A Study of the Aquatic Actinomycetes of Blelham Tarn

by

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INTRODUCTION

In a previous contribution to knowledge of the aquatic actinomycetes detailed consideration was given to forms occurring on allochthonous leaf material which was recovered from lakes and streams of the English Lake District (WILLOUGHBY, 1969). In this further contribution an effort has been made to consider the whole aquatic environment more comprehensively and to this end samples of water and mud from lakes, rivers and streams have been examined. Once again emphasis is on local collections and the Blelham Tarn lake and drainage basin (Fig. 1) was visited repeatedly.

MATERIALS AND METHODS

Surface water samples were collected directly in 500 ml dry sterilized stoppered bootles, leaving a small air space. Profile water samples from the lake were collected in a Friedinger trap and transferred to the appropriate bottle immediately. Littoral and profundal surface mud samples were collected using the Gilson or Jenkin sampler respectively. In the laboratory the mud core samples were carefully pushed through their sampling tubes from below and the surface 1—2 cm spooned off into a plastic bag. Dilutions of these mud samples were made using sterile lake water. Neat water samples and mud dilutions were plated onto dishes of chitin-actidione agar (WILLOUGHBY, 1968). These dishes had been allowed to dry in the laboratory for two days after pouring. Experience showed that a

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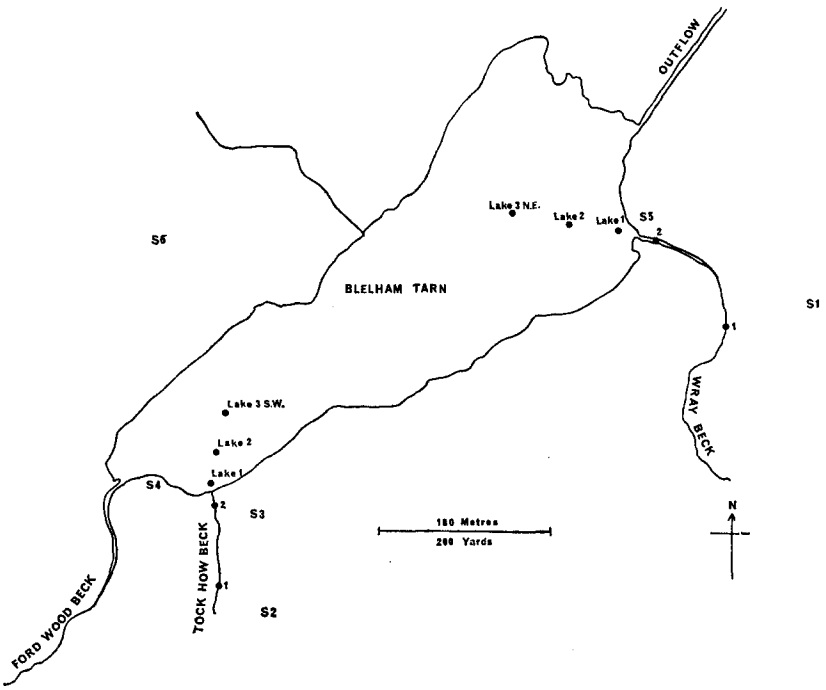

Fig. 1. Plan of Blelham Tarn with its associated streams. On Tock How and Wray Becks the sampling stations of 9.11.67 are indicated. Soil Sites (S 1 – S 6) are those described in Table X.

sample aliquot of 0.2 ml was a convenient one to use and this became standard. During the course of four to six weeks incubation at 25°C the agar surface was scanned and all actinomycete colonies recorded. Myxobacteria populations of certain water samples, particularly those from streams, proved to be so high that the chitin agar used could be completely cleared by their activity alone. In order to curb this activity, and following advice from Dr. N. P. BURMAN, a preliminary chlorination was often administered to the stream water samples in the following manner. Addition of 4 ppm ammonia was followed by 2 ppm Cl as sodium hypochlorite. After thirty minutes reaction time excess chlorine was neutralised with sodium thiosulphate, the correct amount of which had been calculated from titration of a blank sample. The sample was then plated as usual.

ORGANISMS ENCOUNTERED

An abbreviation system has been used for the various taxa distinguished. In the tabulated results these are presented in a standard

order (which is not an alphabetical one) and this follows, together with the abbreviation explanations. Apls, *Actinoplanes*: Apl NI, Actinoplanaceae not identified to genus: Amorph, *Amorphosporangium*: Amp, *Ampullariella*: Strepspm 1, *Streptosporangium* Type 1: Strepspm 2, *Streptosporangium* Type 2: Domed, colony with spore dome releasing motile spores: Microm, *Micromonospora*: Nt, *Nocardia*-type: Lspi, large spored pink irregular: Sspi, small spored irregular: Strep, Streptomycete: Strep M, Streptomycete-type colony releasing motile spores: Sterile, colonies with no fruiting structures observed.

Strepspm 1 produces fairly numerous sporangia dispersed over the colony surface (Fig. 2 a, c), and in their ontogeny an internal coiled structure is clearly distinguished in the later stages. Final maturation and rounding off of the spores becomes particularly apparent after the material is mounted in water (Fig. 2 b). The sporangia soon dehisce readily through wide apical pores (Fig. 2 d, e) and fragments of the coil dis-articulate as chains, become motile, and struggle to free themselves. Chains are clearly constricted into spherical units representing spores, and indeed some single spores may be liberated. However, it seems clear that the liberation of fairly lengthy spore chains is typical. In sub-culture Strepspm 1 is usually deep orange, both on chitin and starch-casein agars. Strepspm 2 comes much closer to Couch's conception of the genus *Streptosporangium* and may in fact represent his species *S. roseum* (COUCH, 1955). On the original isolation dishes, in contrast to Strepspm 1, the whole colony has a pronounced white aerial mycelium which extends from a pinky substrate mycelium. Sporangia are concentrated at the colony centre and are difficult to wet in order to make microscopic mounts. Once again an internal coiled structure is distinguished as the sporangia mature (Fig. 2 g). The latter are slightly smaller than those of Strepspm 1 and apparently contain fewer spores. In contrast to Strepspm 1 mounting in water does not provoke the final rounding off and delimination of the internal spores and seems to have no discernable effect at all. Finally despite numerous efforts Strepspm 2 has never been induced to dehisce, hence COUCH's remarkable account of this aspect cannot be confirmed. Domed is a colony type which has been fully described in the allochthonous leaf study. At the colony centre there is an elevated area where massed phialides produce spores successively from their tips. These spores show strong motility in water.

Nt, Lspi and Sspi are three important colonial types encountered in this study which cannot readily be ascribed to any known taxon. They have in common the absence of any sub-aerial mycelium, the reproductive spores being formed deep in the agar. During growth

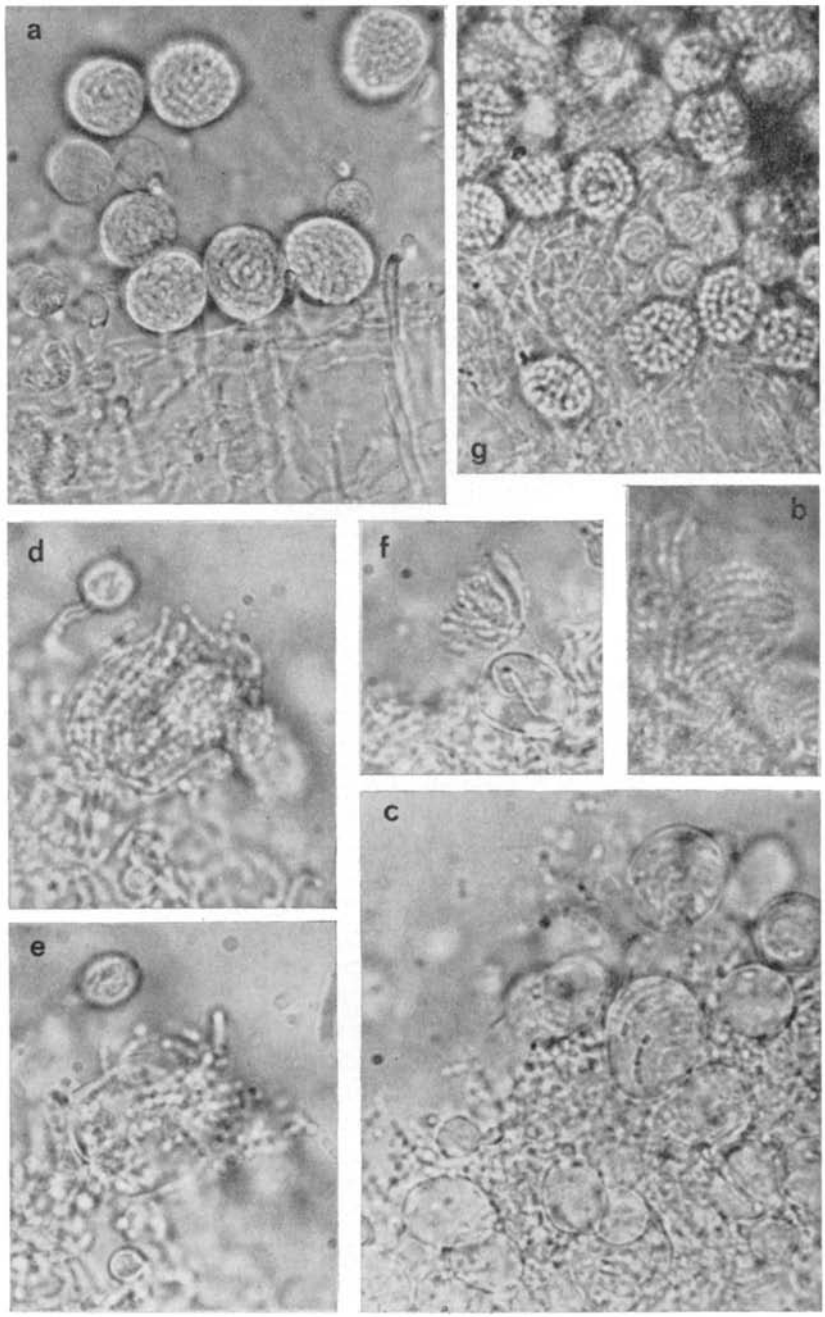

Fig. 2. a—f, *Streptosporangium* type 1: g, *Streptosporangium* type 2. a, b, vertical sections at the colony surface with sporangia immediately prior to dehiscence showing internal coiled spore chains: c, younger sporangia showing internal coils: d, e, two stages in the dehiscence of a single sporangium with a wide open pore distinguished (top : 468 hrough which chains of spores are emerging: f, a dehisced sporangium (and a conidial head type of structure (above) often observed in Strep colonies: g, central area of colony crowded with sporangia, some of which (middle) are young and show clear coiling. All photographs are of living material growing on chitin agar and to the same magnification (X 1,000).

the transformed mycelium produces spores in uniseriate files in Nt but in dense rope-like masses in Lspi and Sspi. Transformation to spores may be so complete in the two latter types that there is often difficulty in observing any undifferentiated mycelium in the whole colony, even at the advancing margin. Lspi and Sspi are considered to show affinity to *Micromonospora* in their production of innumerable small spherical non-motile spores. In their colony morphology and lack of the characteristic pigmentation however they seem distinct from *Micromonospora*, at least as the latter is known from these particular studies. Nt, Lspi and Sspi are illustrated and compared in Figs. 3—5 and Table I. There is an additional point of biological interest concerning Lspi which has been observed occasionally. At the colony centre, which is always conspicuous because of its denser mycelium, a single colony of *Azotobacter* may be present. The exact centring of this bacterium upon the Actinomycete colony beneath and its absence over other parts of the isolation dish suggests some syntrophic association may be in operation. The category Strep refers to any Streptomycete bearing sporing structures on an elevated sub-aerial mycelium and includes mostly *Streptomyces* species. Strep M produces a small colony only 2 mm in diameter which is initially creamy white but may turn black with age. This has a typical Streptomycete appearance with a dry surface which is only made wettable with difficulty. Concentric rings of growth may be present and distinguish sterile and fertile portions or the entire surface may be fertile. At the fertile surfaces short vertical phialides, projecting just

Table I. Comparison of colony types Nt, Lspi and Sspi.

Nt	Lspi	Sspi
Colonies usually 2-5 mm diam. at 6 weeks, but can be up to 13 mm.	Colonies up to 4 mm.	Colonies up to 6 mm.
White on chitin agar, lemon yellow on starch-casein	Pinky on chitin and starch-casein agars, colour deeper at the colony centre	Creamy white on chitin agar, colour deeper at colony centre
Chitin agar not cleared	Chitin agar not cleared	Chitin agar may be cleared
Colony mycelium predominantly running along straight radii	Colony mycelium radiating out in curling strands	Colony mycelium branching irregularly to give a close net-work
Spores cylindrical, variable in size, 0·7 - 3 μ long X 0·7 μ wide.	Spores spherical, large, 1·0 - 1·4 μ diam.	Spores spherical, small, 0·7 μ diam.
Faint odour on starch-casein agar		

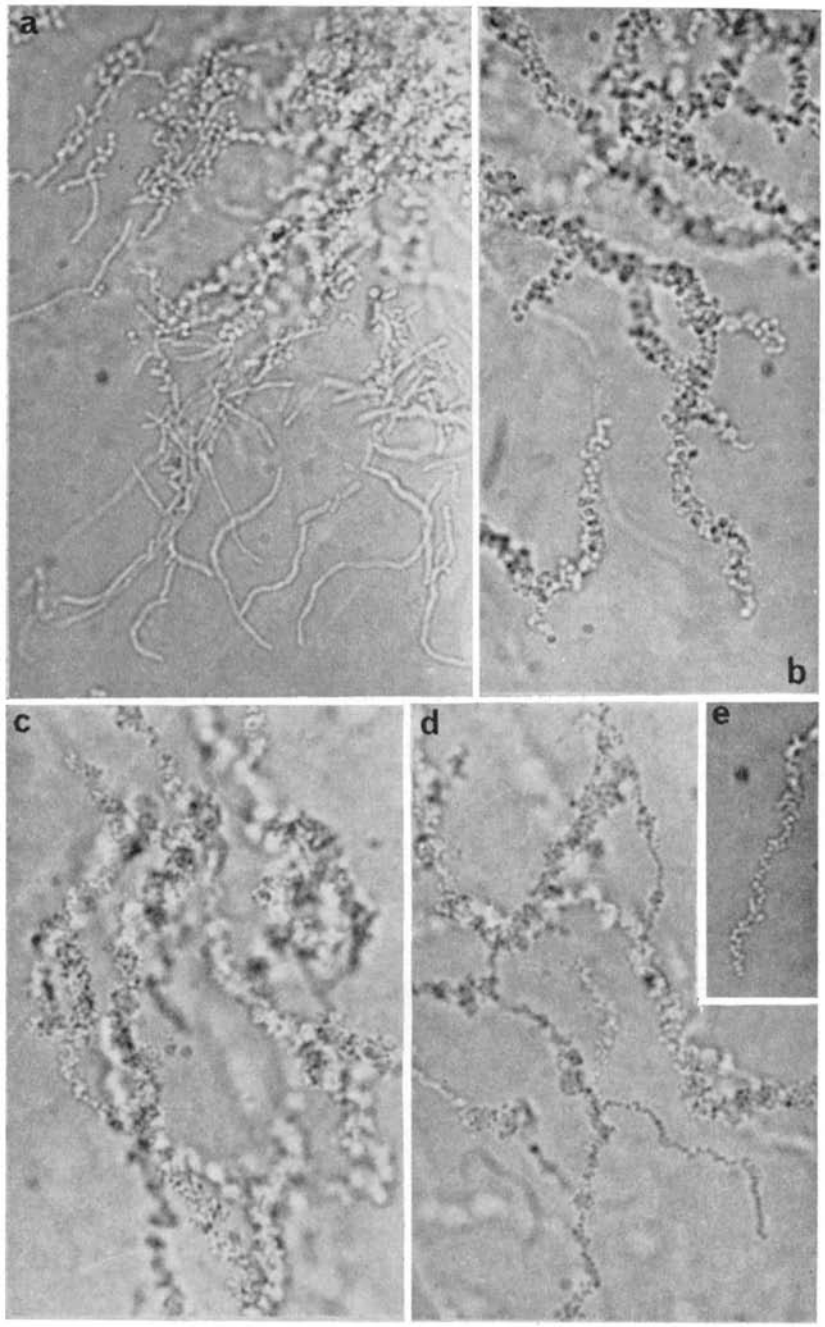

Fig. 3. a, b, large spored pink irregular (Lspi): c–e, small spored irregular (Sspi). a, colony margin showing free mycelium with some dis-articulation and copious spore production in the older portions behind: b, colony margin with mycelium completely transformed to spores: c, portion of colony showing rope-like masses of minute spores interconnected in a complex pattern: d, e, portions of colonies at the colony margin showing uniseriate spore production in the terminal mycelium. All photographs are of living material growing on chitin agar and to the same magnification (X 1,000).

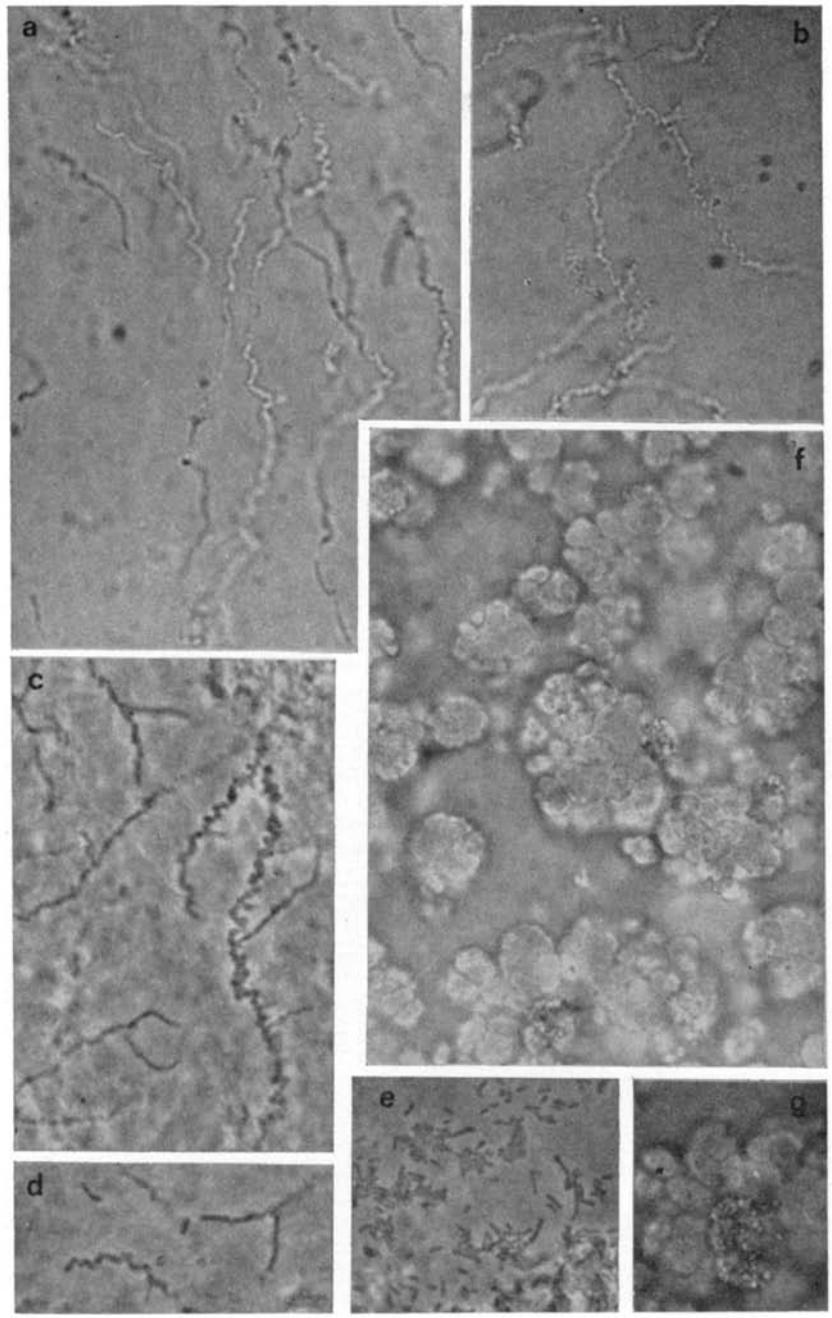

Fig. 4. a, b, *Nocardia*-type (Nt): c, d, forms cultivated as facultative anaerobes apparently resembling Nt: e, *Nocardia* sp. from facultative anaerobe incubation: f, complex sorus-like system of sporangia or spores in a colony from Ford Wood Beck: g, release of non-motile spores from this colony. All photographs are of living material, growing on chitin agar, X 1,000. c—e, phase contrast.

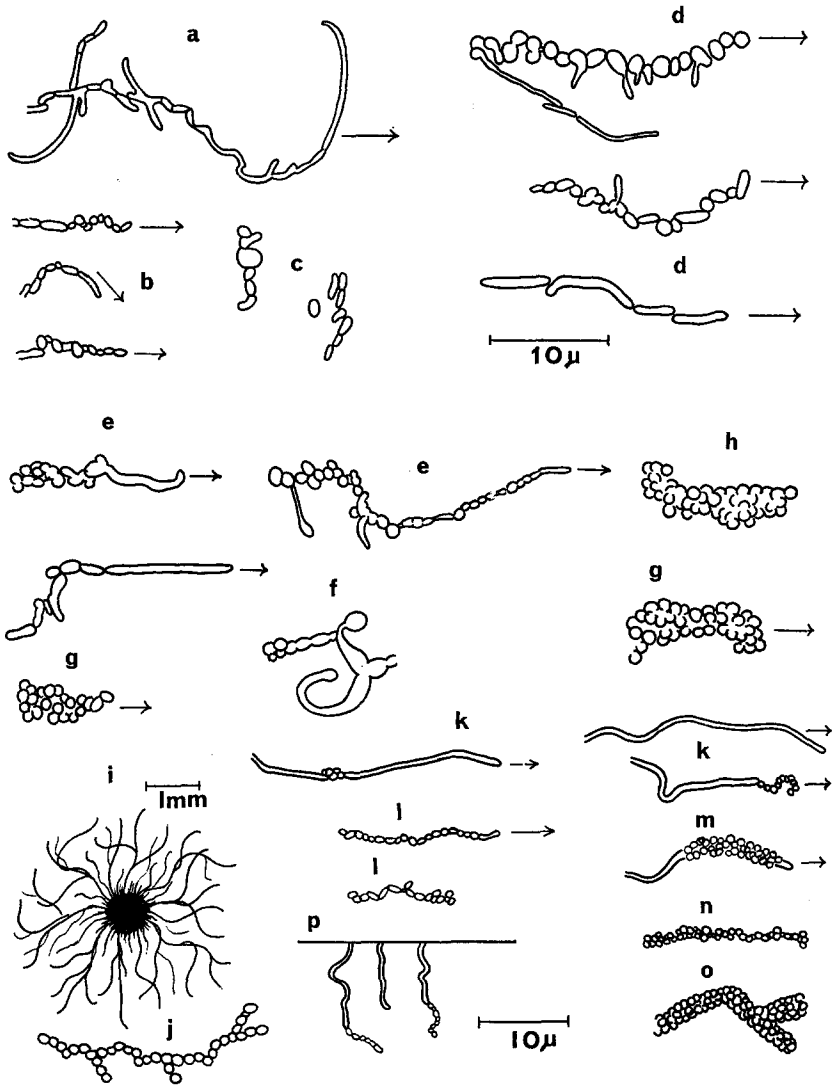

Fig. 5. a—c, *Nocardia*-type (Nt): d, *Nocardia*-type from facultative anaerobe incubations: e—j, large spored pink irregular (Lspi): k—p, small spored irregular (Sspi). a, b, advancing margin of mycelium: c, larger intercalary sections: d, marginal mycelia, some with inflated spore segments: e, uniseriate marginal mycelia: f, larger swollen segments: g, rope-like spore masses at colony margins: h, intercalary spore masses: i, a whole colony at low magnification to illustrate curling strands of mycelium radiating from the dense colony centre: j, portion of i showing how spore arrangement in this particular colony was largely uniseriate. k, marginal mycelia with few spores: l, uniseriate marginal mycelia: m, profuse development of spores just behind colony margin: n, o, intercalary spore chains forming dense rope-like masses: p, vertical section of part of the centre of a colony showing tendency for spores to form more readily at deeper levels. Arrows indicate that the drawing was made at the colony margin and show the general direction of advance. d and i to scales indicated, remainder to scale below p. Growth medium was starch-casein agar for a—c, e—h and chitin for d, i—p.

above the agar, abstrict large oval spores which are 2.5 to 3.6 μ in length and 1.5 μ across. Spore motility ensues following the water addition. Another *Streptomyces*- like Actinomycete which releases motile spores, *Sporichthya polymorpha*, has recently been described (LECHEVALIER, LECHEVALIER & HOLBERT, 1968). However, Strep M seems to differ in some respects from this species, in particular in its production of substrate mycelium. Sterile refers to colonies which were usually small, only attaining 1 mm diameter or less, which produced no clear reproductive structures.

Lake water samples

At Blelham Tarn sampling of both littoral and profundal water was made, the latter up to the maximum depth of 13 metres. These samplings were made on a total of six separate occasions representing the different seasons and corresponding temperature regimes (WILLOUGHBY & COLLINS, 1966), but with emphasis on surface water. Thus there was no investigation of the water which has a greatly reduced oxygen content and occurs below the thermocline during the summer months. Actinomycete estimations per unit volume of water were all very similar, irrespective of depth and season, varying only from 60 to 125 per ml. In all 580 colonies were investigated comprising 6 Apls, 442 Microm, 1 *Nocardia*, 2 Nt, 26 Lspi, 34 Strep and 69 sterile. This was from a total sample aliquot of 7.2 ml. Thus a mean figure of 80 Actinomycetes per ml is derived. The proportion of *Actinoplanes* and Streptomycetes was very low, 1% and 6% respectively, but that of *Micromonospora* was high, being 76%.

Esthwaite Water, Windermere and Thirlmere were each sampled on a single occasion only when these lakes were in full circulation. At Esthwaite Water two 0.2 ml sample aliquots from each of eight selected depths down to 14 metres yielded 1 Apls, 94 Microm, 7 Strep and 6 sterile. Thus there were 108 colonies from a total sample aliquot of 3.2 ml, giving a mean figure of 34 Actinomycetes per ml. Similarly Windermere yielded only 15 Actinomycete colonies from a total sample aliquot of 1.2 ml and Thirlmere even fewer, namely 21 from 4 ml, or 5 per ml.

Stream and river water samples

Small streams in the English Lake District are usually referred to as becks. Early in this study a single water sample from the Tock How inflow beck at Blelham Tarn gave a very high Actinoplanaceae estimation figure of more than 4000 per ml. Although this figure was never approached subsequently, and indeed on that account is somewhat suspect, the evidence from repeated sampling was that

streams and rivers did in fact carry high Actinoplanaceae loads. Data from samplings made on three dates, namely 28.9.67, 31.10.67 and 11.4.68 are presented (Tables II—IV). Comparing recoveries with or without the chlorination treatment the total number of colonies of all kinds was not greatly affected. However, there was clear evidence that within these totals the *Micromonospora* yield was increased with chlorination while the Actinoplanaceae yield was substantially decreased. Clearly therefore there is some competitive balance which

Table II. Actinomycetes in three Blelham Tarn inflows, also the outflow on 28.9.67. For each site total water samples of 1·6 ml were examined, both directly and also following a pre-treatment with chloramine (Cl).

Site and Treatment	Apls	Amorph	Amp	Strepspm 1	Strepspm 2	Domed	Microm	Nt	Lspi	Sspi	Strep	Strep M	Sterile	Totals
Ford Wood	14	2	0	2	2	4	64	12	0	0	98	0	60	258
Ford Wood Cl	12	0	0	6	2	0	156	12	0	0	24	1	47	260
Took How	16	0	4	8	0	0	42	30	0	6	24	0	60	190
Took How Cl	4	0	0	0	0	1	109	22	0	1	9	0	69	215
Wray	10	0	2	0	0	0	44	10	4	0	4	0	32	106
Wray Cl	1	0	0	0	0	0	60	1	0	0	4	0	22	88
Outflow	14	0	6	4	0	10	60	2	2	0	20	0	84	202
Outflow Cl	3	0	0	0	0	1	160	1	0	0	6	0	20	191
Totals	74	2	12	20	4	16	695	90	6	7	189	1	394	1510

Table III. Actinomycetes in three Blelham Tarn inflows, also the outflow on 31.10.67. For each site total water samples of 1·6 ml were examined, both directly and also following a pre-treatment with chloramine (Cl).

Site and Treatment	Apls	Amorph	Amp	Streptospm 1	Domed	Microm	Nt	Lspi	Sspi	Strep	Strep M	Sterile	Totals
Ford Wood	12	0	2	0	2	42	2	0	0	18	0	22	100
Ford Wood Cl	2	0	0	0	4	70	2	2	0	14	2	18	114
Took How	48	2	0	12	0	18	30	0	28	32	0	28	198
Took How Cl	14	0	0	2	0	76	22	0	0	38	0	28	180
Wray	4	0	0	0	2	22	0	2	0	4	0	12	46
Wray Cl	2	0	0	0	0	42	0	0	0	10	0	16	70
Outflow	8	0	5	0	0	131	3	11	0	24	0	27	209
Outflow Cl	5	0	3	0	5	160	0	0	0	3	0	37	213
Totals	95	2	10	14	13	561	59	15	28	143	2	188	1130

Table IV. Actinomycetes in three Blelham Tarn inflows, also the outflow on 11.4.68. For each site total water samples of 1·6 ml were examined, both directly and also following a pre-treatment with chloramine (Cl).

Site and Treatment	Apls	Microm	Lspi	Strep	Sterile	Totals
Ford Wood	4	32	4	4	28	72
Ford Wood Cl	0	42	0	4	8	54
Took How	4	36	0	20	24	84
Took How Cl	4	60	0	52	14	130
Wray	0	32	0	4	56	92
Wray Cl	0	20	0	4	10	34
Outflow	0	140	4	8	12	164
Outflow Cl	0	174	0	6	34	214
Totals	12	536	8	102	186	844

affects the recovery of these two groups. Considering only the non-chlorinated samples on these three dates Actinoplanaceae were 84 out of 756 (11%), 93 out of 553 (17%) and 8 out of 412 (2%) respectively. Considering the two forms of *Streptosporangium* distinguished, on the three dates combined, Strepspm 1 was recovered on 34 occasions as against only 4 for Strepspm 2. Nt, Lspi and Sspi were all represented in at least two of these three collections. Nt was particularly prominent in the September and October ones being 91 out of 1510 (6%) and 59 out of 1130 (5%) on those occasions.

Windermere inflows, and the outflow, some of which are sizeable rivers, notably the Brathay and Leven, also gave high Actinoplanaceae figures (Table V). The accumulated total was 63 colonies out of 245, or 26%. Strepspm 1 and Nt were also well represented, as they had been in the Blelham Tarn inflows.

Table V. Actinomycetes in seven Windermere inflows, also the outflow, on 2.7.68. For each site numbers recorded are from a total water sample of 0·4 ml.

Stream or River	Apls	Amp	Strepspm 1	Domed	Microm	Nt	Lspi	Sspi	Strep	Sterile	Totals
Blelham	6	2	3	0	0	0	0	0	2	22	35
Brathay	6	0	2	0	0	6	3	0	1	14	32
Cunsey	2	0	1	0	0	5	0	0	9	15	32
Holbeck	9	0	0	0	1	4	0	1	3	2	20
Rothay	0	0	2	0	1	0	0	0	1	1	5
Troutbeck	10	0	1	0	0	15	0	0	0	0	26
Wilfin	16	0	1	0	5	15	0	0	27	14	78
Leven (outflow)	2	0	0	1	1	4	1	0	2	6	17
Totals	51	2	10	1	8	49	4	1	45	74	245

Inflow Streams entering Blelham Tarn

Since there had been indications of differences in the species composition of stream and river water as compared with that of the lakes to which they contribute this aspect was investigated further. On a single sampling date, 9.11.67, samples at Blelham Tarn were taken along the course of the Tock How and Wray Beck inflows and following these into the lake (Fig. 1, Tables VI, VII). The total number of colonies recovered from the inflows was approximately three (Wray) to five (Tock How) times as many as in the lake itself. On Tock How Beck Actinoplanaceae were so numerous that a complete analysis to genus could not be made. Most of the Apl NI category were probably *Actinoplanes*. Total Actinoplanaceae were 57 and 65 at the two beck sites as compared with totals of 58, 0 and 2 along a line to the lake centre (S.W.). Thus there was a striking reduction in Actinoplanaceae following mixing in the lake. Similarly the Streptomycete and the *Nocardia*-type content showed a sharp decline in contrast to *Micromonospora* which maintained its level or even increased. Turning to

Table VI. Actinomycetes at Blelham Tarn on 9.11.67 sampled in a sequence through the Tock How inflow beck and into the lake. Lake 1 is the point where the beck first mixes with lake water. Lake 2 is surface water mid-way between this point and the lake centre, while Lake 3 is surface water at the lake centre (South West), see also Fig. 1. For each site Actinomycete numbers recorded are from a total water sample of 0·6 ml.

Site	Apls	Amp	Strepspm 1	Apl NI	Domed	Microm	Nt	Lspi	Sspi	Strep	Sterile	Totals
Tock How Beck 1	8	3	0	46	0	13	58	0	6	88	71	293
Tock How Beck 2	13	3	3	46	0	13	9	0	5	42	105	239
Lake 1	15	3	0	40	0	15	11	0	4	25	79	192
Lake 2	0	0	0	0	2	35	0	0	1	4	13	55
Lake 3	2	0	0	0	0	40	0	0	0	5	12	59

Table VII. Actinomycetes at Blelham Tarn on 9.11.67 sampled in a sequence through the Wray inflow beck and into the lake. Lake 1, 2 and 3 have the same significance as in Table VI. Lake 3 is surface water at the lake centre (North East). For each site Actinomycete numbers recorded are again from a total water sample of 0·6 ml.

Site	Apls	Amp	Strepspm 1	Apl NI	Domed	Microm	Nt	Lspi	Sspi	Strep	Sterile	Totals
Wray Beck 1	8	1	2	15	1	34	3	0	1	28	74	167
Wray Beck 2	9	1	0	21	2	29	0	0	0	38	60	160
Lake 1	35	0	0	0	1	19	1	0	0	33	78	167
Lake 2	1	0	0	0	0	45	0	3	0	5	10	64
Lake 3	1	0	0	0	0	39	0	4	0	3	10	57

the Wray Beck entry a very similar pattern emerged for the Actinoplanaceae although they were numerically fewer. Nt was hardly represented but Streptomycetes once again showed a decline in the lake while *Micromonospora* did not. Thus there were strong similarities between the two opposite ends of the lake in regard to mixing in of Actinomycetes arriving through the inflows.

Blelham Tarn mud samples

It is known from the previous study that Actinoplanaceae may occur in some quantity on the allochthonous leaf material of streams and lakes. However, it is not certain that the high Actinoplanaceae content of stream water which has been described in this contribution is entirely explicable on this basis. Accordingly another aspect of the stream environment was considered, namely the bottom mud. However, the results obtained from mud dilutions plated onto chitin-actidione agar were not suggestive of a major Actinoplanaceae reservoir in this situation (Table VIII) and this work was not persevered with.

A more persistent attempt was made to obtain knowledge of the Actinomycete content of benthic mud. On a number of occasions paired samples of surface mud from the profundal (13 meters depth)

Table VIII. Actinomycetes in the bottom mud of Blelham Tarn inflow streams, also the outflow, on 4.1.68. Numbers recorded are recoveries per 10^{-3} ml of mud. They were derived from 1/100 and 1/1,000 dilutions. + Denotes a recovery was made at some other dilution.

Site	Strepspm 2	Microm	Nt	Lspi	Strep	Sterile	Totals
Ford Wood	3	25	3	80	25	-	136
Tock How	-	10	3	5	58	8	84
Wray	-	3	+	1	8	2	14
Outflow	-	10	+	3	3	3	19
Totals	3	48	6	89	94	13	253

and littoral (up to 2 meters depth) zones were examined. Both consisted of a fine grained mobile material high in organic matter. Considering the results as a whole, the variation between total Actinomycete numbers recorded for the different dates was remarkably small (Table IX). In each set of paired samples there was however a consistent difference between littoral and profundal totals, the latter being the greater. Considering the yields in categories, the dominance of *Micromonospora* (52% of the aggregated totals) and of Streptomycetes (30%) was well marked. Another category which was smaller, but obtained with some degree of consistency, was Lspi. On their face

Table IX. Actinomycetes in the benthos of Blelham Tarn. Numbers recorded are recoveries per 10^{-3} ml of mud. They were mostly derived from 1/1,000 or 1/5,000 dilutions. + Denotes a recovery was made at some other dilution.

Sample Date	Strepspm 2	Domsd	Microm	Nt	Lspi	Sspi	Strep	Sterile	Totals
Littoral 23.6.67	-	4	36	-	3	-	8	8	59
Profundal 23.6.67	+	4	33	-	5	-	77	12	131
Littoral 13.7.67	-	-	27	-	1	-	19	3	50
Profundal 13.7.67	2	-	72	2	+	-	49	5	130
Littoral 21.7.67	-	-	79	-	-	1	12	1	93
Profundal 21.7.67	-	-	64	-	11	6	56	11	148
Littoral 11.8.67	-	-	85	-	-	-	45	-	130
Profundal 11.8.67	5	-	75	+	+	-	70	35	185
Littoral 14.12.67	-	-	45	-	25	+	28	5	103
Profundal 14.12.67	3	-	123	3	60	-	65	15	269
Littoral 17.7.68	-	-	53	-	18	-	20	5	96
Profundal 17.7.68	-	-	193	-	50	-	98	3	344
Littoral 5.9.68	-	-	28	-	3	-	13	10	54
Profundal 5.9.68	-	-	153	-	18	-	63	33	267
Totals	10	8	1066	5	194	7	623	146	2059

value the figures might even suggest that this category is increasing in the lake, but continued study will be necessary to confirm this. Although relating to a minor category the Strepspm 2 recoveries, all from the profundal mud only, were of great interest. On 21.7.67, 11.8.67 and 22.8.68 the physical state of the lake was such (stratification period) that there was a reduced oxygen tension at the profundal mud surface. Values of 0.22, 0.21 and 0.11 mg. O_2/litre respectively were recorded on these dates as compared with much higher values for profundal surface mud in early summer and winter, e.g. 0.68 and 1.1 mg O_2/litre respectively for 23.6.67 and 14.12.67. These latter figures are similar to those obtained from littoral surface mud throughout the year. However, there was no obvious correlation between the different oxygen levels recorded for the different seasons and the Actinomycete yields.

Benthic Actinomycetes as facultative anaerobes

On one occasion, in August 1967, a search was made for facultative anaerobes at Blelham Tarn in deeper benthic mud from that normally sampled. A complete mud core obtained using the Jenkin sampler was brought back to the laboratory. The surface 2 cms was rejected and the more compact mud beneath removed and made up to suitable dilutions with sterile lake water. These were mixed into molten chitin agar and poured into tubes where they were immediately covered with sterile 2% plain agar. Thus the procedure was designed to recover Actinomycetes growing as facultative anaerobes. With a final dilution of 1/5,000 very few Actinomycetes were recovered, even after prolonged incubation at 25°C. Three types of organization were represented. The first resembled *Micromonospora* quite closely except that the spores were somewhat larger and produced on a less conspicuous and more fragmentary mycelium than that observed in purely aerobic isolates. The second resembled the *Nocardia*-type described above but with the dis-articulated spore segments slightly more inflated (Fig. 4, c, d). The third consisted of *Nocardia* colonies (Fig. 4 e).

Soil actinomycetes from the Blelham Tarn drainage basin

There is the possibility that some, perhaps the majority, of Actinomycetes obtained from aquatic situations owe their recovery to the persistent viability of spores washed into the environment from soil. Since the Blelham Tarn drainage basin is such a clearly delimited one, forming a natural steep sided amphitheatre, the opportunity arose to consider this possibility and to this end six soil sites were sampled (Table X). There was considerable variation in the numbers of Actinomycetes recovered per unit volume of soil, a limed pasture

field being the most productive and an acid peat soil the least. Streptomycetes were most numerous, followed by smaller numbers of *Micromonospora*. Nt occurred at one site only, a well drained one, while Lspi was represented only in the two waterlogged soils. The occurrence of Strepspm 2 suggested it was more of a soil organism than an aquatic one.

Table X. Actinomycetes in six soils in the Blelham Tarn drainage basin. For each site numbers recorded are recoveries per 2 x 10^{-4} ml of soil. They were derived from 1/5,000 (top figure) and 1/10,000 (lower figure) dilutions. + Denotes a recovery was made at some other lower dilution.

Site	Description of Site	pH	Strep	Strep M	Strepspm 2	Microm	Nt	Lspi	Sspi	Sterile
1	Pasture field (Limed)	7.0	388 480		+	5 25	58 60			10 15
2	Pasture field	5.8	160 215		+	10 15			5	15 10
3	Pasture field (Flooded)	5.7	148 115			18 15	3	5 40		5
4	Permanent Bog	5.3	33 35			18 45	+	5 10		5 30
5	Alluvial stream soil	4.5	15 25	15		25				10
6	Bracken Peat	4.4	3			+				

Discussion

From this study comes the suggestion that the total number of Actinomycetes in the water of a lake reflects its chemical richness. Considering the total chemical ions present (meq/l) a recent representative set of figures is 0.83 for Blelham Tarn, as compared with 0.81 for Esthwaite, 0.55 for Windermere and 0.35 for Thirlmere. Thus Blelham Tarn which heads this list has also given the highest Actinomycete water counts. Thirlmere illustrates the converse situation. In view of the limited sampling however, and the possibility of seasonal variation, it would be unwise to stress the conclusion too strongly. Considering the Actinomycetes from the water of streams and rivers there seems little doubt that these are sometimes extremely numerous. The sampling programme of 9.11.67 brought out very clearly how numbers fell as stream water mixed in with the main body of the lake. Therefore it must be concluded that some settling out of Actinomycete spores occurs here. Considering the sedimentary zone, the surface mud at the bottom of the lake, there is evidence at Blelham Tarn of a greater viable population in the profundal than in the littoral. A precisely similar result has been obtained for the heterotrophic aerobic bacteria of the same lake (V. G. Collins, personal communication). This is unexpected since

potential growth substrates in the littoral would appear to be more numerous and varied than in the profundal. One possible explanation is that there is a continual slumping of mud down the streep sides of the lake bottom, leading to a central accumulation. On this basis surface mud of the profundal receives a continuous recruitment of propagules from the shallows.

On a numerical basis the categories *Micromonospora* and Streptomycete have been major recoveries in this investigation at Blelham Tarn. In surface mud from the lake, for example, both of the littoral and of the profundal, they showed a particularly striking dominance. It is appropriate to ask, whether these recoveries relate to active populations in these situations or whether they are merely wash-in forms. Past recorders of aquatic *Micromonospora* recoveries, for example CROSS & COLLINS (1966) and UMBREIT & MCCOY (1941) have tended to find the former of these two alternatives the more attractive. UMBREIT & MCCOY maintained that *Micromonospora* was virtually the sole Actinomycete representative in the bottom deposits they examined and hence were thoroughly convinced of its importance. In this current investigation the high proportion of *Micromonospora* in water of the lake (in contrast to Streptomycetes) and the frequent record of very high numbers in the outflow, for example the samplings recorded in Tables II—IV (again in contrast to Streptomycetes) tend to support the thesis that *Micromonospora* spores are indeed produced within the body of the lake and those of Streptomycetes are not. However, *Micromonospora* is clearly a local soil inhabitant and is very well represented indeed in the inflow streams. Therefore some reservation might well be felt about its true ecological status in the aquatic environment. Certainly further work is desirable. For the time being however this type of distribution pattern is taken as being indicative of a form which plays some active part in the metabolic processes of freshwater.

The position regarding the Actinoplanaceae (but excluding Strepspm 2) is slightly clearer. Spores appertaining to this family are readily recovered from the waters of streams and rivers but less so from those of the lakes which these feed. In the case of Blelham Tarn they do not occur with measurable frequency either in the mud of the lake or in the surrounding local soils. This argues for a production predominantly in lotic environments, although there is clearly also some activity on the foreshores of lakes, in both cases presumably mainly on allochthonous leaf material. However, there are hints of a more complicated derivation. At Blelham Tarn the Tock How inflow stream, which has often given particularly high Actinoplanaceae yields, is consistently contaminated by residues from livestock maintenance. The levels of dissolved phosphate and nitrate on

31.10.67 for example were 0.064 mg/l PO_4 P and 2.1 mg/l NO_3 N. On the same day Wray Beck gave figures of only 0.0049 mg/l PO_4 P and 0.44 mg/l NO_3 N while the lake itself stood at 0.0064 mg/l PO_4 P and 0.25 mg/l NO_3 N. Thus there is the suggestion that chemical enrichment may encourage the production of Actinoplanaceae in a suitable environment.

The ecology of Strepspm 2 is rather mysterious. It was the only representative of the Actinoplanaceae to be recovered from the Blelham Tarn soil sites, suggesting it might be more common in local terrestrial environments than *Actinoplanes* for example. However, recovery from an inflow stream was made on only one occasion, at Ford Wood Beck. There were no recoveries from lake water. Finally Strepspm 2 was recovered from the profundal mud of Blelham Tarn on four out of seven sampling occasions, whereas littoral mud never yielded it, and there were also recoveries from stream mud at Ford Wood Beck. Thus in submerged mud, as in soil, it was the sole Actinoplanaceae representative. On their face value these records suggest that distinct Strepspm 2 populations occur in soil and in profundal lake mud. As far as Actinoplanaceae in aquatic environments are concerned one might have guessed that this particular *Streptosporangium*, with its largely unwettable sporangia and mycelium, would be the form least likely to occur. Further information about it would be most welcome.

Nt occurred at three of the soil sites which were sampled. On Blelham Tarn itself it was relatively numerous in the inflow streams but rather less so in the outflow. On the other hand it was barely represented in lake mud or in the water. These records are suggestive of a soil organism the spores of which are readily washed into streams and rivers but which do not develop there.

Considering Lspi and Sspi it was perhaps significant that while both were recovered from soil the former was restricted to the two waterlogged sites. There were fluctuating but persistent records of both in inflow streams and rivers. As far as lake water and mud was concerned Lspi was the most numerous named category after *Micromonospora* and Streptomycete, in contrast to Sspi which was barely represented. In stream mud Lspi was present in even greater proportion, displacing *Micromonospora*. It is concluded that Lspi is a truly aquatic form while the evidence for Sspi is so far inadequate.

In this study on aquatic Actinomycetes, as in the previous one on allochthonous leaf forms, a large number of sterile isolates was obtained. It is known that isolates of this kind may sometimes be induced to yield further information. For example it was shown (WILLOUGHBY, BAKER & FOSTER, 1968) that the application of a humic acids growth medium induced sporangium formation in particular

strains of the Actinoplanaceae which had hitherto remained sterile on standard agars such as chitin and starch-casein. Although the sterile isolates are often small, slow growing and ostensibly unrewarding, to make these studies more complete, it is essential that they should not be totally neglected in favour of the more obvious taxa.

Summary

A study was made of the Actinomycetes occurring in aquatic environments of the English Lake District. Lake water often contained few Actinomycetes; but the number recovered seemed to show a correlation with the total ionic concentration of the water. Stream and river water was more productive and one group, the Actinoplanaceae, was especially prominent. Mild chemical pollution by farmyard washings did not discourage the Actinoplanaceae of streams, on the contrary more were produced under these conditions. The bottom mud of lakes yielded many Actinomycetes, *Micromonospora* and Streptomycetes being predominant. *Nocardia*-type, Lspi and Sspi were three forms, apparently previously undescribed, which were encountered in this investigation. *Nocardia*-type appears to be a soil organism which is washed into the aquatic environment. Lspi was numerous in lake water and mud and seems to be a truly aquatic form. The evidence for Sspi is indecisive.

Zusammenfassung

Die Actinomycetes wurden in wässerichen Umgebungen im Englischen Seengebiet studiert. Seewasser enthält oft einige Actinomycetes; aber die enthaltene Zahl ist von der totalen Ion Konzentration abhängig. Bach- und Flußwasser erzeugte mehr, und eine Gruppe, die Actinoplanaceae, war besonders zahlreich. Milde chemische Verunreinigungen bei landwirtschaftlichen Waschungen verhindern die Actinoplanaceae von Flüssen nicht, im Gegenteil, viele wurden unter diesen Zuständen erzeugt. Der Grundschlamm von Seen enthält viele Actinomycetes, *Micromonospora* und Streptomycetes waren vorherrschend. *Nocardia*-type, Lspi und Sspi waren drei Arten, scheinbar früher unbeschrieben, welche in dieser Untersuchung gebräuchlich waren. *Nocardia*-type scheint organische Erde zu sein welche in den wasserichen Umgebungen gewaschen wird. Lspi war in Seewasser und Schlamm zahlreich und scheint eine wahre wasserliche Art zu sein. Der Beweis für Sspi ist unentscheidend.

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