quilonensis* and *S. olivoreticuli* on individual media were compared, quite a few significant differences in the substrate mycelium colour and to a certain extent in the soluble pigment and aerial mycelium colours and certain significant quantitative and qualitative differences in biochemical reactions were noticed which are presented in Table 4. In view of the differences in verticil formation, in aerial mycelial, reverse and soluble pigment colour characteristics and in view of significant differences in biochemical reactions, mainly tyrosinase and melanoid formation, reaction in milk, reduction of nitrate and differences in the utilization pattern of as many as 7 carbohydrates and due to significant differences in antimicrobial activity, it was considered that our isolate is a new one as it differs quite significantly from all known *Streptoverticillium* species. It is designated as *Streptoverticillium quilonensis* sp. nov. SAMBAMURTHY and ELLAIAH. The species is named after the place from where the soil sample was collected, namely Quilon in Kerala State, India.

The culture is deposited at the culture collection centre "National Collection of Industrial Microorganisms" of the National Chemical Laboratory, Poona, India and the new species is allotted the number NCIM 2641 by the centre.

Acknowledgments

The authors wish to express their grateful thanks to Centraalbureau Voor Schimmelcultures, Nederlands for supplying the cultures of *S. olivereticuli* and *S. olivoverticillatus*.

References

1) MAERZ, A. & M. R. PAUL: A Dictionary of Color, II ed. McGraw Hill Book Co., New York, 1950
2) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bact. 56: 107~114, 1948
3) BREED, R. S.; E. G. D. MURRAY, N. R. SMITH & 94 contributors: BERGEY's Manual of Determinative Bacteriology. 7th ed. The Williams and Wilkins Co., U.S.A., 1957
4) GAUSE, G. F.; T. P. PREOBRAZHENSKAYA, E. S. KUDRINA, N. O. BLINOV, I. D. RJABOVA & M. A. SVESHNIKOVA: Zur Klassifizierung der Actinomyceten. Fischer, Jena, 1958
5) WAKSMAN, S. A.: The Actinomycetes. Vol. 2. Classification, identification and descriptions of genera and species. The Williams and Wilkins Co., Baltimore, 1961
6) KRASSILNIKOV, N. A.: Biologiya Otdel nykh grupp actinomitsetov. Nauk, U.S.S.R. Moscow, 1965
7) SHIRLING, E. B. & D. GOTTLIEB: Species descriptions from first study; Additional species descriptions from first and second studies; species description from the second, third and fourth studies. Intern. J. Syst. Bact. 18: 69~189, 1968; 18: 279~392, 1968; 19: 391~512, 1969
8) LOCCI, R.; E. BALDACCI & E. PETROLINE BALDAN: The genus *Streptoverticillium*—A taxonomic study: Giorn. Microbiol. 17: 1, 1969
9) ARAI, T.; T. NAKADA & M. SUZUKI; Production of viomycin-like substance by a *Streptomyces*. Antibiot. & Chemoth. 7: 435~442, 1957