A New Filamentous, Gliding Bacterium, *Filibacter limicola* gen. nov. sp. nov., from Lake Sediment

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A new, Gram-negative, multicellular, filamentous, gliding bacterium is described. The organism was isolated from the sediment of a eutrophic lake on a dilute peptone medium. The growth habit on solid media was characterized by spreading whorls of growth and spiral colonies. Filaments, 8 to 150 µm long, were composed of cylindrical cells 1.1 µm wide by 3 to 30 µm long. Junctions between individual cells within a filament were marked by constrictions. The organism was not pigmented, and therefore resembled members of the genus *Vitreoscilla*. Comparison with two *Vitreoscilla* strains showed important differences in cytochrome composition, DNA base composition, isoprenoid quinone content, and sensitivity to actinomycin D, which indicated that the isolate was more closely related to the *Flexibacteriaceae* than to *Vitreoscilla* spp. The organism did not resemble any previously described taxon of *Flexibacteriaceae*. On the basis of differences from both *Vitreoscilla* spp. and flexibacteria it is proposed that the organism be placed in a new genus, *Filibacter*, with the type species named as *Filibacter limicola* sp. nov., from its origin in sediment. The type strain of *F. limicola* is 1SS101 (NCIB 11923).

The organism was a strict aerobe capable of growth on defined mixtures of amino acids, and had a requirement for vitamins. Only amino acids served as substrates. The organism required particular combinations of amino acids for growth. No single amino acid or mixture of amino acids from a single biosynthetic family supported growth.

INTRODUCTION

A wide variety of filamentous bacteria are found in freshwater sediments. Being large and distinctive in morphology, they are readily observed and recognized in material from these environments. However, only recently has a methodical ecological survey of the qualitative and quantitative distribution of filamentous bacteria in relation to environmental factors been made (Godinho-Orlandi & Jones, 1981 *a, b*). Also, only a few of these organisms have been isolated and studied, and many are known only by morphological descriptions. Thus, although it is now recognized that filamentous organisms are much more important in terms of biomass than is indicated by their low numbers (Jørgensen, 1977; Godinho-Orlandi & Jones, 1981 *a*), very little is known of their physiological activities and contributions to nutrient cycling processes in sediments. One notable exception to this is *Beggiatoa*. Populations of this organism have been shown to have the potential to oxidize all the sulphides produced in the marine sediments in which they occur (Jørgensen, 1977).

Common among the filamentous bacteria observed in sediment are multicellular gliding organisms which resemble the genus *Vitreoscilla*. Pringsheim (1949, 1951) proposed the family *Vitreoscillaceae* for a group of colourless, multicellular, gliding filamentous bacteria. The genus *Vitreoscilla* was described, with a number of species isolated from dung and ditch mud, and differentiated by morphological features. Only one of these species, *V. stercoraria*, is still available for study (strain ATCC 15218). The only other cultures of *Vitreoscilla* spp. available

are two strains of Costerton *et al.* (1961) (strains ATCC 13981 and 13982). There has been no study of the genus *Vitreoscilla* since that of Pringsheim (1951), and therefore the taxonomic standing of the species described, and the relationship of the genus to other filamentous bacteria, are uncertain. The other genus of the *Vitreoscillaceae* of Pringsheim (1951), *Microscilla*, is now considered to be a member of the *Cytophaga/Flexibacter* group (Lewin, 1969). The only recently published work on vitreoscillas has dealt with the ultrastructure of two strains (Costerton *et al.*, 1961), the physiology of one named strain (*V. stercoraria*; Mayfield & Kester, 1972, 1975), and the cytochrome system of one strain (Webster & Hackett, 1966*a, b*). The occurrence of plasmid DNA in some of these organisms has been reported recently by Minges *et al.* (1983). A recent review of the *Beggiatoaceae* (Wiessner, 1981) mentioned *Vitreoscilla* only briefly, reflecting the complete lack of taxonomic information on the genus. In contrast, members of the family *Flexibacteriaceae*, encompassing the major genera *Cytophaga* and *Flexibacter*, have been well studied (Lewin & Lounsbery, 1969; Soriano, 1973; Christensen, 1977; Reichenbach & Dworkin, 1981). These organisms are also found in lake sediments, and are characterized by unicellular filaments which flex actively. Macroscopic growth is brightly coloured due to the presence of carotenoid and flexirubin-type pigments.

Filamentous bacteria as a group encompass organisms with a variety of physiologies. Most are heterotrophic, utilizing a wide range of organic substrates. *Leptothrix* and *Sphaerotilus* species can use a large number of simple compounds as carbon and energy sources (van Veen *et al.*, 1978), whereas *Cytophaga* spp. are able to degrade polymeric substrates such as cellulose or chitin. *Beggiatoa* utilizes acetate and a small number of other simple compounds (Pringsheim, 1964; Nelson & Castenholz, 1981), and although it is not autotrophic as originally thought, some strains may be mixotrophic (Güde *et al.*, 1981), obtaining some of their energy from the oxidation of sulphides. There is evidence that some strains can assimilate carbon dioxide (Strohl *et al.*, 1979, 1981). Apart from *Beggiatoa* and the filamentous cyanobacteria, there is no evidence for autotrophy or mixotrophy in other filamentous bacteria. Most are strict aerobes with respiratory metabolisms, but some are facultative anaerobes with fermentative and respiratory metabolisms. *Desulfonema* (Pfennig *et al.*, 1981) is an anaerobic, sulphate-reducing, gliding filamentous bacterium. It has been reported to use acetate, higher fatty acids, succinate or fumarate as electron donors and carbon sources (Widdel *et al.*, 1983). *Capnocytophaga* species are strict anaerobes with solely fermentative metabolisms (Reichenbach & Dworkin, 1981).

Some filamentous bacteria may be specialized in the utilization of a small range of substrates. Examples are *Microthrix parvicella* which utilizes only certain fatty acids (Slijkhuys & Deinema, 1982), and *Vitreoscilla stercoraria* which Mayfield & Kester (1972, 1975) reported could use only certain mixtures of amino acids as carbon and energy sources.

A study of filamentous bacteria in sediments (Maiden, 1983) resulted in the isolation of the organism (isolate ISS101) described in this paper. It resembled members of the genus *Vitreoscilla* and was compared with two available strains of *Vitreoscilla* spp. The physiology of the isolate was studied, particularly its carbon metabolism.

METHODS

Source and treatment of sediment. Sediment from Blelham Tarn, the lake in the English Lake District where the greatest numbers and variety of filamentous bacteria had been observed (Godinho-Orlandi & Jones, 1981*a*), was sampled using a Jenkin surface-mud sampler (Ohnstad & Jones, 1982). Profundal (deep water) cores were taken at 13–14 m depth, and the top 1–2 cm layer of sediment was extruded and removed (Ohnstad & Jones, 1982). This sediment was diluted as required in filtered lake water, mixed vigorously with a vortex mixer, and used for the inoculation of isolation media. All inocula were checked microscopically for the presence of filamentous bacteria.

Microscopy. Routine microscopy of sediment and cultures was done on a Leitz Dialux microscope fitted with the interference contrast device T.

Media. TSB was Tryptone soya broth (Oxoid CM129), TSA was Tryptone soya agar (Oxoid CM131), NB was Nutrient broth (Oxoid CM1), and NA was Nutrient agar (Oxoid CM3). Isolation media were based on mineral base M which was the Artificial Esthwaite medium of Jones (1983). The composition was (mg l⁻¹): K₂HPO₄, 28; MgCl₂·6H₂O, 127; KNO₃, 4; (NH₄)₂SO₄, 60; MnSO₄·4H₂O, 8; ferric citrate, 6. The pH of the medium was adjusted with 1 M-KHCO₃ before autoclaving, and the final pH was 7.0–7.2. The media contained yeast extract (YE; Difco, 10 mg l⁻¹) and peptone (Oxoid L37, 100 mg l⁻¹) as follows: MY medium, YE only; MP medium,

peptone only; MYP medium, YE plus peptone. For plates, the media were solidified with 1% (w/v) agar (Oxoid L28).

For defined media, the mineral base used was a modification of the medium L of Leadbetter & Foster (1958). Modified medium L (ML medium) had no NaH_2PO_4 , an increased concentration of Na_2HPO_4 and NaNO_3 replaced with NH_4NO_3 . The composition was as follows (mg l^{-1}): Na_2HPO_4 , 767; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 200; KCl, 40; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 15; NH_4NO_3 , 2000; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.1. For trace metals the solution of Patel *et al.* (1978) was used. The medium was sterilized by autoclaving at 121 °C for 15 min. After autoclaving, ML medium was slightly cloudy but cleared on cooling. The final pH was 7.4. The vitamin solution used was that of Wolin *et al.* (1963). The 19 amino acids were grouped in families based on common biosynthetic pathways (Dawes & Large, 1973) as follows. Glutamate family, glutamate, glutamine, arginine and proline; aspartate family, aspartate, asparagine, lysine, threonine, isoleucine and methionine; aromatic family, tryptophan, phenylalanine and tyrosine; serine family, serine, glycine and cysteine; pyruvate family, alanine, valine and leucine. Histidine, citrulline and ornithine were also used individually. All (except glycine) were L-amino acids (Sigma). Each amino acid was provided at a final concentration of 2 mg atom amino acid carbon l^{-1} , except tyrosine (0.5 mg atom l^{-1}). The complete amino acid mixture contained the 19 amino acids of the five families.

Incubation. Unless otherwise stated, all incubations were at 20 °C, shaken when required in an orbital incubator at 120 r.p.m.

Isolation and purification of cultures. Sediment samples (10 µl) were streaked on the various isolation media and incubated at 10 °C and 20 °C. Plates were examined by eye, and through a 32× long working distance objective. Any filaments developing away from contaminants were subcultured by excizing the surrounding agar as a block, and transferring this to a plate of fresh medium. This was repeated until pure cultures were obtained. Purity was checked on TSA and NA plates, and the cultures were maintained on TSA slopes incubated at 20 °C, and stored at 10 °C.

Culture of *Vitreoscilla* strains. The *Vitreoscilla* strains for taxonomic comparison were *Vitreoscilla stercoraria* (ATCC 15218) and *Vitreoscilla* sp. strain 390 (Costerton *et al.*, 1961). Cultures were maintained on TSA slopes.

Preparation of standard inocula. Exponential phase cultures were prepared from 24 h, 20 °C static broth cultures, and incubated for 20–24 h at 20 °C in an orbital incubator (120 r.p.m.). Defined media and commercial test systems were inoculated with washed cell suspensions. The cells were harvested by centrifugation (1800 g, 10 min), washed twice, and resuspended in sterile mineral medium or buffer.

Antibiotic sensitivity. Samples (0.1 ml) of a 24 h broth culture of the test organism were spread on TSA and NA plates. The antibiotics and inhibitors, and the quantity per disc (µg), used were; chloramphenicol, tetracycline, streptomycin, kanamycin, neomycin, cycloheximide and penicillin G (0.1, 1, 10, 100, 1000); 2,4-dinitrophenol and rotenone (0.1, 1, 10, 100); and actinomycin D (1, 10, 40). The plates were examined after incubation at 20 °C for 2 d and zones of inhibition recorded.

Morphology and cytology. Agar-coated slide mounts were used to examine the gross morphology of filaments and cells. A drop of cell suspension (15 µl) was added to agar (25 µl; 1%, w/v) spread evenly on a clean glass slide, and sealed under a coverslip with Vaseline. Cells and filaments of the isolated strain were also examined by transmission electron microscopy. Drops of a 24 h broth culture were placed onto carbon stabilized Formvar films on 300 mesh copper grids. After 1 h excess liquid was removed and the specimen shadowed with gold/palladium. The electron microscope used was a Corinth 500 (AEI) and the grids were examined with an accelerating voltage of 60 kV. Electron micrographs were taken using Agva F071P Rapidoline Ortho film, with an exposure time of 2 s. The colonial morphology/growth habits of strains on solid media were observed, and wet mounts were examined for evidence of motility. The sodium chloride/Tyler's crystal violet method (Conn & Jennison, 1957) and negative stains using nigrosin (1%, w/v) or drawing ink were used to detect sheaths. Negative stains, the Alcian Blue stain and Hiss's capsule stain (Doetsch, 1981) were used to detect capsules or slime. To detect flagella the stains of Leifson (1951) and Mayfield & Inniss (1977) were used. All other stains used were as described by Norris & Swain (1971) and Doetsch (1981).

Cytochrome spectrophotometry. For each strain, 2 × 250 ml TSB cultures in 1 litre Erlenmeyer flasks were grown at 20 °C, shaken, for 60 h. The cells were harvested (1600 g, 10 °C), washed twice in 0.1 M-phosphate buffer, pH 7.4, and resuspended in buffer. Samples (10 ml) were sonicated with an exponential probe (tip amplitude 84 µm) for a total of 5 min in 30 s bursts with 15 s rest periods. Sonicate (0.6 ml) was further clarified by mixing with sodium deoxycholate (0.03 g in 2.4 ml phosphate buffer). The samples were then placed in an LR ultrasonic bath for 3 min and remixed. Treated sonicates were diluted 10^{-1} and spectra were recorded using a Pye-Unicam SP8-100 UV spectrophotometer. Absolute spectra were recorded with potassium ferricyanide (30 mmol l^{-1}) as oxidizing agent and sodium dithionite (200 mmol l^{-1}) as reducing agent. Dithionite-reduced minus ferricyanide-oxidized difference spectra were also recorded.

Isoprenoid quinones. Freeze-dried samples of isolate ISS101 and *Vitreoscilla stercoraria* were analysed for major isoprenologs as described by Collins & Jones (1981).

Determination of T_m of DNA. Harvested cells were washed twice in saline/EDTA (0.15 M-NaCl, 0.1 M-EDTA, pH 8.0). For extraction of DNA, 2–3 g (wet weight) of cell slurries were treated by the method of Marmur (1961),

as far as the first precipitation. The DNA was purified by the method of Gibson & Ogden (1979), using column chromatography with Sepharose 4B. The spectra (230–300 nm) of the purified DNAs were measured to check their purity. The melting temperature (T_m) of each purified DNA sample was determined using a UV-visible microprocessor controlled Spectrophotometer System 2600 with temperature programmer (Gilford Instruments Laboratories Inc., Corning Glass Works, Corning, NY, USA).

Physiology. The oxygen requirements of the strains were determined using streak plates incubated in air, at low oxygen tension, and anaerobically (BBL Gas Pak system, $H_2 + CO_2$). Agar shake cultures in solid and semi-solid agar deeps (TSB + agar at 0.2, 0.5 and 1.0%, w/v) were also used. Some tubes of each agar concentration were sealed with plugs of 1% (w/v) agar. Optimum growth temperatures were determined from the growth curves of cultures (25 ml TSB in 250 ml Erlenmeyer flasks) incubated at 4, 10, 15, 20, 25, 30, 35 and 40 °C. Casein hydrolysis was tested on TSA plus 1% (w/v) casein. The plates were developed by flooding with 0.1 M-HCl. Gelatin hydrolysis was tested on TSB solidified with agar (Oxoid L28, 0.5%, w/v) plus gelatin (Oxoid L8, 0.5%, w/v), and developed with tannic acid (1%, w/v). Starch hydrolysis was tested on TSA plus 0.2% (w/v) starch, and developed with Gram's iodine. Growth on amino acids only was tested on ML medium plus the complete amino acid mixture and vitamins. Details of tests for the utilization of various carbon sources and amino acid combinations by the isolated strain are given below. Strains were tested for catalase, and for oxidase reaction by the method of Kovacs (1956). A range of biochemical tests were performed using the API 20B commercial identification system. Strips were inoculated with washed cell suspensions, and incubated at 20 °C for 3 d. The organisms were also tested by the API ZYM system which provided assays for 19 enzymes. Test strips were inoculated with dense washed cell suspensions, and incubated at 25 °C for 5 h. The strains were tested for the oxidation of sulphide and the deposition of intracellular sulphur granules. Sodium sulphide solution (10 μ l, approximately 125 mM) plus cell suspension (10 μ l) were mixed on a glass slide, and sealed under a coverslip with Vaseline. Cells were examined for the presence of intracellular sulphur granules immediately and after 0.5 h. Cultures in TSB (5 ml plus 0.1 ml sulphide solution as above) were also examined for sulphur granules after incubation at 20 °C for 2 d.

Measurement of optical density and dry weight. The OD of cultures was measured at 540 nm with a Cecil CE 292 spectrophotometer (Cambridge, UK). When necessary, samples were diluted to give an OD of less than 0.4. OD was calibrated against dry weight (80 °C, 24 h).

Nephelometry. Cell densities in test-tube cultures were determined using an EEL nephelometer head and Unigalv galvanometer. This method was used to test the growth responses of isolate 1SS101 to single amino acids, mixtures of amino acids from a single family, and omissions of single families or pairs of families from the complete mixture. Some of the carbon sources (without amino acids added) were also screened by this method.

Growth yield experiments. These were used to determine the response to pairs and triplets of amino acid families, and to other carbon sources. Cultures were incubated, shaken, in 250 ml Erlenmeyer flasks. Vitamins, amino acids and carbon sources were added as required to ML medium to a total volume of 75 ml. Positive controls contained the complete amino acid mixture, and negative controls contained ML medium plus vitamins, inoculated, but with no amino acids added. To test for the requirement for vitamins, a positive control without vitamin solution added was used.

The other potential carbon sources tested were: glucose, fructose, sucrose, lactose, galactose, mannitol, glucosamine, galacturonic acid, acetate, pyruvate, lactate, citrate, malate, glutamate, butyrate, ethanol, glycerol urea and bovine serum albumin (BSA), at concentrations equivalent to 20 mg atom carbon l^{-1} (organic acids added as sodium salts). All the compounds apart from sucrose, glutamate, urea and BSA were tested in the presence of the complete amino acid mixture at 2 mg atom carbon l^{-1} and 40 mg atom carbon l^{-1} , with amino acids at 0.37 mg atom carbon l^{-1} and 36.5 mg atom carbon l^{-1} , and at 20 mg atom carbon l^{-1} , with amino acids at 3.65 mg atom carbon l^{-1} and 18.25 mg atom carbon l^{-1} . The final pH of all cultures was measured. To enable direct comparison of the numerous amino acid family combinations, and of substrates with and without amino acids, single flasks were used for each combination. Positive controls were always in duplicate, and indicated good reproducibility between replicate flasks.

Auxanography. Plates of solidified ML plus vitamins medium, containing various combinations of amino acid families, were seeded with washed cell suspension, either by spreading, or in a semi-solid overlay. Crystals of individual amino acids were spotted onto the plates with sterile wooden applicator sticks. For testing each family, the basal media used contained complementary families which would support growth in combination with the added family (determined from results of amino acid family utilization experiments). For each plate, all the individual amino acids of the family being tested were applied in spot patterns designed to cater for multiple requirements or to show any synergistic effects.

RESULTS

Isolation

After 14 d incubation, microscopical examination of isolation plates showed the presence of filaments, singly and in small groups, often in circular formations, at some distance from the

Table 1. *Morphological and cytochemical data for isolate ISS101 and the two Vitreoscilla strains*

Characters in common: Gram-negative, non-branching, non-sheathed, non-pigmented, multicellular filaments with clearly constricted cell junctions.

Morphological/cytochemical character	Isolate ISS101	<i>V. stercoraria</i>	<i>Vitreoscilla</i> sp. strain 390
Filament dimensions (width × length)	1.1 × 8–150 µm	1.1–1.2 × 10–200 µm	1.1 × 10–70 µm
Cell dimensions (width × length)	1.1 × 3–30 µm	1.1–1.2 × 1.2–5 µm	1.1 × 1.1–3 µm
Cell shape	Long rods, straight or curved, rounded ends	Short rods, rounded ends	Short rods, rounded ends
Colonial morphology	Spreading whorls and spiral colonies	Spreading whorls and spiral colonies	Discrete, non-spreading colonies
Motility	Gliding	Gliding	—
Capsule	—	—	—
Slime	+	+	+
Endospores	—	—	—
PHB granules	+	—	+
Volutin granules	—	+	±†

* Unstructured, extracellular material staining with carbol fuchsin.

† Not all cells in smear contained stained inclusions.

streak lines. Such filaments were observed on plates of all three media and occurred most abundantly on MYP plates at 10 °C. Filaments were subcultured, and of the strains, all of which appeared identical, only one, coded ISS101, was obtained in pure culture. The organism grew well on complex peptone-containing media such as NA and TSA.

Characterization

Isolate ISS101 was not sensitive to cycloheximide and rotenone, and was inhibited by the other agents, confirming its prokaryotic nature. It was much more sensitive to actinomycin D than were the *Vitreoscilla* strains.

The morphological and cytochemical characteristics of the three strains are given in Table 1. All three strains produced long filaments, those of ISS101 being shorter than those of *V. stercoraria* (Fig. 1). In all cases the widths of cells were constant. The filaments of all the strains were multicellular, with the junctions between cells clearly marked by constriction. This was particularly so with the *Vitreoscilla* strains. With ISS101, the longer filaments were composed of longer cells, though in the longest cells, septa without corresponding constrictions could sometimes be seen. Cell length was not constant within single filaments. The cell lengths of the *Vitreoscilla* strains were one, two or at the most, three diameters. The growth habits on solid media of isolate ISS101 and *V. stercoraria* were similar and very distinctive. Both spread widely over the surface of agar plates producing whorls of growth and spiral colonies (Fig. 2*a, c*). On some occasions, at 20 °C ISS101 grew predominantly as spiral colonies, as shown in Fig. 2(*b*). However, this behaviour was not consistent, and was probably due to the degree of dryness of the agar surface. Strain 390 formed discrete, non-spreading colonies. Cell masses of the three strains were not pigmented. Often cell aggregates of ISS101, particularly the spiral colonies, were very refractile (Fig. 2*b*).

The distinctive growth habit of ISS101, with cells spreading far from the point of inoculum, resembled that of *V. stercoraria* and suggested gliding motility. On observation of plates with a long working distance objective, some filaments could be seen to move slowly (up to 5 µm min⁻¹). The non-spreading habit of *Vitreoscilla* strain 390 suggested that it was not actively motile. The culture was therefore considered to be aberrant as this strain should glide (Costerton *et al.*, 1961). However, in all other morphological details, and biochemical tests, it was virtually identical to *V. stercoraria* and can therefore be considered a genuine *Vitreoscilla* strain. In wet mounts, ISS101 was usually immotile, but cells often showed small vibrating

Fig. 1. Comparison of gross morphologies of isolate ISS101 and the two *Vitreoscilla* strains. (a) Isolate ISS101, (b) *V. stercoraria*, (c) *Vitreoscilla* sp. strain 390. Agar-coated slide mounts; bars, 10 μ m.

Fig. 2. Colonial morphologies/growth habits of isolate ISS101 and *Vitreoscilla stercoraria*. Isolate ISS101 on TSA incubated at (a) 10 °C, (b) 20 °C, and (c) *V. stercoraria* on TSA incubated at 20 °C.

Fig. 3. Transmission electron micrographs of unfixed cells of isolate 1SS101 showing (a) fibrils associated with the cell and (b) fibrillar material sloughing off a capsule-like covering of the cell. Bars, 1 μ m.

Table 2. *Physiological characters of isolate 1SS101 and the two Vitreoscilla strains*

Physiological character	Isolate 1SS101	<i>V. stercoraria</i>	<i>Vitreoscilla</i> sp. strain 390
	Strict aerobe	Strict aerobe	Strict aerobe
Relation to oxygen			
Temperature response (test-tube cultures):			
growth at 4 °C	+	—	—
growth at 30 °C	—	+	+
survival at 4 °C for 8 d	+	—	+
Optimum temperature (°C)	20	25–30	25–30
Hydrolysis of:			
casein	—	—	—
gelatin	+	—	—
starch	—	—	—
Growth on amino acids plus vitamins only	+	+	+
Utilization of other organic compounds	—*	—†	NT‡
Enzymes:			
catalase	+	+	+
oxidase	+	—	—
Oxidation of sulphide	—	—	—

* See utilization tests.

† Mayfield & Kester (1972).

‡ NT, Not tested.

movements which did not seem to be due to Brownian motion. Occasionally, cells 10–20 µm long were seen to rotate slowly through 360°, with one end appearing to be attached to the glass. Very rarely, gliding filaments were observed. Filaments of *V. stercoraria* were never seen to glide in wet mounts. However, when in groups they waved about jerkily, bending at cell junctions. *Vitreoscilla* strain 390 showed similar movements, though not so vigorous. Media low in organic nutrients have been used to encourage gliding motility, but when plated onto NA with the nutrients at one-tenth the normal strength, strain 390 did not spread.

No sheaths or capsules were observed in any of the strains. Heat-fixed smears stained with carbol fuchsin showed the presence of stained material associated with, and between cells. Cell masses grown on agar media were sticky, suggesting that the organisms produced slime or mucilage. Electron micrographs of unfixed cells and filaments of 1SS101 showed many to be covered with fibrillar material which often appeared as a capsule-like layer around the cells (Fig. 3), with the fibrils appearing to be peeling off from the structure. Some cells had very few fibrils, and cells fixed with glutaraldehyde (3% w/v) showed no sign of such material. None of the strains produced spores, nor were flagella observed. All three strains were Gram-negative. If safranin was used as counter stain, the two *Vitreoscilla* strains showed a poorly stained region at the centre of the their cells, whereas isolate 1SS101 stained evenly. Isolate 1SS101 and strain 390 contained one particle of Sudan Black-stained material per cell, but *V. stercoraria* contained none. Isolate 1SS101 was poly-β-hydroxybutyrate (PHB) negative when tested by the method of Oste & Holt (1982). Isolate 1SS101 was volutin negative, *V. stercoraria* was positive (one particle per cell), and strain 390 was positive, though not all cells in the smears had stained particles.

The physiological characteristics of the strains are summarized in Table 2. All were strict aerobes, growing well at the medium surface in shake cultures, with some growth to 2 mm depth. Isolate 1SS101 grew to a slightly greater depth (3–4 mm). In tubes with an agar plug, 1SS101 showed very slight growth at the surface of the semi-solid agar, but the other two strains did not. It is possible that 1SS101 may be able to grow at lower oxygen concentrations than the *Vitreoscilla* strains. Qualitative assessment of the growth of isolate 1SS101 suggested that it was more tolerant of low temperatures, and more sensitive to higher temperatures than the *Vitreoscilla* strains. This was confirmed by the temperature response curves of the three strains; isolate 1SS101 had an optimum temperature in the region of 20 °C, and responded better to low temperatures than the other strains which had optimum temperatures around 25–30 °C.

None of the strains produced zones of clearing on media containing casein or starch. Only isolate 1SS101 gave a narrow clear zone (1–2 mm wide) on TSA plus gelatin. All three strains

Table 3. *Spectral data for cytochromes from various bacterial sources*

Cytochrome/source	Wavelengths of major peaks in reduced, room temperature spectra (nm)		
	Alpha	Beta	Soret
Isolate 1SS101*	552	522	417
<i>V. stercoraria</i> *	559	—	—
<i>Vitreoscilla</i> sp. strain 390*	558.5	—	—
<i>Vitreoscilla</i> sp.†	560	—	427
Cytochrome <i>c</i> , various sources‡	550	521	415
Cytochrome <i>c</i> ₄ , <i>Azotobacter</i> ‡	551	522	416
Cytochrome <i>b</i> ₁ , bacteria‡	560	530	426

—, Not determined.

* Experimental data.

† Published data: Webster & Hackett (1966*a*).

‡ Published data: Neilands (1958).

grew on a medium which contained purified amino acids as the only substrates. Isolate 1SS101, like *V. stercoraria* (Mayfield & Kester, 1972) failed to grow on any of a range of common substrates (see below). All strains were catalase positive, isolate 1SS101 was oxidase positive, but the two *Vitreoscilla* strains were oxidase negative. None of the strains deposited intracellular sulphur globules in the presence of sulphide, either in wet mounts or in broth cultures.

When tested by the API 20B system the three strains gave mainly negative results. Isolate 1SS101 was urease and oxidase positive, the two *Vitreoscilla* strains were urease and oxidase negative. All three strains were catalase positive. The lack of response to the other tests can be attributed to lack of growth due to the absence of amino acids in the test medium. When tested by API ZYM, the *Vitreoscilla* strains were virtually identical in their responses. They both gave a strong reaction for esterase lipase (isolate 1SS101 did not), and they were negative for chymotrypsin whereas isolate 1SS101 gave a strong reaction. All three strains were positive for phosphoamidase, and negative for all the sugar metabolism enzymes. This agrees with previous observations of the nutritional indifference of these organisms to carbohydrates.

The oxidase test is commonly used to test for the presence of cytochrome *c* in the respiratory chain. The work of Webster & Hackett (1966*a, b*) has shown that *Vitreoscilla* strains possess *b*-type cytochromes as major components of their respiratory chain, and lack *c*-type cytochromes. The *Vitreoscilla* strains were oxidase negative, whereas isolate 1SS101 was oxidase positive. The cytochrome spectra were determined to further compare the respiratory chain compositions of the three strains. The absolute spectra for isolate 1SS101 showed a Soret peak at 409 nm in the oxidized spectrum, which increased and shifted to about 417 nm when reduced. The absolute reduced spectrum also showed the characteristic cytochrome beta and alpha peaks at approximately 520 nm and 550 nm, respectively, indicating the presence of *c*-type cytochromes. In reduced minus oxidized difference spectra of the alpha/beta bands of the three strains, the two *Vitreoscilla* strains both had alpha peaks at about 559 nm, characteristic of *b*-type cytochromes, and isolate 1SS101 had an alpha peak at 552 nm and a beta peak at 522 nm, characteristic of *c*-type cytochromes. In isolate 1SS101, small shoulders in the alpha and beta peaks (at about 565 nm and 530 nm, respectively) indicated the presence of lesser amounts of *b*-type cytochromes. The spectral properties of the cytochromes of the three strains are summarized in Table 3, together with published data for various cytochromes for comparison.

Analyses for isoprenoid quinones showed the major isoprenolog of *Vitreoscilla stercoraria* to be ubiquinone (Q-8), and of isolate 1SS101, menaquinone (MK-7). The results of the mol % G + C determinations are given in Table 4. The values for the two *Vitreoscilla* strains were almost identical, but that for 1SS101 was 6% lower.

Utilization of carbon sources

Isolate 1SS101 grew well on TSB, following a normal growth curve, with a maximum growth rate equivalent to a generation time of 6.6 h. It also grew with Casamino acids or the complete amino acid mixture, plus vitamins. In the absence of vitamins there was no growth.

Table 4. DNA base ratios of isolate ISS101 and the two *Vitreoscilla* strains

Organism	DNA melting temperature, T_m (°C)*	DNA base ratio (mol % G + C)†
Isolate ISS101	87.45	44.3 (± 0.1)
<i>Vitreoscilla stercoraria</i>	90.0	50.5
<i>Vitreoscilla</i> sp. strain 390	89.55	49.4 (± 0.1)

* Means of two determinations.

† Mol % G + C = $2.44 (T_m - 69.3)$.

None of the organic compounds tested supported growth of strain ISS101 in the absence of amino acids. There was no growth on BSA. In medium containing urea, the pH of the culture rose to over 9 after 12 h incubation, indicating an active urease. The potential carbon sources were also tested in the presence of amino acids at both substrate and growth factor level. Molar growth yields were calculated in terms of yield per mole of carbon (Y_c) of amino acids, added substrate, and amino acid plus substrate (enabling comparison of mixtures of substrates), and the more conventional Y_s (yield per mole of substrate). Many of the compounds tested were inhibitory, causing decreased yields, or in some cases, preventing any growth. No changes in pH which might account for these effects were detected. The only compounds which produced significantly enhanced yields were acetate, lactate, butyrate and glycerol, at 2 mg atom carbon l^{-1} with amino acids at 36.5 mg atom carbon l^{-1} . The Y_s values for these compounds (17.1, 17.9, 30.8 and 12.8 g mol^{-1} , respectively) were of the same order as, but lower than, published values for a variety of other organisms (see Payne, 1970). In all cases, stimulation of growth decreased at higher substrate concentrations and lower amino acid concentrations. This was particularly so with butyrate.

Isolate ISS101 grew well on the complete amino acid mixture as sole carbon and energy source. The relationship between yield and amino acid concentration was linear over the range 20–70 mg atom carbon l^{-1} , but was not linear at very low concentrations. This was probably due to growth limitation by certain required amino acids. No single amino acid alone supported the growth of isolate ISS101, neither did any of the mixtures of amino acids from single families. Mixtures of amino acid families, omitting each family in turn, were used to determine whether any one family was essential for the growth of isolate ISS101. The results indicated that histidine was inhibitory, and that no single family (or single amino acid) was essential for growth. The aromatic family was also slightly inhibitory. Omission of the glutamate or aspartate families reduced the growth response, but not to the same extent as the omission of the serine or pyruvate families. However, the maximum growth rates and final yields were not greatly different. For further investigation of amino acid requirements, pairs of families were omitted. Absence of the glutamate or aspartate families decreased the yield, and omission of the serine or pyruvate families often produced long lag phases. In the absence of both the aspartate and serine families there was no growth.

To determine which families of amino acids could support the growth of isolate ISS101, media containing pairs and triplets of families were used. The molar growth yields on pairs of families are given in Table 5. The only combinations which supported growth were the glutamate family plus the aspartate or serine families, and the aspartate family plus the pyruvate, serine or aromatic families. Best growth was obtained with the aspartate plus pyruvate families. The aspartate plus serine families gave a similar yield and growth rate, but with a much longer lag. After the aspartate plus pyruvate families, the next best growth was with the glutamate plus serine families, with a similar lag, but slightly lower yield and growth rate. The glutamate plus aspartate families gave a reasonable yield, but after a fairly long lag, and with a low growth rate. There was very slow growth with the aspartate plus aromatic families after a long lag. The aspartate plus pyruvate families gave the highest growth rate, but the glutamate plus serine families gave the highest molar growth yield. When the aspartate family was present, the absence of the pyruvate family again produced long lags as seen in the double omission experiments. The requirement for the aspartate or serine families for growth with the glutamate

Table 5. *Growth data of isolate ISS101 on pairs and triplets of amino acid families*

Amino acid family added*	Lag phase (h)	Growth rate constant, μ (h^{-1})	Molar growth yield, Y_c [$\text{g (mol carbon)}^{-1}$]
All five (positive control)	<5	0.107	8.37
Glutamate + aspartate	94	0.012	3.14
Glutamate + serine	25	0.026	4.77
Glutamate + pyruvate	—	No growth	0.00
Glutamate + aromatic	—	No growth	0.00
Aspartate + serine	115	0.035	4.00
Aspartate + pyruvate	27	0.041	4.21
Aspartate + aromatic	160	0.007	1.46
Serine + pyruvate	—	No growth	0.00
Serine + aromatic	—	No growth	0.00
Pyruvate + aromatic	—	No growth	0.00
Glutamate + aspartate + serine	175	0.055	5.79
Glutamate + aspartate + pyruvate	<5	0.024	5.46
Glutamate + aspartate + aromatic	102	0.022	2.43
Glutamate + serine + pyruvate	22	0.055	7.23
Glutamate + serine + aromatic	42	0.028	4.04
Glutamate + pyruvate + aromatic	—	No growth	0.00
Aspartate + serine + pyruvate	30	0.058	5.38
Aspartate + serine + aromatic	80	0.041	3.38
Aspartate + pyruvate + aromatic	19	0.032	3.74
Serine + pyruvate + aromatic	—	No growth	0.00

* Each amino acid was added at a concentration equivalent to 2 mg atom carbon l^{-1} . The control was the complete amino acid mixture.

family (previously indicated by the double omission experiments), was again demonstrated. The molar growth yields of isolate ISS101 on triplets of amino acid families are also given in Table 5. The highest molar growth yield was obtained with the glutamate plus serine plus pyruvate families. A similar growth rate, but with a lower Y_c was obtained with the aspartate plus glutamate plus serine families, and the lag was much longer. The highest growth rate was obtained with the aspartate plus pyruvate plus serine families, though there was a short lag and the yield was slightly lower than in the above cases. The shortest lag was with the glutamate plus aspartate plus pyruvate families, with a similar yield, but only 50% of the growth rate of the combinations discussed above.

Auxanography was used to determine the individual amino acids required by isolate ISS101. Overlay plates were superior to spread plates in that they overcame the problem of the irregular spreading growth habit of isolate ISS101. Neither of the methods gave sufficiently clear results to determine precisely the individual amino acids required. However, there was some evidence that: of the glutamate family, glutamate, glutamine and proline were stimulatory but arginine was not; of the aspartate family, aspartate, asparagine, methionine and threonine were stimulatory; of the serine family, serine and glycine were stimulatory; and of the pyruvate family, alanine and possibly leucine were stimulatory. There was no observable stimulation or inhibition of growth by acetate, pyruvate or lactate spotted onto plates of the complete amino acid mixture.

DISCUSSION

Characterization

Isolate ISS101 has been characterized as a Gram-negative, non-pigmented, gliding, multicellular filamentous bacterium. As such it is differentiated from the unicellular, carotenoid-containing *Flexibacteriaceae*, and the multicellular carotenoid-containing *Herpetosiphon*. It did not oxidize sulphide and is thus differentiated from *Beggiatoa* and *Thioploca*. Its

gliding motility and lack of a sheath differentiates the organism from the genera *Halicomonobacter*, *Sphaerotilus* and *Leptothrix*. It was very different from *Leucothrix* spp. which are larger, solely marine organisms (but see Poffé *et al.*, 1979), and reproduce via gonidia. Skuja (1956) described colourless, multicellular, gliding filaments of the genus *Achroonema*. Organisms resembling *Achroonema* spp. are often observed in Blelham Tarn sediment (Godinho-Orlandi & Jones, 1981*b*), and differ in appearance from isolate 1SS101, being larger with characteristically granular cytoplasm. No *Achroonema* spp. have been isolated, so that further comparison is not possible.

In terms of morphological characteristics, isolate 1SS101 resembled members of the genus *Vitreoscilla*. The characteristic growth habit on solid media and the lack of flagella indicated that the organism was a true gliding bacterium. Electron micrographs and other observations indicated that the cells were surrounded by a slime layer. Most gliding bacteria produce slime, which has been suggested to have a role in gliding motility (Burchard, 1981). The fibrillar nature of slime has been reported in several gliding bacteria, for example *Herpetosiphon* (Reichenbach & Golecki, 1975). The specialization of the organism in the utilization of amino acids as sole carbon and energy sources resembled that of *V. stercoraria* (Mayfield & Kester, 1972, 1975). The better response of isolate 1SS101 to lower temperatures may reflect its origin in aquatic sediment; *V. stercoraria* and strain 390 were both isolated from dung. Comparative characterization tests showed that in terms of cytochrome composition, isoprenoid quinones, DNA base ratios, and sensitivity to actinomycin D, isolate 1SS101 differs significantly from the *Vitreoscilla* strains. The cytochrome and isoprenoid quinone compositions of bacterial respiratory chains are of increasing importance in bacterial taxonomy (Jones, 1980; Collins & Jones, 1981), with major differences usually observed at generic or family level. On the basis of the observed differences, isolate 1SS101 does not belong in the same family as *Vitreoscilla*. Possession of menaquinone, unusual in Gram-negative aerobes, indicated affinity with the *Flexibacteriaceae*, which share this peculiarity (Collins & Jones, 1981). The marked sensitivity of isolate 1SS101 to actinomycin D also indicated a close relationship to the *Flexibacteriaceae*, which appear to be characterized by sensitivity to this agent (Dworkin, 1969).

Isolate 1SS101 thus appears to be related to members of the *Flexibacteriaceae*. However, as it lacks pigmentation and actively flexing filaments, it does not resemble any described taxon of this family. On the basis of differences from *Vitreoscilla* strains, and from the *Flexibacteriaceae*, it is proposed that isolate 1SS101 be placed in a new genus, *Filibacter* gen. nov., and it is named as the type strain of the type species, *Filibacter limicola* sp. nov. A culture of this strain has been deposited in the National Collection of Industrial Bacteria (NCIB no. 11923). To further compare the genus *Filibacter* with the *Flexibacteriaceae* it would be desirable to have data on fatty acid profiles and genetic homology. Also, analysis of isolate 1SS101 for capnoids would be useful. These unusual sulphonolipids have been reported to occur only in members of the *Flexibacteriaceae* and closely related organisms (Godchaux & Leadbetter, 1983).

It is suggested that the definition of the genus *Vitreoscilla* be amended to include the characteristic cytochrome composition: oxidase negative with *b*-type cytochromes as major respiratory chain components.

Physiology

Isolate 1SS101 has been shown to be a strict aerobe, with a temperature optimum of 20 °C. It grew well on peptone-containing media at pH 7.4, and had a requirement for vitamins.

None of the organic compounds tested could serve as sole carbon and energy source for isolate 1SS101. The list of compounds tested was limited, but included all those most commonly found in lake sediments as major substrates of the bacterial flora. Also, most are intermediates in the main metabolic pathways. The organism, growing on amino acids, was able to produce more cell material in the presence of acetate, lactate, butyrate, or glycerol. However, the overall low values of Y_p , particularly at higher concentrations, indicated that any carbon/energy input from the substrates tested was of only minor importance.

Isolate 1SS101 grew well on a defined mixture of purified amino acids as sole carbon and energy source, and had a complex requirement for amino acids at substrate level. Histidine was

inhibitory (possibly due to inhibition of glutamine synthetase), and the aromatic family was also inhibitory in most, but not all, cases. No single amino acid was essential for growth, but all were required for maximum growth. The failure of isolate 1SS101 to grow in the absence of both the aspartate and serine families showed that one or both of these was required for the utilization of the other components (glutamate, pyruvate and aromatic families). The long lags followed by growth at normal rates in the absence of the serine or pyruvate families suggested that these amino acids were required for growth, but when absent from the medium could be synthesized by inducible enzyme systems. For example, it was possible that isolate 1SS101 possessed the enzyme L-aspartate 4-decarboxylase (EC 4.1.1.12) which converts L-aspartate to L-alanine, and that this was induced in the absence of L-alanine (pyruvate family).

From the results of all the amino acid experiments, the following conclusions can be drawn: (1) the glutamate and aspartate families were the major carbon sources; (2) utilization of the glutamate family, (a) required the presence of the serine family, (b) for maximum growth also required the pyruvate family and (c) was inhibited by the presence of the aspartate family (possibly catabolite repression); (3) utilization of the aspartate family, (a) required the presence of the pyruvate family, (b) in the absence of the pyruvate family required the necessary amino acid(s) to be synthesized by an inducible enzyme, and (c) required the presence of the serine family for maximum growth rate, and the glutamate family for maximum growth yield.

The nutrition of *Filibacter limicola* strain 1SS101 with respect to amino acids as substrates is thus very similar to that of another filamentous gliding bacterium, *Vitreoscilla stercoraria*. Mayfield & Kester (1972, 1975) reported that this organism required amino acids from more than one family at substrate level. As with strain 1SS101, the glutamate and aspartate families were the major carbon sources, but there were some differences. For example, *V. stercoraria* had a requirement for L-arginine as growth factor, and also required the aromatic family. The serine family was also important, but the pyruvate family was not. The serine family was required for maximum growth on the glutamate family, as with strain 1SS101. Results for growth on the aspartate plus aromatic plus pyruvate families were not given and therefore it is not known whether the pyruvate family was required for growth on aspartate. These requirements for particular combinations of amino acids indicate that the biochemistry of their utilization by these organisms may be novel and worthy of further study. The interdependence of the glutamate and serine families, and the aspartate and pyruvate families, suggests that pairs of amino acids from different families may be involved in specific coupled reactions. These may be transaminase reactions, or coupled oxidation-reduction reactions as in the Stickland reaction in anaerobes. The similarity of nutrition of strain 1SS101 and *V. stercoraria* is also interesting in the context of the important differences between them discussed above. Further strains will have to be isolated and studied in order to determine whether other *Filibacter* and *Vitreoscilla* spp. have the same mode of nutrition.

Ecologically, it is possible that *Filibacter*, *Vitreoscilla* and similar organisms specialize in the utilization of amino acids and may be important in the aerobic mineralization of amino acids in the lake sediments in which they occur. The specialized utilization of amino acids by these organisms is in contrast with the very versatile use of organic nitrogen compounds by planktonic unicellular heterotrophs (Sepers, 1981). A nutrient-rich environment such as sediment may support organisms with very limited substrate ranges, whereas in the water column a greater substrate versatility is one of the adaptations of planktonic bacteria for survival in a nutrient-poor environment (Poindexter, 1981). Amino acid concentrations in sediment may be of the order of 35 mg glycine equivalents l^{-1} (Simon & Jones, 1982), as compared to concentrations in surface waters of the order of 35 $\mu g\ l^{-1}$ (Sepers, 1981). Amino acid mineralization is the least studied part of the nitrogen cycle, and it would be of interest to investigate further the possible importance of filamentous bacteria in this process in lake sediment. The nutritional pattern of isolate 1SS101 may be related to the relative abundance of individual amino acids in sediment. Jørgensen *et al.* (1981) reported that glutamic acid and members of the serine and pyruvate families were the most abundant amino acids in the sediments they examined. The urease activity of strain 1SS101 is interesting in the context of the importance of urea as a product of the decomposition of phytoplankton (Sato, 1980).

Description of Filibacter gen. nov.

Fi.li.bac'ter. L. n. *filum* a thread; M.L. masc. n. *bacter* masculine form of Gr. neut. n. *bactrum* a rod; M.L. masc. n. *Filibacter* thread rod.

Filaments composed of cylindrical cells, straight or curved. Cell junctions clearly marked by constriction. Filaments are flexible, but do not show active flexing, are not sheathed and do not branch. Motile by gliding, giving characteristic spreading growth and spiral colonies on solid media. Cell masses not pigmented. Resting stages not observed. Gram-negative. Strictly aerobic, catalase positive. Intracellular sulphur granules not formed in the presence of hydrogen sulphide.

Oxidase positive, major cytochrome component *c*-type. Major quinone isoprenolog is menaquinone (MK-7 in type strain).

The G + C content of the type strain is 44 mol % (T_m).

Type species: *Filibacter limicola* gen. et sp. nov.

Description of Filibacter limicola sp. nov.

li.mi'co.la L. n. *limus* mud; L. suff. verbal n. *cola* dweller; M.L. masc. n. *limicola* mud dweller.

Filaments 1.1 μm $\times$ 8–150 μm , cells 1.1 μm $\times$ 3–30 μm , rarely less than three diameters in length. Gliding motility about 5 $\mu\text{m min}^{-1}$. Grows well at pH 7.4. Optimum temperature 20 °C, slow growth at 4 °C and no growth above 26 °C. Gelatin hydrolysed, but casein and starch not hydrolysed. Sugars and organic acids not utilized, amino acids only are used as carbon and energy sources. Good growth on peptone-containing media.

Urease positive.

Isolated from lake sediment.

Type strain: ISS101 (NCIB 11923).

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