MYXOBACTERIA

J. V. BHAT AND NIRMALA SUNDARRAJ
Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore-12

THE existence of an aesthetically fascinating group of bacteria, the fruiting myxobacteria, was first recognized by Thaxter[1]. That the characteristic properties of these organisms, like, (1) their gliding non-flagellar motility, (2) conspicuous production of slime, and (3) the life-cycle consisting of a vegetative stage followed by a fruiting stage, were quite unique among the Schizomycetes was at the same time recognised. In the succeeding few years Thaxter collected several of these forms[2-4] and Jahn[5] described a few more species and placed all the then known species in a new order 'Myxobacterales' ; this represents the first attempt at classifying myxobacteria[5].

Organisms closely related to the above forms but lacking the capacity to form fruiting structures had been encountered frequently in decomposing cellulose. Stanier[6-8] recognized their relation to fruiting myxobacteria and placed them in two new genera *Cytophaga* and *Sporocytophaga*. In the latter genus were included those organisms which formed microcysts (resting cells) but not fruiting bodies. He[6] redefined the order 'Myxobacterales' to include five families, *viz.*, *Archangiaceae*, *Sorangiaceae*, *Polyangiaceae*, *Myxococcaceae* and *Cytophagaceae*. Thus Jahn's system of classification revised by Stanier is currently in vogue[9]. The lower myxobacteria will be in this review referred to as 'cytophagas' and the fruiting myxobacteria as 'myxobacters'.

The information accumulated over the years on myxobacters has been ably reviewed by Dworkin[10] in 1966. It may however be pointed out that since Stanier's review[8] on cytophagas published in 1942, only a little has been contributed to their study and all that is known has been discussed in a recent symposium[11-17].

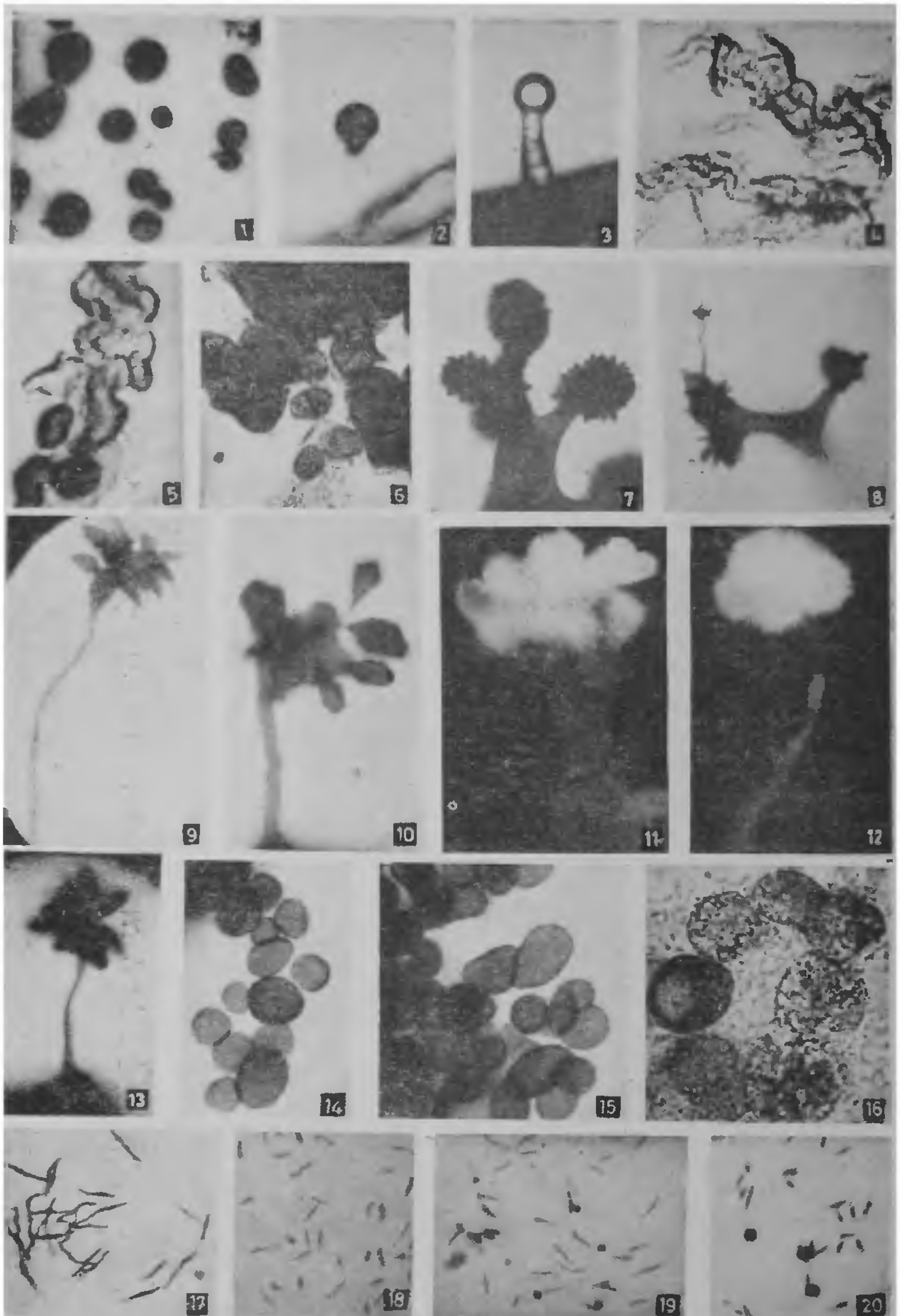
Ecology.—Myxobacteria are widely distributed in nature, in soil, composts, decaying plant debris, animal dung, bark of living trees and in marine environments. Although earlier workers thought them to be common inhabitants of animal dung, they do not seem to be part of the animal excretory microflora prior to contact of the animal dung with soil. The fact that myxobacters can lyse and grow on intact bacterial cells may explain their frequent appearance in dung constituting, as it does, of innumerable bacterial cells. Available information[1-4, 18-48] on the occurrence of myxobacteria in various parts of the world indeed point them to be ubiquitous in nature. Although not many have investigated on the occurrence of myxobacteria in the tropical countries, published reports[44,49,50] show the organisms to be widely distributed in the Indian soils.

Life-Cycle, Morphology and Morphogenesis.—Fruiting body formation involves the aggregation of cells. In other words, vegetative cells start moving towards definite points and heap up in masses embedded in slime. The fruiting bodies thus formed vary in morphology from a simple spheroid or ovoid group to elaborate brightly coloured structure with a stalk, branching cystophores and cysts containing the resting cells (Figs. 1 to 16). The colour of fruiting bodies usually varies from green, yellow, orange and red to brown.

Recently McCurdy[51] demonstrated that in the fruiting bodies of *Chondromyces crocatus* the cysts are bound by discrete layer of slime enclosing the closely packed resting cells. The slime stalk was shown to consist of vertical parallel empty tubules through which the cells got migrated.

To explain myxobacterial gliding motility various hypotheses have been put forth, *e.g.*, rhythmic cellular contraction[52], the excretion of slime which pushes along the cells[53], flexious nature of the cells due to a mucilagenous band encircling the cells[54,15] ; a possible role by rhapidosomes has also been postulated[10]. However, the experimental evidences are not convincing to support any of the above hypotheses. Stanier[7] observed a marked inclination of cytophagas to move along the lines of physical stress on the surface, a phenomenon referred to as elasticotaxis.

That the aggregation process leading to fructification is directed by chemotaxis was first suggested by Bonner[55]. Subsequent reports[56-61] have convincingly proved the association of chemotactic substance(s) in the aggregation process in *Myxococcus* species, but the chemical nature of the substance(s) has not yet been established.



FIGS. 1–20. Fruiting bodies of : (1) *Myxococcus fulvus*, × 180; (2) *M. ovalisporus*, × 200; (3) *M. stipitatus*, × 200; (4) *Archangium gephyra*, × 200; (5) *Archangium primigenium*, × 250: (6) *Polyangium fuscum* (cysts surrounded by slime envelope), × 250; (7) *Chondromyces crocatus*; × 250; (8) *Ch. crocatus*, showing an abnormal branching at the apex of the mass of cysts, × 180; (9) *Ch apiculatus*, × 270; (10) *Ch. auranliacus* × 300; (11) *Ch. cylindricus*, × 320; (12) *Ch. thaxteri*, × 250; (13) *Ch. pediculatus*, × 200; (14) *Chondrococcus megalosporus*, × 180; (15) *Chondrococcus* species, × 180; (16) *Angiococcus* species, × 320; (17) Vegetative cells of *Chondrococcus* species, × 2,500; (18) Vegetative and resting cells of *Chondromyces crocatus*, × 1,500; (19) Vegetative cells and microcysts of *M. stipitatus*, × 1,500; (20) Vegetative cells and microcysts of *M. virescens*, × 1,500.

Electron micrographs of thin sections of vegetative cells of *M. xanthus* indicate that its fine structure grossly resembles that of eubacterial cells[62], except that the former shows the presence of subcellular particles composed of RNA and proteins, called rhapidosomes. Rhapidosomes have been detected in *Archangium* species[63], *Chondrococcus columnaris*[64], *Sporocytophaga myxococcoides*[54], *M. xanthus* and *Cytophaga* sp[10]. Presence of peptidoglycans, similar in composition to that found in the eubacteria, has been reported in cell walls of both fruiting and non-fruiting myxobacteria[65]. An increased cross linking of the peptidoglycans through diaminopimelic acid during microcyst formation has been observed[66].

According to Thaxter[3] microcyst formation takes place by direct transformation of each vegetative cell by gradual swelling with corresponding shortening of the rod in the direction of the long axis, finally getting converted into a thick-walled refractile spore. However, the recent electron micrographic studies on *M. xanthus*[67] and *Stigmatella aurantiaca*[68] indicate the absence of a thick wall surrounding the microcysts, though they seem to be surrounded by an adherent capsule. Capsule formation has been shown to limit expansion of the growing cell[69]. Though the chemical nature of the capsular material has not yet been determined, the water soluble slime produced by *M. xanthus* was shown to contain protein, polysaccharide, lipid and nucleic acid.[70] The extracellular slime of *Chondrococcus columnaris* was determined to be of high molecular weight and made up of homopolymer of galactosamine[71].

Microcyst formation is a feature generally associated with fructification occurring in deficient media but not in rich nutrient media. Accordingly, Adye and Powelson[72] reported a method for converting within about 2–3 days vegetative cells into microcysts. Dworkin's group[73-74] developed an excellent method for the rapid conversion of vegetative cells to microcysts, which consists essentially of using glycerol as an inducer in the presence of divalent cations, Ca and Mg. Later, they showed that glycerol could be substituted by a variety of primary alcohols[75]. Recently, Sadler[76] proved that even monovalent cations like Li, NH_4, K and Rb also could induce morphogenesis in *Stigmatella aurantiaca*.

With a suitable method like glycerol induction, it was possible to study the structural and molecular changes taking place in the cell during morphogenesis. Sadler and Dworkin[77] demonstrated that both RNA and protein syntheses were obligatory for microcyst development in *M. xanthus*. Likewise, Bacon and Rosenberg[78] established that all classes of RNA including *r*-RNA were synthesized during microcyst formation.

Employing hybridization technique, Okano *et al.*[79] demonstrated that whereas certain *m*-RNA species were synthesized only during vegetative growth, some other types were synthesized only during microcyst formation. Detailed information on the rates of RNA synthesis, protein synthesis and growth during division cycle have been recently reported by Rosenberg and co-workers[80-83]. They also studied uptake of K^+ during microcyst formation and showed that there occurred a considerable binding of K^+ to the surface of microcysts[84].

Thaxter[9] accurately described the process of germination of microcyst to consist of slight enlargement of microcyst followed by a rod emerging out of the microcyst, which then elongates and slips out leaving an empty sheath. Recent electron microscopic studies[62-67] have confirmed the remarkably accurate observation of Thaxter. Microcyst germination has been shown to be induced by protein hydrolysates, certain amino acids like glycine, alanine, valine, aspartic acid and also by inorganic ions like PO_4^{---}, Mg^{++}, Ca^{++} and NH_4^+[85].

Quehl[86] was the first to show that nutrients controlled fructification in myxobacters. He noticed that high peptone concentration inhibited fructification in some myxobacters. This effect was demonstrated to be due to specific inhibitors like phenylalanine and tryptophan in *Myxococcus*[87-88] and in *Chondromyces crocatus*[92]. Similar effect was demonstrated to be due to methionine also[89-90]. Requirement of divalent cations like Ca, Mg and Sr for fructification has been also reported[91-92].

Isolation.—Most of the cytophagas isolated so far are able to utilise at least one polysaccharide like cellulose, chitin, starch or agar as the sole source of carbon and energy ; hence enrichment culture technique is the method of choice to make the cytophaga population predominant from a source comprised of mixed population. For another group of cytophagas, consisting of facultatively anaerobic species as occurring in freshwater and marine environments, the same procedure may not yield result as they do not attack polysaccharides[47-93]. The fish pathogen, *Cytophaga columnaris*, is,

for example, non-polysaccharolytic, and was isolated by merely plating the material from gills or lesions of diseased fish, on a medium containing complex carbon and nitrogen sources in low concentrations[18,94-96]. Various other methods of isolating cytophagas have been presented in two recent symposia[13,97].

Myxobacters can be obtained in gross cultures very easily because of their tendency to swarm, and form fruiting bodies when suitable conditions are provided. Such a method was first developed by Krzemieniewskis[29] who used sterile rabbit dung pellets to enrich myxobacteria from soil.

The observation that myxobacters can lyse and grow on several eubacterial cells was made by Singh[43] who developed a new method by placing heavy circles of *Aerobacter* cells on non-nutrient agar and placing small samples of soils on them. Because myxobacteria swarm across the circles, lyse the *Aerobacter* cells, and finally form fruiting bodies, their isolation becomes easy. By incubating bark of trees and decaying wood (good sources of myxobacters) in humid atmosphere also myxobacters can be obtained in gross cultures[41].

Purification of myxobacters can be achieved by the transference of the fruiting bodies on suitable media followed by repeated transfer of the advancing swarms on fresh media.

Often it is difficult to separate myxobacters from the contaminants which remain embedded in myxobacterial slime. McCurdy[98] developed a method to disperse the myxobacterial cells and then purified by streaking. Contaminating fungi, when present, were eliminable by using antifungal antibiotic like 'Actidione' (Cyclohexamide)[20].

Nutrition.—The cytophagas, which solubilise polysaccharides, can use the products of polysaccharide-hydrolysis as the carbon and energy source. Earlier workers thought that cellulolytic cytophagas can use only cellulose and that other sugars were inhibitory. Stanier[8] showed this inhibitory effect to be due to some toxic products formed from the sugars while autoclaving. He in fact succeeded in growing cytophagas on media containing various sugars sterilised by filtration. Most cytophagas can use inorganic salts or organic compounds as nitrogen source for their growth; some agar decomposing cytophagas however demand a complex nitrogen source like peptone or yeast extract.

All of the chitinolytic and cellulolytic cytophagas are obligate aerobes, except a chitinolytic *Cytophaga*, viz., *C. johnsonii* variant which can respire anaerobically using nitrate as electron acceptor[45].

Myxobacters are nutritionally more complex than cytophagas. They require complex nitrogen and carbon sources like peptone, casein hydrolysate, dung extract or living and dead cells of eubacteria.

To a large extent, attempts to define nutrients of myxobacters were unsuccessful due to the lack of a good method for their quantitative estimation. Myxobacters generally do not grow in a dispersed state in liquid media but grow as thin film on the inner surface of the container, either at the bottom or on the sides. General method used for measuring myxobacterial growth consisted of comparing the diameter of the swarm on the agar surface or the thickness of the film formed on the inner surface of the tubes with liquid media: this yielded conflicting results. Recently, Schurmann[99] and the authors independently developed almost identical methods to get dispersed growth of myxobacteria in liquid media and these have proved useful for studying nutritional requirements as well as other biochemical aspects of myxobacters (unpublished data).

Much of the earlier information was based on the study carried out with the dispersed growing variants of *Myxococcus* species. Dworkin[100] and Hemphill and Zahler[101], using one such variant of *M. xanthus*, showed that doubling time of this species in a synthetic medium containing amino acids could be reduced if protein hydrolysates were provided. They also showed that leucine, isoleucine, valine, phenylalanine and methionine were essential for the growth of *M. virescens*.

Data on carbohydrate utilization are very conflicting. Although sugars like arabinose, galactose and polysaccharides like starch and glycogen were shown to stimulate the growth of a few myxobacters[38,100,102,103], their role in the nutrition appears to be a minor one. Similarly no absolute requirement of any vitamin was demonstrated, although a few vitamins have been shown to stimulate the growth of *M. virescens*[103] but not *M. xanthus*[100], *Archangium* sp.[102] and *Chondrococcus columnaris*[104].

Metabolism.—As pointed out earlier, most of the polysaccharolytic cytophagas are obligate aerobes and they seem to bring about

hydrolysis of cellulose to glucose in two stages. The cellobiose formed first is then hydrolysed by a cellobiase to glucose[105-107].

Veldkamp[46] could detect N-acetylglucosamine and glucosamine in culture filtrates of chitin-grown *C. johnsonii*. The present authors were able to detect, besides those two products, glucosaminic acid, gluconic acid and 2-keto-gluconic acid (unpublished data). Another important observation made by the authors was that, unlike other chitinolytic bacteria, some strains of *C. johnsonii* do not liberate chitinase extracellularly but must necessarily be in close contact with the chitin particles in order to hydrolyse it.

Of interest in this context is the reported ability of *Cytophaga albogilva, C. deprimata* and *C. johnsonii* to attack polygalacturonic acid[108]. The enzyme responsible for its breakdown was characterised as an endo-polygalacturonate lyase in all the three species studied[109].

The facultatively anaerobic cytophagas have been shown to convert glucose to pyruvate through the EMP pathway[93,110]. While *C. succinicans* fermented glucose to succinate, acetate and formate[110], *C. fermentans* formed propionate and lactate as well[111].

The presence of citric acid cycle enzymes in vegetative cells and microcysts of *M. xanthus* was demonstrated by Watson and Dworkin[112]. The level of alcohol dehydrogenase was much higher in microcysts than in vegetative cells. Later, they reported that all the enzymes of EMP pathway, except the hexokinase and pyruvate kinase, were also present both in vegetative cells and microcysts[112].

Acetate was shown to be metabolised at a lower rate by microcysts than by vegetative cells. The label from acetate was found to be incorporated in glutamate, aspartate and proline of the protein fraction[10].

Dworkin and Niederpruem[113] studied the terminal electron transport system of *M. xanthus* and reported that both vegetative cells and microcysts have cytochrome system with cytochromes *a*, a_3, *b* and *c* type components.

Scarcely any attempts have been made to study intermediary metabolism of amino acids. The authors' study on the breakdown of phenylalanine by *Myxococcus* species revealed that myxococci hydroxylated phenylalanine to tyrosine, thence transaminated to yield *p*-hydroxyphenylpyruvate before it was further broken down. Some of the identified intermediates in its breakdown were *p*-coumarate *p*-hydroxyphenylacetate, *p*-hydroxyphenylmandelate, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoate and homoprotocatechuate (unpublished data).

Lytic activity.—Ever since Pinoy in 1913 described the bacteriolytic activity in *Chondromyces crocatus*[114], it has been well established that a large number of myxobacteria can lyse other bacteria[43,115-124]. Lysis of both living and heat-killed cells has been repeatedly demonstrated[103,118-121,125-128]. Although mechanism of lysis is still not clear, various reactions involved in the lysis have been demonstrated in several myxobacteria.

Some myxobacters were shown to grow more vigorously on living cells than on heat-killed cells[43,127]. The lytic factor produced by different myxobacteria against living cells was shown to pass through cellophane and to be heat-stable[43,123]. As pointed out by Dworkin[10], it is likely that this factor is a polypeptide antibiotic.

Lysis of heat-killed eubacterial cells and of cells disrupted by organic solvents and EDTA was shown to be due to proteolytic enzymes[89,116,119,129]. The findings that this lytic factor could be concentrated by $(NH_4)_2SO_4$ precipitation, was heat-labile, and was non-dialysable, further supported the view that it is an enzyme(s)[116,120].

Ensign and Wolfe[130,131] purified a lytic enzyme from a *Cytophaga* capable of lysing a large number of gram positive and a few gram negative bacteria. The purified homogenous enzyme brought about the lysis of intact and heat-killed *Arthrobacter* cells, as well as their isolated cell walls. It had both cell wall lytic and proteolytic activity. Cell wall was lysed by cleaving the peptide bond in the glycosamino peptides[132-134]. In addition, this enzyme was able to lyse isolated cell walls of *Staphylococcus aureus* and *Micrococcus lysodeicktieus*[133]. Physiological properties, amino acid composition and substrate specificity of this enzyme were studied by Jackson and Wolfe[135]. Strominger and his colleagues, who have examined extensively bacterial cell walls, recently used the above *Cytophaga* enzyme for characterising peptidoglycans of different bacteria[134-136].

In contrast to the *Cytophaga* sp. a *Sorangium* sp. was shown to liberate at least two extracellular enzymes, a protease and lysin[117]. Lysin of *M. xanthus* was also separable from protease[137]. Some other myxobacteria have been shown to excrete ribonuclease and

deoxyribonuclease at low levels[117-138]. Sudo and Dworkin[139], who analysed extracellular cell walls lytic system of *M. xanthus*, demonstrated the presence of at least three activities—acetyl glucosamidase, acetyl-muramidase and peptidase in it.

The culture filtrates of *M. xanthus* were able to bring about lysis of yeast cells sensitised earlier by heat, acetone, or ethylacetate treatment[140]. The product of lysis were non-dialysable polysaccharides. Bacon *et al.*,[141] demonstrated the lysis of isolated yeast cell wall by a *Cytophaga johnsonii* enzyme.

Recent reports indicate lysis of blue-green algae by myxobacteria[142-144]. Shilo[145] drew attention to the essentiality of close contact between myxobacterial cells and algal cells for the lysis indicating thereby that the lytic system was probably non-diffusible.

Antibiotic activity.—Antibiotic production was first recognized by Oxford[121] in *M. virescens*. This antibiotic and antibiotics from other species were active only against gram positive bacteria. Kato[26] tested about 400 cultures of myxobacters and found that two strains of *M. fulvus* produced antibiotics which were active against both gram positive and gram negative bacteria. Kletter and Henis[146] were not able to detect any antibiotic in culture filtrates of *M. fulvus* or *M. virescens* strains tested by them.

Peterson *et al.*,[138] isolated an antibiotic from a *Sorangium* sp. to a high degree of purity. It was a broad spectrum antibiotic, stable in organic solvents at 4 C and was active against several gram positive and gram negative species.

Extracts of *M. virescens* were also reported to inactivate influenza A and B and mumps virus[147]. The active principle was dialysable and was partially purified by paper chromatography and electrophoresis[148].

Pigments.—Both cytophagas and myxobacters are brightly pigmented. The pigments are carotenoid in nature and non-diffusible[149-152]. Their formation and nature are influenced by the presence or absence of light during incubation[151-153] Burchard and Dworkin[149] showed that both photoinduced carotenoids and the diffusible photosensitising pigment protoporphyrin IX[154] did not appear until the end of exponential growth in *M. xanthus*. They also reported that the carotenoids have a photoprotective role in this organism. Richembach and coworkers[155-158] have carried out detailed studies on characterization of myxobacterial carotenoids. They showed that *M. fulvus* synthesized about 50–60 different carotenoids of which at least four contained one or more secondary hydroxyls not common in bacterial carotenoids. They also revealed the presence of several more carotenoids, not encountered in bacteria, especially the carotenoid-fatty acid ester. It was observed that from chemosystematic point of view pigments of *Sorangium compositicum* differed from that of *Stigmatella* and *Myxococcus*. Ketocarotenoids present in the latter two species were totally absent in the former which was shown to contain a number of non-polar carotenoids and four new carotenoid rhamnosides not described in any other bacteria previously.

Pathogenicity.—A cytophaga species, *C. columnaris* and *Chondrococcus columnaris* have been reported to be pathogenic to fish[11,94,95,159]. Garnjobst[94] isolated *Cytophaga columnaris* from infected bull-heads. Graf[160-162] isolated an anaerobic myxobacterium from human oral cavity and showed its ability to induce cytopathogenic effects in tissue cultures.

Bacteriophage.—Bacteriophages have been reported only for a few species of myxobacteria, *i.e.*, *Chondrococcus columnaris*, *M. xanthus*[163-167] and *C. marinoflava*[168]. They are double stranded DNA phages. The *M. xanthus* phage (MX-1), isolated by Burchard and Dworkin[165] is similar to other DNA phages. They also reported that the cells lose their ability to adsorb phage during glycerol induction of microcyst formation.

Taxonomy.—As pointed out earlier, Stanier revised the scheme of 'Myxobacterales' in *Bergey's Manual*[9] to include *Cytophaga* and *Sporocytophaga*. In his view cytophagas were quite different from other gliding filamentous bacteria belonging to Beggiatoaceae and closely resembling some blue-green algae. However, the genus *Flexibacter*, described by Soriano and Lewin[169], consisting of nutritionally unspecialised gliding bacteria, share many common features with cytophagas.

Stanier's definition of the order 'Myxobacterales' needs a little modification. Firstly, electron micrographs have shown the presence of a rigid cell wall in myxobacteria[62], considered earlier as absent. Secondly, the property of division by constriction, thought to be restricted to myxobacteria, is now recognised in other organisms like *Flexibacter*, *Oscillatoria* and *Microcoulla*[167,170]. Because of wide difference in their base compositions, relationship of cytophagas to myxobacters has been chal-

lenged by Marmur *et al.*,[171] Myxobacters have G + C values of 65–70%[10,172] as compared to that of cytophagas 35–40%[173]. However, Follet and Webley[174] found many common properties in the fine structure of vegetative cells of *Cytophaga* and fruiting myxobacteria.

Since the publication of the last *Bergey's Manual,* a few new species have been added to the genus *Cytophaga.* Some of the newly described species are facultatively anaerobic fresh and sea-water forms. Graf[160,175] and Bauer[176] described some new genera and species mainly on the basis of their ability to form 'spheroids'. The classification seems to be invalid because *Cytophaga* are known to form involution forms in old cultures. In Dworkin's[10] opinion creation of new genera and species on the basis of spheroplast formation and faulty morphological observations can only lead to confusion of an already difficult taxonomic situation.

Classification of fruiting myxobacteria is mainly based on pigmentation, morphology of fruiting bodies and the shape of resting cells. A careful observation under specified conditions is necessary to record the above details. Pigmentation is shown to vary with the physical and nutritional variations. Myxobacters frequently lose their ability to fructify on cultivation. However, use of biochemical characteristics, base composition, and phage typing techniques which are now in use for other bacteria, cannot be employed easily for myxobacters which are rather difficult to cultivate despite some attempts[98,122,177]. Recently McCurdy[178-180] has discussed the reorganisation of some of the species grouped under *Polyangium, Podangium, Sorangium* and *Chondromyces.*

Conclusions.—From what has been discussed above it is clear that although earlier workers were mainly concerned with morphology and taxonomy of myxobacteria recent interests have been centred around their fine structure, nutrition, biochemical and molecular aspects of morphogenesis and so on. The difficulty experienced in growing myxobacters dispersedly in liquid medium had the effect of confining the work to a dispersed growing variant of *M. xanthus.* Handling of the other species now appears to be simplified in view of suitable methods recently developed to get them in dispersed state in liquid cultures. Another obstacle in applying routine microbiological techniques in their study is the excretion of large amounts of slime in which the cells remain embedded—a difficulty which does not appear to be formidable if a solution can be found to remove the slime without affecting the cells.

Myxobacteria, with a complex developmental cycle, offer a new tool in the developmental biology which has been an exciting field of research in recent years. So far, these investigations have been carried out mainly on myxomycetes which have a similar life-cycle.

More investigations on myxobacterial metabolism are likely to unravel new pathways and enzymes which may prove unique to this group. With more attention one may expect myxobacteria to provide answers to some of the basic questions in Microbiology.

1. Thaxter, R., *Botan. Gax.,* 1892, 17, 389.
2. —, *Ibid.,* 1893, 18, 29.
3. —, *Ibid.,* 1897, 23, 395.
4. —, *Ibid.,* 1904, 37, 405.
5. Jahn, E., *Kryptogamen flora der Mark Brandenberg,* 1911, 5, 187.
6. Stanier, R. Y., *J. Bact.,* 1940, 40, 619.
7. —, *Ibid.,* 1942, 44, 405.
8. —, *Bact, Rev.,* 1942, 6, 143.
9. Breed, R. S., Murray, E. G. D. and Smith, N. R., *Bergey's Manual of Determinative Bacteriology,* 7th ed., Williams and Wilkins, Co., Baltimore, Md., 1957.
10. Dworkin, M., *Ann. Rev. Microbiol.,* 1966, 20, 75.
11. Anderson, J. I. W. and Conroy, D. A., *J. Appl. Bact.,* 1969, 32, 30.
12. Coucke, P., *Ibid.,* 1969, 32, 24.
13. Mitchell, T. G., Hendrie, M. S. and Shewan, J. M., *Ibid.,* 1969, 32, 40.
14. Peterson, J. E., *Ibid.,* 1969, 32, 5.
15. Schmidt-Lorenz, W., *Ibid.,* 1969, 32, 22.
16. Shewan, J. M., *Ibid.,* 1969, 32, 1.
17. Weeks, O. B., *Ibid.,* 1969, 32, 13.
18. Anacker, R. L. and Ordal, E. J., *J. Bact.,* 1959, 78, 25.
19. Brockman, E. R., *Ibid.,* 1967, 94, 1253.
20. — and Boyd, W. L., *Ibid.,* 1963, 86, 605.
21. Geitler, L., *Arch. f. Protistenk.,* 1925, 50, 67.
22. Imshenetsky, A. A. and Solntseva, L., *Bull. Acad. Sci., U.S.S.R. Ser. Biol.,* 1936, No. 6, 1115.
23. and —, *Microbiologya,* 1937, 6, 3.
24. Jeffers, E. E., *Int. Bull. Bact. Nomencl. Taxon.,* 1964, 14, 115.
25. Johnson, D. E., *J. Bact.,* 1932, 24, 335.
26. Kato, H., *Ecol. Rev.,* 1955, 14, 25.
27. Krzemieniewska, H., *Arch. Mikrobiol.,* 1933, 4, 394.
28. — and Krzemieniewski, S., *Acta Soc. Botan. Polon.,* 1926, 4, 3.
29. — and —, *Ibid.,* 1927, 5, 79 and 102.
30. — and —, *Ibid.,* 1928, 6, 46.
31. — and —, *Ibid.,* 1930, 7, 250
32. — and —, *Bull. Int. Acad. Cracovie (Acad. Pol. Sic.) Ser. B. Sci. Nat.,* 1937, 75, 11.

33. McCurdy, H. D., *Can. J. Microbiol.*, 1969, **15**, 1453.
34. Mishustin, E. N., *Mikorbiologiya.*, 193?, 6, 12?4.
35. —, *Ibid.*, 1938, ?, 427.
36. Noren, B., *Sv. Bot. Tidskr.*, 1950, 44, 108.
37. —, *Nature (Lond.)*, 1951, **167**, 225.
38. —, *Botan. Tidskr.*, 1952, 46, 325.
39. Peterson, J. E., *Mycologia.* 1958, **50**, 628.
40. —, *Ibid.*, 1959, 51, 1.
41. —, *Ibid.*, 1959, 51, 163.
42. Sabado, S. A., *Res. Knj.*, 1957, 312, 5.
43. Singh, B. N., *J. Gen. Microbiol.*, 1947, 1, 1.
44. —, Mathew, S. and Sreenivasaya, M., *Symp. Antibiotics.* Saraswathy Press Ltd., Calcutta, 1958.
45. Stanier, R. Y., *J. Bact.*, 194?, 53, 297.
46. Veldkamp, H., *Meddedelingen Landbouwhogesched Wageningen*, 1955, 55, 127.
47. —, *J. Gen. Microbiol.*, 1961, 26, 331.
48. Yoshii, Y., *Sci. Rept. Tohoku Imp. Univ. Biol.*, 1926, **1**, 2??.
49. Kamat, N. K., "Investigations of fruting Myxobacteria, their occurrence and importance," *Master's Thesis*, Univ. of Bombay, India, 1966.
50. Warke, G. M. and Dhala, S. A., *J. Gen. Microbiol.*, 1968, 51, 43.
51. McCurdy, H. D., *Arch. Mikrobiol.*, 1969, **65**, 380.
52. Kuhlwein, H., *Ber. Deut. Botan. Ges.*, 1957, 70, 227.
53. Jahn, E., In *Beitgrage zur Botanischen Protistologie Leipzig Geb. Borntraeger*, 1924, p. 1.
54. Graf. W., *Arch. Hyg. Bakteriol.*, 1965, **149**, 518.
55. Bonner, J. T., In *Morphogensis* Atheneum, New York, 1963, p. 296.
56. Fluegel, W., *J. Bact.*, 1963, **81**, 99.
57. —, *Minn. Acad. Sci., Proc.*, 1963, 30, 120.
58. —, *Ibid.*, 1964, 31, 114.
59. Jenning, J., *Nature, (Lond).*, 1961, **190**, 190.
60. Lev, M., *Ibid.*, 1954, 173, 501.
61. McVittie, A. and Zamler, S. A., *Ibid.*, 1962, **194**, 1299.
62. Voelz, H. and Dworkin, M., *J. Bact.*, 1962, **84**, **943.**
63. Reichenbach, H., *Arch. Mikrobiol.*, 1965, 50, 246.
64. Pate, J. L. and Ordal, E. J., *Bacteriol. Proc.*, 1965, p. 32.
65. Verma, J. P. and Martin, H. H., *Arch. Mikrobiol.*, 1967, 59, 355.
66. Johnson, R. and White, D., *Bacteriol. Proc.*, 1971, p. 40.
67. Voelz, H., *Arch. Mikrobiol.*, 1966, 55, 110.
68. Reichenbach, H., Voelz, H. and Dworkin, M., *J. Bact.*, 1969, **97**, 905.
69. Gonda, S. R. and Voelz, H., *Bacteriol. Proc.*, 1971, p. 39.
70. Holt, J. G. and Powelson, D. M., *Ibid.*, 1960, p. 59.
71. Johnson, J. L. and Chilton, W. S., *Science*, 1966, **152**, 1?47.
72. Adye, J. C. and Powelson, D. M., *J. Bact.*, 1961, 81, 780.
73. Dworkin, M. and Gibson, S. M., *Science*, 1964, **146**, 243.
74. — and Sadler, W., *J. Bact.*, 1966, **91**, 1516.
75. Sadler, W. and Dworkin, M., *J. Bact.*, 1966, 68, 405.
76. —, *Ibid.*, 1970, **101**, 325.
77. — and Dworkin, M., *Ibid.*, 1966, **91**, 1520.
78. Bacon, K. and Rosenberg, E., *Ibid.*, 1967, **97**, 1883.
79. Okano, P., Bacon, K. and Rosenberg, E., *Ibid.*, 1970, 104, 275.
80. Zusman, D. and Rosenberg, E., *Ibid.*, 1970, 96, 981.
81. — and —, *J. Mole. Biol.*, 1970, **49**, 609.
82. — and —, *J. Bact.*, 1971, **105**, 801.
83. —, Gottlieb, P. and Rosenberg, E., *Ibid.*, 1971, 105, 811.
84. Giberman, E. and Rosenberg, E., *Ibid.*, 1970, **104**, 87.
85. Ramsey, W. S. and Dworkin, M., *Ibid.*, 1968, **95**, 2249.
86. Quehl, A., *Zbl. Bakt. Zabt.*, 1906, 16, 9.
87. Dworkin, M., *J. Bact.*, 1963, 86, 67.
88. Leadbetter, E. R., *Nature*, (*Lond.*), 1963, 200, 1127.
89. McCurdy, H. D., *Can. J. Microbiol.*, 1964, **10**, 935.
90. Hemphill, H. E. and Zahler, S. A., *J. Bact.*, 1968, **95**, 1018.
91. Witkin, S. S. and Rosenberg, E., *Ibid.*, 1970, **103**, 641.
92. McCurdy, H. D. and Khou, B. T., *Can J. Microbiol.*, 1969, **15**, 731.
93. Anderson, R. L. and Ordal, E. J., *J. Bact.*, 1961, **81**, 130.
94. Garnjobst, L., *Ibid.*, 1945, **49**, 113.
95. Ordal, E. J. and Rucker, R. R., *Proc. Soc. Expt. Biol.*, 1944, 56, 15.
96. — and —, *J. Bact.*, 1946, **51**, 579.
97. Veldkamp, H., *Anreicherungskultur und Mutantenauslese.* Ed. Schelegel, H. Zentbt. Bakt. Parasitkde. Supplement Eftl. Stuttgart : Gustav Fischer Verlag. 1965, p. 81.
98. McCurdy, H. D., *Can. J. Microbiol.*, 1963, 9, 282.
99. Schurmann, C., *Appl. Microbiol.*, 1968, 15, 971.
100. Dworkin, M., *J. Bact.*, 1962, **84**, 250.
101. Hemphill, H. E. and Zahler, S. A., *Ibid.*, 1968, **95**, 1011.
102. McDonald, J. C. and Peterson, J. E., *Mycologia.*, 1962, 54, 368.
103. Noren, B., *Botan. Notiser*, 1955, **108**, 81.
104. Chase, I. M., In *Nutrition of Some Equatic Myxobacteria,* Master's Thesis, Univ. Washington, Seattle, Wash., 1965.
105. Charpentier, M., *Compt. Rend.*, 1963, **256**, 4123.
106. —, *Ann. Inst. Pasteur.*, 1963, **105**, 194.
107. Fahraeus, G. *Symbolae Botanicae Upsaliensis.* 1947, **9**, 2, 5.
108. Kamat, N. K. and Bhat, J. V., *Curr. Sci.*, 1967, 36, 486.
109. Sundarraj, N. and Bhat, J. V., *Arch. Microbiol.*, 1971, 77, 155.
110. Anderson, R. L. and Ordal, E. J., *J. Bact.*, 1961, 81, 139.
111. Bachmann, B. J., *J. Gen. Microbiol.*, 1955, 13, 541.
112. Watson, B. F. and Dworkin, M., *J. Bact.*, 1968, 96, 1465.
113. Dworkin, M. and Niederpruem, D. J., *Ibid.*, 1964, **87**, 316.

114. Pinoy, M. E., *C.R. Acad. Sci.* (*Paris*), 1913, **157**, 77.
115. Beebe, J. M., *Iowa State Coll. J. Sci.*, 1941, **15**, 307.
116. Bender, H., *Arch. Mikrobiol.*, 1962, **43**, 262.
117. Gillespie, D. C. and Cook, F. D., *Can. J. Microbiol.*, 1965, **11**, 109.
118. Noren, B., *Sv. Bot. Tidskr.*, 1955, **49**, 282.
119. —, *Ibid.*, 1960, **54**, 550.
120. —, *Botan. Notiser*, 1960, 113, 320.
121. Oxford, A. E., *J. Bact.*, 1947, **53**, 129.
122. Snieszko, S. F., McAllister, J. and Hitchner, E. R., *Quart. Bull. Polish. Inst. Arts Sci. Amer.*, 1943, 1, 651.
123. Solntzeva, L. I., *Mikrobiologiya*, 1939, **8**, 700.
124. —, *Ibid.*, 1941, **10**, 505.
125. Kamat, N. K. and Dhala, S. A., *Indian J. Microbiol.*, 1968, 8, 69.
126. Noren, B., *Sv. Bot. Tidskr.*, 1953, **47**, 402.
127. Oetker, H., *Arch. Mikrobiol.*, 1953, **19**, 206.
128. Oxford, A. E. and Singh, B. N., *Nature* (*Lond.*), 1946, **158**, 745.
129. Huang, L. Y. and Ensign, J. C., *Bacteriol. Pro.*, 1971, p. 127.
130. Ensign, J. C. and Wolfe, R. S., *J. Bact.*, 1965, **90**, 395.
131. — and —, *Ibid.*, 1966, **91**, 524.
132. Katz, W. and Strominger, J. L., *Biochemistry*, 1967, **6**, 930.
133. Tipper, D. J., Katz, W., Strominger, J. L. and Ghuysen, J. M., *Ibid.*, 1967, **6**, 921.
134. —, Strominger, J. L. and Ensign, J. C., *Ibid.*, 1967, **6**, 906.
135. Jackson, R. L. and Wolfe, R. S., *J. Biol. Chem.*, 1968, **243**, 879.
136. Strominger, J. L. and Ghuysen, J. M., *Science*, 1967, **156**, 213.
137. Hart, B. A. and Zahler, S. A., *J. Bact.*, 1966, **92**, 1632.
138. Peterson, E. A., Gillespie, D. C. and Cook, F. D., *Can. J. Microbiol.*, 1966, 12, 221.
139. Sudo, S. Z. and Dworkin, M., *Bact. Proc.*, 1968, p. 20.
140. Bender, H., *Arch. Mikrobiol.*, 1963, **45**, 407.
141. Bacon, J. S. D., Milne, B. D., Taylor, I. F. and Wobley, D. M., *Biochem. J.*, 1965, **95**, 18C.
142. Howe, H. B., *Bacteriol. Proc.*, 1968, p. 48.
143. Stewart, J. R. and Brown, R. M., Jr., *Science*, 1969, **164**, 1523.
144. Wu, B., Handy, M. K. and Howe, H. B., *Bact. Proc.*, 1968, p. 48.
145. Shilo, M., *J. Bact.*, 1970, **104**, 453.
146. Kletter, B. and Henis, Y., *Can. J. Microbiol.*, 1963, **9**, 577.
147. Katzenberger, I., Kuhlwein, H. and Kausche, G. A., *Zentr. Bakteriol. Parasitenk; Abt.* 2, 1956, **109**, 478.
148. Katzenberger, I. and Kaushe, G. A., *Naturwissenschaften*, 1957, **46**, 607.
149. Burchard, R. P. and Dworkin, M., *J. Bact.*, 1966, **91**, 535.
150. Greene, J. M. and Leadbetter, E. R., *Bacteriol. Proc.*, 1962, p. 34.
151. — and —, *Ibid.*, 1963, p. 54.
152. — and —, *Ibid.*, 1964, p. 17.
153. Batra, P. P. and Rilling, H. C., *Arch. Biochem. Biophys.*, 1964, **107**, 485.
154. Burchard, R. P., Gordon, S. A. and Dworkin, M., *J. Bact.*, 1966, **91**, 896.
155. Kleinig. H., Egger, K. and Reichenbach, H., *Arch. Mikrobiol.*, 1969, **68**, 210.
156. —, —, — and Achenbach, H., *Ibid.*, 1970, 74, 223.
157. Reichenbach, H. and Klenig, H., *Ibid.*, 1971, **76**, 364.
158. Kleinig, H., Reichenbach, H., Achanbach, H. and Stadler, J., *Ibid.*, 1971, **78**, 224.
159. Borg, A. F., *Wilde Dis.*, 1960, **8**, 85.
160. Graf, W., *Arch. Hyg. Bakteriol.*, 1961, **145**, 405.
161. —, *Ibid.*, 1962, **146**, 481.
162. —, *Ibid.*, 1962, **146**, 492.
163. Anacker, R. L. and Ordal, E. J., *J. Bact.*, 1955, **70**, 738.
164. Burchard, R. P. and Dworkin, M., *Bacteriol. Proc.*, 1964, p. 118.
165. — and —, *J. Bact.*, 1966, **91**, 1305.
166. Kingsbury, D. T. and Pacha, R. E., *Bacteriol. Proc.*, 1964, p. 118.
167. — and Ordal, E. J., *J. Bact.*, 1966, **91**, 1327.
168. Colwell, R. R., Citarella, R. V. and Chen, P. K., *Can. J. Microbiol.*, 1966, **12**, 1099.
169. Soriano, S. and Lewin, R. A., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 1965, 31, 66.
170. Steed, P. and Murray, R. G. E., *Can. J. Microbiol.*, 1966, **12**, 263.
171. Marmur, J., Falkow, S. and Mandel, M., *Ann. Rev. Microbiol.*, 1963, **17**, 329.
172. Mandel, M. and Leadbetter, E. R., *J. Bact.*, 1965, **90**, 1795.
173. McCurdy, H. D. and Wolf, S., *Can. J. Microbiol.*, 1967, 13, 1707.
174. Follett, E. A. C. and Webley, D. M., *Antonie van Leeuwenhoek. J. Microbiol. Serol.*, 1965, **31**, 361.
175. Graf, W., *Arch. Hyg. Bakteriol.*, 1962, 146, 114.
176. Bauer, L., *Ibid.*, 1962, 146, 392.
177. Grilione, P., *J. Bact.*, 1968, **95**, 1202.
178. McCurdy, H. D., *Intern. J. Syst. Bacteriol.*, 1970, **20**, 283.
179. —, *Ibid.*, 1971, **21**, 40.
180. —, *Ibid.*, 1971, **21**, 50.