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Thermoactinomyces candidus, a New Species of Thermophilic Actinomycetes

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Thermoactinomyces candidus, a new species isolated from home environments and other sources, is reported. *T. candidus* differs from *T. vulgaris* in that the former species hydrolyzes esculin, splits arbutin, and does not attack tyrosine, hypoxanthine, or starch, whereas the latter does. *T. candidus* differs from *T. sacchari* by producing fast-growing colonies, abundant aerial mycelia, and hemolysis in blood agar, by decomposing esculin and arbutin, and by failing to hydrolyze starch. The type strain of *T. candidus* is T-106 (= ATCC 27868).

A number of actinomycetes were isolated during the course of a study on the occurrence of thermophilic actinomycetes in home environments of individuals suffering from allergic pneumonitis. The actinomycetes isolated included *Thermoactinomyces vulgaris* Tsiklinsky, *Saccharomonospora viridis* (Schuurmans, Olson, and San Clemente) Nonomura and Ohara, occasional strains of *Streptomyces* species, and a group of unidentified thermophiles. These strains had some features in common with those of the three recognized species of *Thermoactinomyces* (5) — *T. vulgaris*, *T. sacchari*, and *T. dichotomica* — but differed sufficiently enough from these species to warrant a study to determine the systematic position of these and similar strains isolated from other sources.

MATERIALS AND METHODS

Bacterial strains. Twenty-five of the new strains, four strains of *T. vulgaris*, and four strains of *T. sacchari* were studied. The strains and their sources are given in Table 1. All cultures, except those of *T. sacchari*, were grown in Trypticase soy agar at 55 C for 3 to 7 days. *T. sacchari* cultures were grown in half nutrient agar at 45 C. Stock cultures were maintained both at 4 C and at room temperature, and subcultures were made at intervals of 8 to 12 weeks. Fresh subcultures were used for all the tests.

Morphology. The morphology of the isolates was studied in Trypticase soy agar (TSA), TSA with 0.2% yeast extract, TSA with 5% sheep blood, nutrient agar, and Trypticase soy broth. Cultures of all the strains were incubated at 50 and 55 C for up to 1 week and studied for rate of growth, aerial mycelial production, sporulation, pigment production, hemolysis in blood agar, and pellicle formation in broth.

Incubation. Unless otherwise stated, all of the cultures used in the physiological tests were incubated at 50 C for 7 days. During incubation, the agar cultures were stored in plastic bags to avoid drying.

Decomposition of casein. This was determined by the method described by Gordon and Smith (10).

Decomposition of tyrosine, xanthine, hypoxanthine, and adenine. The media and methods used were as described by Gordon et al. (7) and Kurup and Schmitt (12), except that TSA was used for the basal medium. Cultures were inoculated at the center of the plate, incubated up to 1 week, and examined for clearing of tyrosine, xanthine, hypoxanthine, or adenine crystals from around and underneath the colonies.

Decomposition of gelatin. TSA supplemented with 0.4% (wt/vol) of gelatin was inoculated and incubated for 5 days. The plates were then flooded with a reagent having the following composition (9): mercuric chloride, 15 g; concentrated HCl, 20 ml; and distilled water, 100 ml. A clear zone around the colony indicated gelatin hydrolysis.

Hydrolysis of starch. Ten grams of potato starch suspended in 100 ml of cold, distilled water was added to 900 ml of TSA, autoclaved, and poured into plates. After inoculation and incubation at 50 C for 7 days, the plates were flooded with Gram's iodide solution (3). A clear zone around the colony indicated starch hydrolysis.

Hydrolysis of esculin. Esculin (0.1% wt/vol) and ferric citrate (0.05% wt/vol) were added to TSA. Plates were inoculated and incubated for 2 weeks. Hydrolysis of esculin was evidenced by the blackening of the medium around the colony (3).

Splitting of arbutin. The medium used was the same as that used for esculin hydrolysis except that arbutin (0.1% wt/vol) was substituted for esculin. The results were interpreted as in hydrolysis of esculin (12).

Hydrolysis of hippurate. The medium and method used were the same as described by Gordon and Horan (8) except that the inoculated broth was incubated at 50 C for 2 weeks.

Resistance to lysozyme. TSB containing 0.005% (wt/vol) of lysozyme was inoculated with each strain and incubated at 50 C for 2 weeks. A plain Trypticase soy broth tube inoculated with each strain served as a control. The presence or absence of growth, in com-

TABLE 1. *List of strains studied and their sources*

Authors' strain designation	Identified as:	Source
T-102, T-118	*Thermoactinomyces candidus*	Humidifier
T-103, T-104, T-110, T-111, T-112, T-113, T-115, T-116, T-120, T-121, T-122, T-123	*T. candidus*	House dust
T-106 (= ATCC 27868), T-129, T-130, T-131, T-133	*T. candidus*	Heating units and air conditioners
T-143, T-144	*T. candidus*	Wood shavings
T-124	*T. candidus*	Mushroom compost
T-108	*T. candidus*	Moldy milo
T-125, T-132	*T. candidus*	Unknown
T-126	*T. vulgaris*	House dust
T-147 (= ATCC 15733)	*T. vulgaris*	ATCC
T-101, T-151	*T. vulgaris*	Received from Marshfield Clinic, Marshfield, Wis.
T-145 (= ATCC 27349)	*T. sacchari*	ATCC
T-171	*T. sacchari*	PriM-received from H. A. Lechevalier
T-198 (= ATCC 27376)	*T. sacchari*	ATCC
T-199 (= ATCC 27375)	*T. sacchari*	ATCC

parison with control growth, was recorded. The lysozyme solution was prepared by the method of Gordon (6).

Production of deoxyribonuclease. Deoxyribonuclease test agar (BBL) was prepared according to the manufacturer's instructions. The medium was inoculated and incubated for 1 week. The plates were then treated with 5 to 8 ml of normal HCl, and a clear zone around the colony indicated the production of deoxyribonuclease (3).

Urease production. Urease production was tested by inoculating urease test medium (4) and by incubating it for up to 1 week. An uninoculated tube also was incubated as a control. The development of a pink color indicated the production of urease.

Resistance to novobiocin. Novobiocin (Albamycin, Upjohn Co.) was incorporated in Trypticase soy broth to a final concentration of 100 μg/ml, and tubes of the medium were inoculated with the test strains. Controls without the antibiotic were inoculated and incubated in the same manner. Resistance to novobiocin was evidenced by the production of apparently similar growth in both the test and control tubes.

Sensitivity to antimicrobial agents. TSA plates were uniformly swabbed with a light suspension of spores. Disks impregnated with ampicillin, streptomycin, chloramphenicol, or gentamicin were placed over the inoculated plates and incubated for 18 to 24 h. The zones of inhibition were measured, and the results were recorded as "sensitive" or "resistant" (2).

Growth at different temperatures of incubation. Each strain was inoculated onto a set of five TSA plates, which were then incubated at 25, 37, 45, 50, and 60 C for up to 1 week and were examined for growth and gross morphology.

Resistance to heating at 100 C for varying periods of time. Spores of the thermophilic actinomycetes were suspended in distilled water and heated in boiling water. Cultures were transferred from the heated suspension onto TSA plates after 10, 30, 60, or 120 min of heating. The plates were examined for 1 week for evidence of growth.

Hydrolysis of tributyrin. The hydrolysis of tributyrin was tested by the method described by Kurup and Schmitt (12).

Hydrolysis of chitin and cellulose. The hydrolysis of chitin and cellulose was determined in TSA with 0.1% wt/vol chitin or cellulose. After 1 week of incubation, plates were observed for clearance of chitin or cellulose from around and underneath the colony.

Carbohydrate utilization. The method used was the one that Lacey employed to study sugar utilization by *T. sacchari* (13), with the exception that TSA was used as the basal medium instead of nutrient agar. One percent (wt/vol) of each of the following sugars was incorporated separately into the media: fructose, glucose, sucrose, arabinose, melibiose, inositol, or mannitol. Results were recorded in the manner described by Lacey.

Acid from carbohydrates. Ten of the new strains and four each of *T. vulgaris* and *T. sacchari* were tested for their ability to produce acid from carbohydrates. The medium and method used were the same as those described by Gordon and Mihm (9) and Kurup and Schmitt (12).

Cell wall and whole-cell analyses. Cell wall analyses of representative strains were made by the method described by Suput et al. (14).

RESULTS

Morphology. All of the strains tested, with the exception of those of *T. sacchari*, grew well in all media. Regardless of the medium used, *T. sacchari* strains grew slowly and produced only minimal aerial mycelium. *T. vulgaris* strains and the other 25 strains tested produced elaborate primary and secondary mycelia. Both the

substrate mycelium and aerial mycelium branched enormously and showed septation. The mycelium in young cultures stained gram positive, but in older cultures the staining varied from gram positive to gram negative. Some of the substrate mycelia broke into arthrospores measuring 0.5 to 0.75 by 2.0 to 4.0 μm and invariably stained gram positive. Unicellular spores, measuring 0.5 to 1.5 μm in diameter, formed on substrate and aerial mycelia and were attached directly on the filaments or on short stalks. All of the strains, except those of *T. sacchari*, grew on and produced hemolysis in blood agar. *T. sacchari* strains grew very slowly in blood agar and failed to produce any hemolysis. All three species produced surface growth with some sediment in broth cultures.

The results of the physiological and biochemical tests are given in Table 2. Casein and gelatin were decomposed by all of the strains tested, but none attacked xanthine, adenine, chitin, or cellulose. Sugar utilization and acidification tests were inconclusive, as some of the strains grew equally well in the basal medium or failed to grow in both the basal and supplemented media.

All nine strains of *T. candidus* tested for their cell wall composition were found to be of type III. Mesodiaminopimelic acid was detected in the whole-cell hydrolysate, but no arabinose, xylose, or madurose.

DISCUSSION

All of the strains studied grew at 55 to 60 C but failed to grow at room temperature. Thermoresistant unicellular spores, produced on both the substrate and aerial mycelia, unusual resistance to high concentrations of novobiocin, and cell wall composition of type III place these organisms in the family *Thermoactinomycetaceae* Baldacci and Locci. Cross and Goodfellow (5) recognized three species in this monogeneric family.

In addition to the morphological features as described by Lacey (13), *T. sacchari* can be differentiated from the new strains by its failure to hemolyze blood agar and to decompose esculin and arbutin and by its ability to hydrolyze starch. The yellow aerial and substrate mycelia and long, dichotomously branching sporophores, measuring 5.0 to 10.0 μm, distinguish *T. dichotomica* (*Actinobifida dichotomica*) from other species (11). In the production of abundant aerial mycelia, in the size and shape of spores, in other characteristics, as the hydrolysis of gelatin and casein, and in sensitivity to lysozyme and antibiotics, all 25 strains studied showed complete accordance with *T. vulgaris*. On prolonged incubation, colonies of *T. vulgaris* on TSA were yellowish-white, whereas those of the new strains were white. The new strains produced spores on short sporophores and occasionally on the filaments, unlike strains of *T. vulgaris*, which produce spores mostly on the filaments and only occasionally on sporophores. The new strains can be differentiated from those of *T. vulgaris* by the failure of the former to hydrolyze tyrosine, hypoxanthine, and starch, and by their ability to hydrolyze esculin and to split arbutin. The difference between the new strains and those of *T. vulgaris* is also indicated by the repeated observation that the antiserum produced against one of the strains isolated from a furnace humidifier (T-118) failed to cross-react with *T. vulgaris* antigens (1). It was also found that antigens from several of the new strains reacted strongly with the antiserum to T-118, while the same antigens gave very little or no cross-reaction against *T. vulgaris* antiserum. Based on these findings, we propose that the new strains belong to a new species of *Thermoactinomyces*, for which we propose the name *Thermoactinomyces candidus* (L. adj. *candidus*, shining white).

Thermoactinomyces candidus sp. nov. (i) Morphology. The width of the aerial and substrate hypha varied from 0.5 to 1.0 μm. Spores (Fig. 1) are round and unicellular and are produced mostly on small sporophores measuring 1.0 to 3.0 μm (average 1.5 μm) in diameter. The spores measure from 0.5 to 1.5 μm (average 0.75 μm) in diameter and are produced both on aerial and substrate hyphae. In TSA, the colony is white and fast growing with a gradually fading margin (Fig. 2). Aerial mycelium is produced in nutrient agar, TSA, TSA with 0.2% yeast extract, and in TSA with 5% sheep blood. In Trypticase soy broth culture, a pellicle is produced with some deposit.

(ii) Physiological characteristics. All of the strains examined failed to grow at 25 C and showed slight growth at 37 C and very good growth at 45, 50, 55, and 60 C. Spores resisted heating at 100 C for 1 h. Growth occurred in media containing 100 μg of novobiocin per ml and in broth containing 0.005% (wt/vol) of lysozyme. Hemolysis was produced in blood agar. Casein, esculin, and gelatin were hydrolyzed, and arbutin was decomposed. Tyrosine, xanthine, hypoxanthine, adenine, cellulose, chitin, hippurate, starch, and tributyrin were not attacked; nitrates were not reduced; urease and deoxyribonuclease were not produced. Most of the strains were sensitive to chloramphenicol, ampicillin, streptomycin, and gentamicin. Cell wall composition is of type III; whole-cell hydrolysate contains mesodiaminopimelic acid

TABLE 2. *Comparison of the physiologcal characteristics of Thermoactinomyces candidus sp. nov., T. vulgaris, and T. sacchari*

Test	*T. candidus* (25 strains; includes type strain)	*T. candidus* type strain T-106 (= ATCC 27868)	*T. vulgaris* (4 strains)	*T. sacchari* (4 strains)
Hydrolysis of:				
Casein	25[a]	+[b]	4	4
Tyrosine	0	−[b]	4	0
Xanthine	0	−	0	0
Hypoxanthine	0	−	4	0
Gelatin	25	+	4	3[c]
Starch	0	−	4	4
Esculin	25	+	0	0
Arbutin	25	+	0	0
Adenine	0	−	0	0
Production of:				
Deoxyribonuclease	16[d]	−	2[d]	0
Urease	5[e]	−	2[e]	1[e]
Nitrate reduction	0	−	0	0
Hydrolysis of hippurate	0	−	0	0
Decomposition of:				
Cellulose	0	−	0	0
Chitin	0	−	0	0
Resistance to lysozyme	25	+	4	ND[b]
Growth at (C):				
25	0	−	0	0
37	7[f]	−	2[f]	3[f]
45	25	+	4	4
50	25	+	4	4
55	25	+	4	4
60	25	+	4	2[g]
Resistance to novobiocin (100 μg/ml)	25	+	4	4
Resistance to heating at 100 C for (min):				
10	25	+	4	4
30	25	+	3[h]	2[h]
60	23[i]	+	2[i]	1[i]
120	15[j]	−	1[j]	0
Sensitivity to:				
Ampicillin	23[k]	+	4	4
Chloramphenicol	19[l]	+	4	4
Streptomycin	24[m]	+	4	4
Gentamycin	25	+	4	4
Hemolysis in blood agar	25	+	4	0

[a] Number of positive strains.

[b] +, Positive; −, negative; and ND, not done.

[c] T-198 did not hydrolyze gelatin.

[d] T-104, T-118, T-120, T-121, T-123, T-130, T-132, T-143, T-126, and T-147 failed to produce deoxyribonuclease.

[e] T-112, T-113, T-121, T-143, T-144, T-126, T-151 and T-198 produced urease.

[f] T-103, T-104, T-111, T-121, T-130, T-143, T-144, T-126, T-147, T-145, T-198, and T-199 grew at 37 C.

[g] T-145 and T-199 failed to grow at 60 C.

[h] T-151, T-145, and T-171 failed to withstand heating at 100 C for 30 min.

[i] T-118, T-131, T-126, T-147, T-145, T-171, and T-199 failed to withstand heating at 100 C for 60 min.

[j] T-102, T-103, T-110, T-113, T-118, T-129, T-131, T-143, T-144, T-126, T-147, and T-151 failed to withstand heating at 100 C for 120 min.

[k] T-110 and T-111 were resistant to ampicillin.

[l] T-102, T-103, T-113, T-115, T-130, and T-122 were resistant to chloramphenicol.

[m] T-111 was resistant to streptomycin.

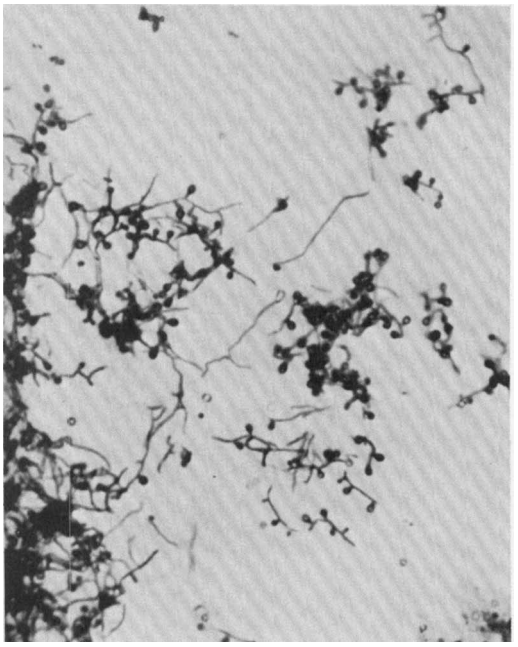

FIG. 1. *Spores of T. candidus from 5-day-old TSA culture incubated at 50 C. ×1250.*

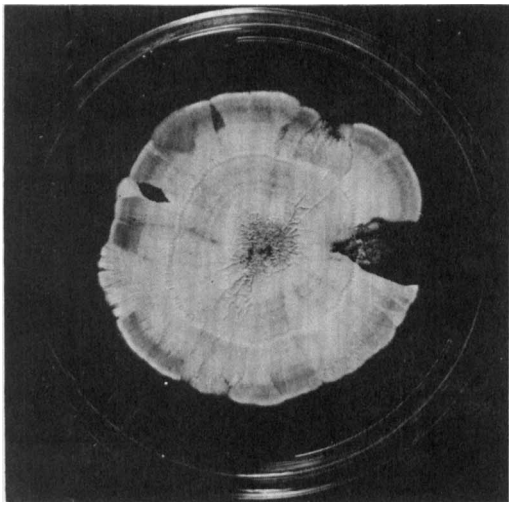

FIG. 2. *One-week-old colony of T. candidus in TSA at 50 C. ×1.*

but no arabinose, xylose, or madurose.

(iii) Source. Strains belonging to the species have been isolated from air conditioners, humidifiers, house dust, mushroom compost, etc.

(iv) Type strain. T-106 was isolated from an air conditioner. This strain has been deposited in the American Type Culture Collection (ATCC) under the number 27868.

The cellular and colonial morphology of the type strain and the other strains in conformity with the description given above for the species. Physiological features of the type strain and other strains included in the study are given in Table 2.

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REPRINT REQUESTS

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