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AND
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Preface

This volume is number 15 in the Technical Series of the Society for Applied Bacteriology. It contains papers that are based on contributions made at the Autumn Demonstration Meeting of the Society which was held at the Polytechnic of the South Bank in London on 18 October 1978.

The meeting aimed to bring together current research and modern techniques that are concerned, not with aspects of the growth of micro-organisms under optimal conditions, but rather with growth and survival under conditions that are far from optimal. Such conditions vary greatly in type, but have in common that they are widely regarded as imposing some sort of additional 'stress' on the microbial cell, and can all be regarded as more or less 'extreme'.

Extreme conditions covered by the various demonstrations included physical extremes, e.g. of heat, ionizing radiation, hyperbaric oxygen, reduced water activity and raised osmotic pressure. Other demonstrations were concerned with chemical and biochemical extremes, e.g. of low and high pH values, high concentrations of sulphur dioxide, nitrite, heavy metals, phenol, other disinfectants and chemicals, detergents and oils, as well as considering the somewhat neglected, but ecologically most important, environmental extreme represented by the very low nutrient concentrations in oligotrophic waters.

The organisms covered encompassed vegetative and spore forms of bacteria yeasts and moulds, and included organisms of public health significance as well as those involved in biodeterioration and those of ecological importance in the natural environment.

Thus, whilst not covering every possible environmental extreme to which micro-organisms have become adapted, the topics contributed to the meeting were sufficiently diverse to give a comprehensive overview, with some topics treated in considerable depth. This is reflected in the present volume, which contains papers based on the majority of the demonstrations that were shown.

We therefore thank all those who contributed to the meeting and who so promptly prepared the manuscripts on which this book is based. We also thank Mr Arnold Fox, and other members of the staff of the Department of Applied Biology at the South Bank Polytechnic for the hard work involved in hosting the meeting.

March 1980

G. W. Gould
Janet E. L. Corry

Contents

Microbial Growth and Survival in Oligotrophic Freshwater Environments

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The many microbial habitats and complex microbial communities found in the aqueous environment are continually changing in response to biotic and abiotic stress. The micro-organisms inhabiting such ecosystems are, consequently, under severe selective pressure to evolve means of coping with the changing nutrient and physiological conditions. This article is concerned with microbial adaptation to one of these stresses, low-level nutrient situations (i.e. oligotrophic freshwater systems) and concentrates primarily on the so-called budding and prosthecate bacteria and their responses to changes in nutrient status.

Micro-organisms possessing appendages which are an integral part of the cell have been collectively termed prosthecate bacteria, the term prostheca having been proposed by Staley (1968) and defined as follows: 'a semi-rigid appendage extending from a prokaryotic cell with a diameter which is always smaller than that of the mature cell and which is bounded by the cell wall'. Prosthecate bacteria can be sub-divided into two groups (Table 1): (i) those in which the prostheca(e) play an apparently obligate role in reproduction; (ii) those in which the prostheca(e) may be induced or repressed in response to environmental stimuli and/or play no part in the reproductive process.

These organisms are not characterized by a common physiological or nutritional property (Table 1) they are, however, indigenous to aqueous environments, particularly oligotrophic waters.

These bacteria seem to have come to terms with the nutrient variability and severity of an oligotrophic aqueous environment by the following means: (i) movement by flagella and gas vacuole production, i.e. chemotactic and phototactic responses; (ii) adhesion to surfaces; (iii) colonization of favourable ecological niches; (iv) specialized dispersal phases; (v) formation of prosthecae.

TABLE 1
Sub-division of the prosthecate bacteria and their nutritional status

Prosthecate bacteria	Group (i) prostheca have an obligate role in reproduction	Group (ii) prostheca non-obligate/ not involved in reproduction	Nutritional status
Hyphomicrobium	+	—	aerobic heterotroph
Rhodomicrobium	+	—	photoheterotroph
Rhodopseudomonas palustris	+	—	photoheterotroph
Rhodopseudomonas viridis	+	—	photoheterotroph
*Caulobacter**	+	(+)	aerobic heterotroph
*Asticcacaulis**	+	(+)	aerobic heterotroph
Ancalomicrobium	—	+	aerobic heterotroph
Prosthecomicrobium†	—	+	aerobic heterotroph
Prosthecochloris†	—	+	obligate photoautotroph

**Caulobacter* and *Asticcacaulis* form prostheca as part of the obligate temporal sequence of events occurring during the vegetative cell cycle. However, recent reports (Osley & Newton 1977) suggest that prostheca formation is not an obligate requirement for reproduction.

†Whether *Prosthecomicrobium* (Staley 1968) and *Prosthecochloris* (Gorlenko 1970) will suppress prostheca formation in situations of nutrient excess, in a similar manner to *Ancalomicrobium* (Whittenbury & Dow 1977) is not known.

The prosthecate bacteria, to varying degrees, incorporate all of these adaptations.

Enrichment and Isolation of Prosthecate Bacteria

Group (i) prosthecate bacteria (Table 1)

(prosthecae have an obligate role in reproduction)

This group of prosthecate bacteria present few problems with respect to enrichment and isolation, the procedures outlined below being simple and highly selective.

Hyphomicrobium

The selective pressures used to enrich hyphomicrobia from a wide range of habitats are their preference to grow on one-carbon compounds, particularly methanol, and their potential to use nitrate as an alternative to oxygen as terminal electron acceptor (Attwood & Harder 1972).

Medium. K_2HPO_4 1·74 g; $NaH_2PO_4.H_2O$ 1·38 g; $(NH_4)_2SO_4$ 0·5 g; $MgSO_4.7H_2O$ 0·2 g; $CaCl_2.2H_2O$ 0·025 mg; $FeCl_2.4H_2O$ 3·5 mg per litre of distilled water. This basic medium is supplemented with 0·5 ml of a trace element solution (Harder & Veldkamp 1967), KNO_3 (0·2% w/v) and methanol (0·5% v/v). The pH is adjusted to 7·0 with N NaOH.

Enrichments are carried out in liquid medium under an atmosphere of N_2 at 30°C in the dark (care must be taken to release the gas pressure which increases dramatically as a consequence of vigorous denitrification). Subsequent isolation and purification is performed on solid medium, lacking nitrate, under aerobic conditions at 30°C.

Rhodomicrobium *and* Rhodopseudomonas palustris

These photoheterotrophic bacteria can best be enriched by employing a malate salts medium of pH 5·5 for *Rhodomicrobium* and pH 7·0 for *Rhodopseudomonas palustris* (Whittenbury & Dow 1977). The basic medium consists of: NH_4Cl 0·5 g; $MgSO_4.7H_2O$ 0·4 g; $CaCl_2.2H_2O$ 0·05 g; NaCl 0·4 g; sodium hydrogen malate 1·5 g per litre of distilled water. The pH is adjusted with potassium hydroxide prior to autoclaving and 50 ml of 0·1 M phosphate buffer (of the desired pH) is added aseptically per litre. Incubation is in glass bottles, under N_2 at 30°C with an incident light intensity (tungsten lamps) of 1000 lx. Subsequent isolation and purification is by dilution of the enrichment cultures into agar deeps of the above medium. Alternatively, spread plates, incubated aerobically in the dark, may be used.

Group (ii) prosthecate bacteria (Table 1)

(prostheca not involved in reproduction and may or may not be non-obligate)

Multiappendaged prosthecate bacteria (*e.g.* Ancalomicrobium)

These organisms are the most difficult to enrich and isolate in pure culture *unless* they are severely stressed with nutrient limitation.

Enrichment. Oligotrophic freshwater samples are incubated static at room temperature for a period of 3–4 months—*no nutrient addition.* At the end of this time interval multiappendaged organisms should be observed by microscopy (preferably electron microscopy).

Isolation. Spread plates are prepared from the enrichment sample using both the pellicle and the body of the sample. The latter is important since many of the multiappendaged prokaryotes do not adhere to surfaces and are most abundant in this phase. Plates are incubated at room temperature over a period of a few weeks and as the colonies

appear they are patch plated on to fresh medium and examined by microscopy.

The major problem encountered with these organisms is that an increase in nutrient concentration will repress prostheca formation and morphologically the organism reverts to a rod-shape (this may not, of course, be true of all multiprosthecate bacteria).

Medium. (a) filter sterilized pond water

(b) filter sterilized pond water +0·001 % (w/v) peptone.

The enrichment of *Caulobacter* and *Asticcacaulis* is essentially as described above, these organisms predominate in the pellicle after 2–3 weeks incubation. Isolation and purification is best facilitated on the following medium, incubation being at 30°C: peptone 2 g; yeast extract 1 g; $MgSO_4.7H_2O$ 0·2 g per litre of filtered pond water. A defined medium suitable for the growth of some caulobacters is: Na_2HPO_4 1·74 g; KH_2PO_4 1·06 g; NH_4Cl 0·5 g and 10 ml of concentrated base per litre of distilled water. After sterilization 0·2 % (w/v) glucose is added as carbon and energy source.

Concentrated base: nitrilotriacetic acid 20 g; $MgSO_4.7H_2O$ 59·16 g; $CaCl_2.2H_2O$ 6·67 g; $(NH_4)_6Mo_7O.4H_2O$ 18·5 mg; $FeSO_4.7H_2O$ 198 mg; 'metals 44' 100 ml per litre of distilled water (the nitrilotriacetic acid is dissolved separately and neutralized with potassium hydroxide).

Metals 44: EDTA 2·5 g; $ZnSO_4.7H_2O$ 10·95 g; $FeSO_4.7H_2O$ 5·0 g; $MnSO_4.H_2O$ 1·54 g; $CuSO_4.5H_2O$ 392 mg; $Co(NO_3).6H_2O$ 250 mg; $Na_2B_4O_7.10H_2O$ 177 mg per litre distilled water (a few drops of concentrated H_2SO_4 added to retard precipitation) (Poindexter 1964).

Microscopy

Light micrographs were taken using high power ($\times$100) oil immersion phase optics. We have found that high contrast, low grain Kodak Panatomic x (32ASA) 35 mm film gives good reproduction (developed in Kodak D-19 developer (20°C) for 3 min).

To enhance the quality of light micrographs it is advisable to cast a uniform agar surface on a slide and inoculate this with the appropriate organism. This is done, as for a slide culture, by placing a few drops of molten agar onto a slide and applying a coverslip immediately. When the agar has set the coverslip is carefully removed leaving behind a flat uniform surface. Inoculation of such a surface reduces the number of organisms out of focus at any one time.

Electron micrographs were taken on an AEI Corinth 275. Sample preparation was either by negative staining or by casting a gold/palladium metal shadow (shadow angle of 65°) (Hayat 1972).

Functions of Prosthecae

What function(s) do the prosthecae serve? Although prosthecate bacteria, *Caulobacter* and *Hyphomicrobium* in particular, were first observed almost fifty years ago (Henrici & Johnson 1935) the question of prostheca function still remains unanswered. Suggested functions range from the prostheca acting as a "sucking proboscis" which enables the micro-organism (*Caulobacter* in this instance) to absorb nutrients from, and destroy, the host bacterium (Houwink 1951), to a floatation organelle to retard sedimentation and so maintain aerobic heterotrophs at the air-water interface (Poindexter 1964), and finally as structures which have evolved to enhance uptake by the cell of nutrients from low nutrient ecosystems. Several observations point to the last suggestion being the primary function; however, the consequences of having evolved prostheca(e) are far reaching, particularly with respect to the group (i) (see Table 1) prosthecate bacteria, all of which have assumed an obligate polar mode of growth. These consequences are discussed in detail later on.

It has long been realized that these particular micro-organisms are capable of growth and survival in extremely low nutrient aqueous environments (Henrici & Johnson 1935; Houwink 1952) and furthermore that prostheca length reflects the nutrient status of the ecosystem (Boltjes 1934). This variation in prostheca length in response to nutrient levels is most apparent with *Caulobacter* spp. grown in the presence and absence of phosphate (Schmidt & Stanier 1966), i.e. at different growth rates (Fig. 1). A similar correlation has been shown for *Hyphomicrobium* (Harder & Attwood 1978) (Table 2), *Rhodomicrobium vannielii* (Whittenbury & Dow 1977; Fig. 1) and an *Ancalomicrobium* isolate (Whittenbury & Dow 1977; Fig. 2).

The close correlation of prostheca length with nutrient concentration has prompted the suggestion that prosthecae serve to enhance nutrient

TABLE 2

Relationship between mother-cell size, mean prostheca length and growth rate in Hyphomicrobium X *grown in a methanol-limited continuous culture*

Growth rate (h^{-1})	Mean dimensions of mother cell (μm)	Mean prostheca length (μm)
0·02	0·7 × 0·4	3·9
0·05	0·9 × 0·6	2·6
0·11	1·0 × 0·6	1·2

Taken from Harder & Attwood (1978).