Hyphomonas oceanitis sp. nov., *Hyphomonas hirschiana* sp. nov., and *Hyphomonas jannaschiana* sp. nov.

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Various characteristics of seven recently isolated bacterial strains were determined and compared with the characteristics of the three extant strains of the genus *Hyphomonas* (ex. Pongratz 1957) Moore et al. 1984. On the basis of these results, three new species, *Hyphomonas oceanitis* sp. nov., *Hyphomonas jannaschiana* sp. nov., and *Hyphomonas hirschiana* sp. nov., are proposed for inclusion within this genus.

The recently revived genus *Hyphomonas* includes the aerobic, gram-negative, non-photosynthetic, prosthecate, budding bacteria that preferentially utilize amino acids for growth (15). These organisms are nonsporeforming, normally possess only a single polar hypha, and do not deposit iron or manganese salts on the cell surface. Unlike members of the genus *Hyphomicrobium*, the size and shape of the main cell body varies markedly during the normal cell cycle (13).

The genus *Hyphomonas* currently contains only two species, *Hyphomonas polymorpha*, the type species, and *Hyphomonas neptunium*. The cell cycle of the latter has been examined in considerable detail (4, 13, 20). Several additional strains which are morphologically similar to these species have recently become available for study. These include strain SCH89^T (T = type strain), which was isolated from the Baltic Sea by Schlesner, and strain H13, which was isolated from a slurry of oysters that were gathered from an estuary of Chesapeake Bay (22). Strains VP1, VP2^T, VP3, VP4, and VP5^T were isolated from shellfish beds near hydrothermal vents on the floor of the Pacific Ocean (8). The results of morphological, nutritional, biochemical, and serological tests of these seven new strains, the type strain of *Hyphomonas neptunium*, and two strains of *Hyphomonas polymorpha* are reported here.

MATERIALS AND METHODS

Bacteria and media. The three reference strains used in this study were *Hyphomonas neptunium* LE670^T (= IFAM LE670^T = ATCC 15444^T) and smooth strain PS728^T (= IFAM PS728^T = ATCC 33881^T) and rough strain PR727 (= IFAM PR727 = ATCC 33880) of *Hyphomonas polymorpha*. The five vent strains, strains VP1 (= ATCC 33882), VP2^T (= ATCC 33883^T), VP3 (= ATCC 33884), VP4 (= ATCC 33885), and VP5^T (= ATCC 33886^T), were provided by H. Jannasch and J. Poindexter. Strain SCH89^T (= IFAM 1325^T = ATCC 33879^T) was obtained from R. Gebers, and strain H13 was isolated by one of us (R.M.W.) in a previous study (22).

Zobell marine medium (Difco Laboratories) (23) was used for routine culturing of all strains. The pH, temperature, and NaCl requirements were examined with this medium. Growth was measured by determining turbidity, direct counts, or plate counts or by combinations of these methods,

as previously described (21). Solid medium contained 15 g of agar (Difco) per liter. All cultures were incubated aerobically at 35°C except strain SCH89^T cultures (22°C) and strain VP5^T cultures (31°C). Growth under anaerobic conditions was examined by using the GasPak system (BBL Microbiology Systems). Blood agar contained 5% sheep blood in Zobell marine medium. Mannitol-salt agar (BBL) was used unaltered. All of the other test media were adjusted to pH 7.2 and brought to a final sodium chloride concentration of 3.5% (wt/vol). The procedures used for the biochemical tests (10, 19) and for the characterization of pigment (1, 7) have been described previously.

A modification of the minimal medium of Havenner et al. (6) was used to investigate nutritional requirements. This medium contained 411 mM NaCl, 13.0 mM MgCl₂ · 6 H₂O, 6.6 mM Na₂SO₄, 4.9 mM CaCl₂, 0.572 mM NaHCO₃, 0.202 mM KBr, 0.107 mM H₃BO₃, 0.065 mM SrCl₂, 0.012 mM NaSiO₃ · 9H₂O, 0.188 mM NH₄NO₃, 0.0555 mM FeCl₃ · 6H₂O, 125 mM L-glutamate, 125 mM L-aspartate, and 125 mM L-serine, and, in certain cases (see below), 2 mM methionine. A buffer solution (0.46 ml) containing 0.10 M KH₂PO₄ and 0.12 M K₂HPO₄ was added to the medium (100 ml). In certain cases (see below) biotin or a mixture of vitamins was added to give the following final concentrations of vitamins: biotin, 0.20 µM; folic acid, 0.05 µM; pyridoxine, 0.15 µM; riboflavin, 0.10 µM; *para*-aminobenzoic acid, 0.20 µM; thiamine, 0.10 µM.

All inorganic salts were obtained from J. T. Baker Chemical Co. Vitamins and amino acids were purchased as the highest purity grade available from Sigma Chemical Co. Chemical analyses of polyhydroxybutyrate and polyphosphate were performed according to Smibert and Krieg (19).

Morphology. Cellular morphology was examined by phase-contrast microscopy (3). Flagella were stained by the procedure of Mayfield and Inness (12). Cellular fine structure was investigated by transmission electron microscopy of thin sections obtained in the following manner. Culture pellets were suspended in 10 ml of phosphate-buffered saline (6), washed four times in phosphate-buffered saline, suspended in a mixture containing 9.5 ml of Kellenberger buffer, 5 ml of Veronal-acetate buffer (2.94 g of sodium Veronal, 1.94 g of sodium acetate, 3.40 g of NaCl, 100 ml of water), 13 ml of distilled water, 7 ml of 0.1 M HCl, 0.25 ml of 1.0 M CaCl₂, (pH 6.0), and 0.5 ml of Kellenberger fixative (100 ml of Kellenberger buffer, 0.5 g of OsO₄), and centrifuged immediately. The pellets were suspended in full-strength fixative, left at room temperature for 20 to 30 min, and then

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TABLE 1. Characteristics of the species of the genus *Hyphomonas*

Characteristic	<i>Hyphomonas polymorpha</i> PS728 ^T and PR727	<i>Hyphomonas neptunium</i> LE670 ^T and H13	<i>Hyphomonas jannaschiana</i>		<i>Hyphomonas hirschi</i> VP5 ^T	<i>Hyphomonas oceanitis</i> SCH89 ^T
			Strains VP1 and VP3	Strains VP2 ^T and VP4		
Type of growth in broth	Turbid	Turbid	Granular	Ropy	Pellicle	Turbid
Optimal temp (°C)	30–37	30–37	37	37	25–31	20–30
NaCl concn for maximum growth (%)	0.5–3.5	2.0–5.0	3.5–7.5	3.5–7.5	3.5–7.5	2.0–5.0
NaCl concn for growth (%)	0.5–5.0	1.0–7.5	2.0–15.0	2.0–15.0	2.0–15.0	1.0–7.5
Optimum pH	7.0–7.4 ^a	8.0 ^b	7.6	7.6	7.6	7.6
Microaerophilic growth	–	–	Slight	Slight	–	Slight
Requires methionine	+	+	–	–	+	+
Requires biotin	–	–	+	+	–	+
Film formation on growth vessel	– or slight	Slight	+	Slight	Slight	–
Rosette formation	–	–	+	–	–	–
Brown pigment at 31–37°C ^c	–	–	+	+	–	–
Resistance to mechanical lysis (min) ^d	2–5	<2	≥9	≥9	≥9	6–8
Hemolysis on sheep blood agar	γ/α	α	α	α	γ	γ
NO ₃ [–] → NO ₂ [–]	–	+	+	+	+	+
Susceptibility to: ^e						
NP-40 (0.01%)	S	S	S	S	S	S
Tween 80 (0.01–1.0%)	R	R	R	R	R	S
Novobiocin (30 µg)	S	S	S	S	S	R
Penicillin (10 IU)	S	S	S	S	I	R
Ampicillin (10 µg)	S	S	S	S	S	R
Streptomycin (10 µg)	I	S	S	S	I	R
G + C content of DNA (mol%)	60 and 61 ^f	62 and 60 ^g	60 and 60 ^g	60 and 60 ^g	57 ^g	59 ^g

^a Data from reference 17.^b Data from reference 9.^c With Zobell marine medium.^d Time required for 99% lysis by a Brownwill Biosonik IV sonicator (low probe at full power).^e S, Susceptible (diameter of inhibition zone, >20 mm); R, resistant (diameter of inhibition zone, <10 mm); I, intermediate (diameter of inhibition zone, 10 to 20 mm). All values quantity of substance per disk, except the NP-40 and Tween 80 values, which are final concentrations.^f Data from reference 11.^g Data from a study by Gebers et al. (in press).

stored at 4°C overnight. They were then diluted with 8 ml of Kellenberger buffer, centrifuged, washed twice in distilled water, suspended in 1 to 2 drops of 10% molten Noble agar (Difco) in distilled water, and cooled on ice. Agar-encased cells were soaked in 0.5% (wt/vol) uranyl acetate for 2 h, rinsed in distilled water, cut into 1-mm pieces, and dehydrated with successively increasing concentrations of acetone (0 to 100% [vol/vol] in 5% increments). The fixed, dehydrated specimens were embedded in Epon 812 and sectioned with a Sorvall Porter-Bum model MT2B microtome. The sections were stained with 2% (wt/vol) aqueous uranyl acetate for 3 min and with a 0.2% (wt/vol) lead citrate solution for 30 s. All specimens were examined with an RCA model EMU-3H transmission electron microscope.

Inhibitors. The standard technique (16) for antibiotic susceptibility testing had to be modified since Mueller-Hinton agar, which is usually used to determine antibiotic susceptibility profiles, supported the growth of only the two *Hyphomonas polymorpha* strains, even if this medium was supplemented with 2.5% NaCl. Consequently, samples of exponential phase cultures were adjusted to a standard turbidity (16) and swabbed onto modified Zobell marine agar

(18.7 g of Difco type 2216 marine broth and 15 g of Noble agar per liter of water). Antibiotic-containing Sensi-Disks (BBL) were placed at least 30 mm apart on the agar surface. Strain SCH89^T was incubated for 24 h before placement of the Sensi-Disks. Other potentially inhibitory substances were tested by using filter paper disks (diameter, 7 mm). The diameters of the zones of inhibition were determined after 48 h of incubation on marine agar. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, which were obtained from P. Criscente, were used as standard controls.

Modified Zobell marine medium produced good growth of all strains and the control bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*). The levels of susceptibility of the controls were consistent with previously reported standard levels (16).

Inhibition by the nonionic detergents Nonidet P40 (NP-40) and Tween 80 was determined by incorporating these compounds into Zobell marine agar and comparing the amount of growth observed with the amount in media lacking detergent.

Serology. Rabbit antiserum to *Hyphomonas neptunium* was produced with live cells harvested in the exponential

TABLE 2. Differential characteristics of species of the genus *Hyphomonas*

Characteristic	<i>Hyphomonas polymorpha</i> PS728 and PR727	<i>Hyphomonas neptunium</i> LE670 [†] and H13	<i>Hyphomonas oceanitis</i> SCH89 [†]	<i>Hyphomonas jannaschiana</i> VP1, VP2 [†] , VP3, and VP4	<i>Hyphomonas hirschiana</i> VP5 [†]
Nutritional requirements: ^a					
NaCl (%)	0.5–5.0	1.0–7.5	1.0–7.5	2.0–15.0	2.0–15.0
Methionine	+	+	+	–	+
Biotin	–	–	+	+	–
Biochemical properties: ^a					
Nitrate reduction	–	+	+	+	+
Hemolysis	α/γ	α	γ	α	γ
Brown pigment (31–37°C)	–	–	–	+	–
Growth at 37°C	+	+	–	+	–
Susceptibility to: ^a					
Mechanical lysis (min) ^b	2–5	<2	6–8	≥9	≥9
Tween 80 (0.01–1.0%)	R ^c	R	S	R	R
Novobiocin (30 µg)	S	S	R	S	S
Penicillin (10 IU)	S	S	R	S	I
Ampicillin (10 µg)	S	S	R	S	S
Streptomycin (10 µg)	I	S	R	S	I
Level of DNA homology (%) with: ^d					
<i>Hyphomonas polymorpha</i> PS728 [†]	100 and 111	23 and 25	6	29, 8, 36 and 10	0
<i>Hyphomonas neptunium</i> LE670 [†]	21 and 20	100 and 110	3	15, 0, 0, and 0	0

^a Data from Table 1.^b Time required for lysis by a sonicator.^c See Table 1, footnote e.^d Data from a study by Gebers et al. (in press).

growth phase (18). Antisera to the remaining strains were produced with Formalin-killed cells harvested after 3 to 5 days of growth in Zobell marine medium. The antisera were prepared by procedures similar to those described by Evans (5, 18).

FIG. 1. Phase-contrast micrograph of *Hyphomonas oceanitis*. Bar = 1.0 µm.

Microtiter plate agglutination (2) was used to test the antigenic similarities of the various strains. Antigen at a concentration which gave an absorbance at 560 nm of 0.05 to 1.0 was mixed with heterologous and homologous serially diluted antisera, and the preparations were incubated at 37°C for 2 h. After an additional 12 h of incubation at 4°C, the results were scored by using a microtiter mirror.

RESULTS AND DISCUSSION

The characteristics of strains in the genus *Hyphomonas* are given in Table 1. All of these strains reproduced by budding from the tips of hyphae which grew from the main cell body. The sizes of the cells fell into the range (0.5 to 1.0 by 1.0 to 3.0 µm) reported for previously described members of the genus *Hyphomonas* (15). The hyphae were unbranched and originated from one of the poles of the mother cell.

All 10 strains were gram negative, non-acid fast, catalase positive, and oxidase positive, and all contained polyphosphate and polyhydroxybutyrate. Spores were never observed. None of the strains produced acid from glucose, from lactose, from mannitol, or in triple sugar iron agar. No strain grew on MacConkey agar. No strain hydrolyzed gelatin or starch or produced indole from tryptophan. Nitrite was not reduced to either molecular nitrogen or ammonia. No strain had deoxyribonuclease, ornithine decarboxylase, arginine dehydrolase, lysine decarboxylase, coagulase, or urease activity.

All strains grew well in Zobell marine medium under aerobic conditions, with generation times ranging from 2 to 4 h. The optimal temperature range for strains VP5[†] and SCH89[†] was somewhat lower than that for the other strains, but all strains exhibited a very broad growth range, with

TABLE 3. Serological relationships among strains on *Hyphomonas*

Antiserum to strain:	Reciprocals of titers									
	<i>Hyphomonas polymorpha</i> strains		<i>Hyphomonas neptunium</i> strains		<i>Hyphomonas jannaschiana</i> strains				<i>Hyphomonas hirschiana</i> VP5 ^T	<i>Hyphomonas oceanitis</i> SCH89 ^T
	PS728 ^T	PR727	LE670 ^T	H13	VP1	VP3	VP2 ^T	VP4		
PS728 ^T	1,280	1,280	— ^a	20	—	—	—	—	20	40
PR727	1,280	1,280	—	320	—	—	—	—	—	—
LE670 ^T	—	—	320	1,280 ^T	—	—	—	—	40	40
VP1	—	—	—	—	160	320	320	320	160	—
VP2 ^T	—	—	—	—	320	320	320	640	80	—
VP3	—	—	—	—	80	160	320	160	—	40
VP4	—	—	—	—	160	160	320	320	80	—
VP5 ^T	—	—	—	—	20	20	40	20	320	20
SCH89 ^T	—	—	—	—	—	—	—	—	40	1,280

^a —, No precipitate detected at a dilution of 1:20.

visible colonies formed after 7 days at temperatures as low as 8°C.

Growth under favorable conditions by all of the strains except strain SCH89^T and the two strains of *Hyphomonas polymorpha* consistently resulted in attachment of the cells to the bottom and sides of the growth vessel, producing a film. The cells of strains VP1 and VP3 produced the thickest films and also attached to one another to produce rosette-like cell arrangements.

All strains produced a yellow chromagen, possibly a derivative of oxalacetic acid, when they were grown in amino acid synthetic medium. This pigment had a molecular weight of less than 2,000 and was soluble in water, insoluble in xylene and acetone, and slightly soluble in ethanol.

All strains were susceptible (see Table 1, footnote e, for criteria) to 0.1 mg of tellurite, 40 µg of crystal violet, 40 µg of brilliant green, 2.0 mg of methylene blue, 0.2 mg of sodium lauryl sulfate, 16 mg of Teepol 610, and 80 µg of pyronin, had intermediate susceptibility to 2.0 mg of ox bile, and were resistant to 80 µg of neutral red and 8.0 µg of methyl violet.

The *Hyphomonas* strains were susceptible to 15 µg of erythromycin, 5 µg of rifampin, 30 µg of cephalothin, 30 µg of kanamycin, and 30 µg of chloramphenicol. Trimethoprim, tetracycline, and nalidixic acid were inactivated in modified marine agar; therefore, the effects of these antibiotics could not be examined. The differential responses of the strains to other antibiotics and growth-inhibiting compounds are shown in Table 1. The characteristic guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) (Table 1) and DNA homologies (Table 2) of the strains which we studied (R. Gebers, R. L. Moore, and P. Hirsch, Syst. Appl. Microbiol., in press) indicate that the new strains are members of the genus *Hyphomonas* (15). For new isolates VP1, VP2^T, VP3, VP4, VP5^T, and SCH89^T, we propose three new species, *Hyphomonas oceanitis*, *Hyphomonas jannaschiana*, and *Hyphomonas hirschiana*. Differential characteristics of these species are given in Table 2.

***Hyphomonas oceanitis* sp. nov.** *Hyphomonas oceanitis* (o. cean. i' tis. N.L. fem.n. *oceanitis* daughter of the ocean) cells are round to oval and ca. 0.9 µm in diameter and usually have one hypha which is up to three times the length of the mother cell (Fig. 1). Under favorable growth conditions, a small proportion of cells produce two hyphae, one at each of the poles. Both hyphae are capable of producing buds concurrently. Buds (daughter cells) are pear shaped and become larger and rounder when they are mature (mother cells) and producing buds. Intercalary buds (i.e., two or more daughter cells remaining attached to the distal

pole[s] of the stalk[s] of the mother cell) are common. Buds are motile until the hyphae form.

Gram-negative. Not acid fast. No endospores. Aerobic or microaerophilic.

On agar, the colonies are colorless, raised, semitranslucent or opaque, and, after 3 days, up to 1.5 mm in diameter.

In liquid media, growth produces uniform turbidity without a pellicle or sediment. The best growth occurs in media containing sea salts. Does not grow on the mineral salts medium used for *Hyphomicrobium vulgare*. A mixture of L-glutamic acid, L-aspartic acid, L-serine, and L-methionine is required for growth. Requires biotin.

The temperature range for optimum growth is 20 to 30°C. The NaCl concentration for optimum growth is 1.0 to 7.5%. The optimum pH is 7.6. Nitrate is reduced. Sheep erythrocytes are not hemolyzed. Pigment is not formed. Under optimal conditions in marine broth, growth is slower than the growth of other species of *Hyphomonas*.

Susceptible to NP-40 (0.01%) and Tween 80 (0.1 to 1.0%). Resistant to novobiocin, penicillin, ampicillin, and streptomycin. Requires 6 to 8 min of treatment with a Brownwill Biosonic IV sonicator (low probe at full power) to produce 99% lysis of cells. Other nutritional and biochemical characteristics are listed in Table 1.

Hyphomonas oceanitis has marginal antigenic relatedness to *Hyphomonas polymorpha*, *Hyphomonas neptunium*, *Hyphomonas hirschiana*, and *Hyphomonas jannaschiana* (Table 3). The levels of genetic relatedness to *Hyphomonas polymorpha* and *Hyphomonas neptunium*, as determined by DNA homology studies (in solution with endonuclease treatment) (Gebers et al., in press), are 6 and 3%, respectively.

Isolated from the Baltic Sea in 1979 by H. Schlesner.

The G+C content of the DNA is 59 mol%.

Type strain: strain SCH89 (= IFAM 1325 = ATCC 33879).

***Hyphomonas jannaschiana* sp. nov.** *Hyphomonas jannaschiana* (jan' nasch. i. an. a. L. fem. adj. *jannaschiana* named for Holger Jannasch for his contributions to marine microbiology) mature mother cells are 0.5 to 0.8 by 1.0 to 3.0 µm, and the main body of the cell tends to be elongated and bullet or spindle shaped. Young swarmer cells are motile and pear shaped and resemble those of other members of the genus. On blood agar, colonies are small, dull grey, dry, and circular to irregular with dimpled elevations and lobate margins (strains VP1 and VP3) or grey, circular, convex, entire, glistening, and mucoid (strains VP2^T and VP4), resembling *Hyphomonas neptunium* colonies, which have a similar shape and are white. In broth, growth appears granular, and a film of tan cells covers the sides and bottom of the culture vessel (strains VP1 and VP3), or growth may

FIG. 2. Transmission electron microscopy of thin sections of *Hyphomonas jannaschiana* strains VP1 (V1), VP2^T (V2), and VP4 (V4). Bars = 0.5 μ m.

appear ropy and produce only a slight film (strains VP2^T and VP4). Strains VP1 and VP3 tend to form rosette-like cell aggregates.

A brown pigment with chemical properties characteristic of melanins (1, 7) is produced in type 2216 marine broth at 31 to 37°C. A mixture containing glutamic acid, aspartic acid, and serine as substrates and the cofactor biotin is required for chemoorganotrophic growth. The temperature for optimum growth is 37°C. The NaCl concentration for optimum growth is 3.5 to 7.5%. The optimum pH is 7.6.

Nitrate is reduced. Produces α -hemolysis on Zobell marine medium supplemented with 5% sheep blood. Susceptible to NP-40 (0.01%), novobiocin, penicillin, ampicillin, and streptomycin. Resistant to Tween 80 (1.0%). Very resistant to breakage by sonication. Other nutritional and biochemical test results are shown in Table 1.

Possesses some surface antigens in common with *Hyphomonas hirschiana*, but none in common with *Hyphomonas oceanitis*, *Hyphomonas polymorpha*, or *Hyphomonas neptunium* (Table 3).

Four strains, strains ATCC 33882 (= VP1), ATCC 33883^T (= VP2^T), ATCC 33884 (= VP3), and ATCC 33885 (= VP4) of *Hyphomonas jannaschiana* were isolated in 1979 (8) from shellfish beds near hydrothermal vents on the floor of the mid-Pacific Ocean.

The fine structure of the vent strains is shown in Fig. 2. The appearance is typical of gram-negative bacteria. The only notable difference among the strains is the consistent presence of large dark granules in strains VP1 and VP3. The occurrence of these granules is independent of the medium used for culturing.

The G+C contents of the DNAs of strains VP1, VP2^T, VP3, and VP4 are 60 mol%.

Low levels of DNA homology occur between all four isolates and *Hyphomonas polymorpha* PS728^T (Gebers et al., in press) (Table 2).

Strains cannot be differentiated on the basis of morphological, serological, nutritional, or biochemical tests.

Type strain: strain VP2 (= ATCC 33883).

***Hyphomonas hirschiana* sp. nov.** *Hyphomonas hirschiana* (hir' schi. an. a. L. fem. adj. *hirschiana* named for Peter Hirsch for his contributions to the study of prosthecate, budding bacteria) cellular morphology is like that of *Hyphomonas polymorpha*. Colonies are small (1.0 mm in diameter after 48 h), dull grey, dry, and circular to irregular, with dimpled elevations and lobate margins. Growth in broth produces a pellicle at the surface and a light film of growth on the sides and bottom of the culture vessel.

A mixture of L-glutamic acid, L-aspartic acid, L-serine, and L-methionine is required for growth. The temperature range for optimum growth is 25 to 31°C. The NaCl concentration for growth is 2.0 to 15.0%. The optimum pH is 7.6.

Nitrate is reduced. Sheep erythrocytes are not hemolyzed. Pigment is not formed. Susceptible to 0.01% NP-40, novobiocin, and ampicillin. Resistant to 1.0% Tween 80. Intermediate susceptibility to penicillin and streptomycin. Treatment for 9 min or more with a Brownwill Biosonic IV sonicator (low probe at full power) is required to produce 99% lysis of cells. Other nutritional and biochemical characteristics are shown in Table 1.

Hyphomonas hirschiana possesses some surface antigens in common with other *Hyphomonas* species but no DNA homology to *Hyphomonas polymorpha* or *Hyphomonas neptunium* in solution with endonuclease treatment (Gebers et al., in press).

The type strain was isolated in 1979 from shellfish beds

near hydrothermal vents on the floor of the mid-Pacific Ocean by H. Jannasch.

The G+C content of the DNA is 57 mol%.

Type strain: strain VP5 (= ATCC 33886).

Our observations concerning both nitrate and nitrite reduction and indole production from tryptophan (Table 1) by *Hyphomonas polymorpha* disagree with the original findings of Pongratz (17). It is unclear at the present time whether the loss of these properties can be attributed to the 27 years of laboratory storage and culture transfers which have occurred since the original description.

The serological relationships among the strains of *Hyphomonas* are shown in Table 3. As in a previous study, no cross-reactions were observed between the type strains of the two previously described species of *Hyphomonas* (18). Strains VP1, VP2^T, VP3, and VP4 also failed to cross-react with these two species, but showed high degrees of similarity among themselves. Strains VP5^T and SCH89^T exhibited low levels of cross-reaction with each other, with the two previously described species, and with some of the following strains: strains VP1, VP2^T, VP3, and VP4.

Although it was isolated 3,000 miles (4827 km) away and 10 years later than *Hyphomonas neptunium* strain LE670^T, strain H13 was identical to strain LE670^T in every characteristic examined in this study except its amino acid requirements (strain H13 required histidine, proline, and arginine in addition to glutamic acid, aspartic acid, serine, and methionine) and its serological cross-reactivity. Strain H13 was also identical to strain LE670^T in DNA homology studies (Gebers et al., in press). We conclude that strain H13 is a second strain of *Hyphomonas neptunium*.

In every character examined, strain VP1 was identical to strain VP3, and strain VP2^T was identical to strain VP4. The closeness, but nonidentity, of these two pairs of strains found in this present study is supported by DNA homology results (Gebers et al., in press). The levels of homology of their DNAs with the DNA of *Hyphomonas polymorpha* PS728^T were 29% (strain VP1), 8% (strain VP2^T), 36% (strain VP3), and 10% (strain VP4) (Gebers et al., in press) (Table 2). However, these two pairs of strains are not sufficiently different to warrant placing them into separate taxa (and we consider them as two isolates, VP1/3 and VP2/4, of the same species).

Strains SCH89^T and VP5^T each represent unique taxa at the species level. The inability to discern DNA homology between strain VP5^T and the two previously described species should not preclude placement of strain VP5^T in the genus *Hyphomonas*, since the stringent hybridization conditions used (Gebers et al., in press) do not rule out low levels of relatedness. The levels of relatedness among the new strains were not determined and also remain to be assessed by ribonucleic acid homology and 5S RNA sequences.

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