Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

New Dimensions in Microbiology: An Introduction

A. T. Bull and J. R. Quayle

Phil. Trans. R. Soc. Lond. B 1982 **297**, 447-457
doi: 10.1098/rstb.1982.0054

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1982 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B **297**, 447–457 (1982)
Printed in Great Britain

New dimensions in microbiology: an introduction

By A. T. Bull†[1] and J. R. Quayle, F.R.S.‡

† *Department of Applied Biology, University of Wales Institute of Science and Technology, Cardiff CF1 3NU, U.K.*

‡ *Department of Microbiology, University of Sheffield, Sheffield S10 2TN, U.K.*

Microbiology – a century on from Koch's pioneering development of monoculture techniques – is steadily changing from traditional studies of pure cultures and their growth on single substrates to those in which new dimensions are being added. These new dimensions include the analysis of mixed substrates, mixed cultures and multiphase systems in which microorganisms grow on or within solid substrates. While it has long been recognized that metabolism of complex substrate mixtures, such as the contents of the rumen, may require a complex community of microorganisms, a new concept is the metabolism of single substrates, whether complex or simple, by stable communities of different organisms, so stable that some have been handled for years as monocultures and named accordingly. Finally, the widespread occurrence of genetic exchange between microorganisms has introduced an entirely new dimension to the older ideas of stable organisms only altering their metabolic potentialities by rare mutational events.

Introduction

The year 1981 marks a major anniversary in the history of microbiology, namely the centenary of the publication of Robert Koch's famous paper 'Zur Untersuchung von pathogenen Organismen' (Koch 1881), a paper which, in Brock's estimation (Brock 1966) was the most significant for the rise of microbiology as a distinct scientific discipline. The technical jewel set in this paper was Koch's description of a proven procedure for the isolation of pure cultures of bacteria, a problem that had taxed the ingenuity and skill of all the great pioneering microbiologists of the nineteenth century. Koch's achievement revolutionized microbiology – first medical bacteriology, but rapidly thereafter the whole gamut of microbiological investigation – and the pure culture approach signalled a spectacular blossoming of the subject. Much has been written about Koch the man and the scientist: that 'ablest of technicians' as he was dubbed by Marjory Stephenson (Stephenson 1949). But whatever epithet history ultimately provides for Robert Koch, one thing is beyond question, that the experimental tradition which he established a century ago has had the most profound influence on the subsequent course of microbiology.

The Koch tradition of microbiology

It is our opinion that Koch's technological innovation has led to a tradition of microbiology that has focused on pure, that is monospecies, culture studies and further has fostered, directly or otherwise, a disproportionate emphasis on single substrate, aerobic, batch systems in which environmental conditions have been made constant and the physical state of the experimental system has been kept as homogeneous as possible (Bull 1980). We would argue that the danger of such an approach is the acceptance, usually unwitting, of too limited a perspective of microbial behaviour. Of course, a great deal has been achieved by following the Koch tradition, but

[1] Present address: Biological Laboratory, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ, U.K.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.*
www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

on the debit side it has engendered a too simplistic view of microbial life and may have obscured several areas of essential enquiry. Thus, it is only under exceptional circumstances that mono-species populations of microorganisms growing at the expense of a single substrate develop naturally. In the past decade or so a radical reappraisal of experimental microbiology has begun, as the papers presented at this Discussion Meeting will help to reveal.

There were many compelling reasons for the development of pure cultures. A major concern of mycologists in the eighteenth and nineteenth centuries was the elucidation of reproductive mechanisms and morphogenetic development of fungi. Thus, in the early years of the eighteenth century Micheli made an extensive microscopic survey of a wide range of fungi in an attempt to discover the counterparts of angiosperm seeds; he assembled a wealth of evidence to support the view that reproductive bodies occur in fungi. However, Micheli's talent lay in following up such observation with experimentation and in devising cultural methods that enabled him to prove that such 'seeds' developed into colonies of the same species that produced them. In the following century the controversies surrounding the species concept in and pleomorphism of bacteria, and the microbial causation of disease, provided the greatest impetus to develop pure culture techniques. Although Koch's early mentor at the University of Göttingen, Jacob Henle, was one of the first to recognize the necessity of isolating the putative pathogen from its host in order to prove that microorganisms could cause disease, it was only 40 years later that the pupil devised a routine practical means of enabling such a test to be made.

The progress towards pure culture

Several historical accounts have been written of pure cultivation but in this centenary year some of the significant landmarks warrant restatement (table 1). Micheli's researches on fungi provide the most definitive starting point for such an analysis and in this context A. H. Reginald Buller's scholarly Presidential Address to the Royal Society of Canada in May 1915 (Buller 1915) is essential reading. Pier' Antonio Micheli (1679–1737), 'happy although in moderate circumstances, an expert in natural history, a leading botanist of Tuscany, well-known everywhere for his researches and writings, and much loved by all the worthy men of his age on account of his wisdom, sweetness of disposition and modesty'†, became botanist to Cosmo III, Grand Duke of Tuscany, and curator of the public gardens in his native Florence. In order to test his 'seed' hypothesis Micheli, by using a soft brush, sowed what we now refer to as spores of *Aspergillus* (probably *A. glaucus*), *Botrytis* (probably *B. umbellata*) and *Mucor* (probably *M. mucedo*) onto the freshly cut surfaces of pieces of melon, pear and quince. After a few days spore-bearing mycelia developed, and when these new spores were transferred to similar fruit substrata, fruit-bodies of the same species from which they had been collected were observed. (Micheli records that he 'always observed the same mode of growth in (these moulds), not in one trial only, but however often and whenever I attempted it'.) These historically important experiments were made in 1718 and Micheli's 'Observations' are contained in his *opus magnum Nova Plantarum Genera* of 1729. The book contains many fine plates, the cost of which was borne by nearly two hundred patrons including the then President of the Royal Society, Hans Sloane. Plates 91 and 95 illustrate the culture of the above-mentioned moulds and are reproduced in Buller (1915).

The use of gel-forming materials on which to grow microorganisms was also first introduced by microbiologists working with fungi. Gelatin was used by Vittadini in 1852 during his

† Commemorative tablet in the Church of Santa Croce, Florence.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

attempts to culture the muscardine disease fungus *Beauvaria* (Bulloch 1938) and several years later the great German mycologist Oscar Brefeld also advocated the use of gelatin, and the algal polysaccharide carragheenan, for solidifying liquid nutrient media. In a landmark paper of 1875 Brefeld enunciated a series of principles that provide the *sine qua non* for obtaining pure cultures and in doing so he advanced Micheli's methodology several stages further on. To Brefeld, successful pure culture of fungi involved (*a*) the use of a growth medium that was transparent and could be sterilized, (*b*) the insemination (inoculation) of the growth medium with a single fungal spore, and (*c*) protection of the culture from contamination by other organisms. Brefeld (1875) devised a simple but elegant procedure for isolating single spores, which involved dilution of a spore suspension in sterile diluent, location of a single spore under

Table 1. Landmarks in the development of pure culture methods

1718	Micheli	'observations' on fungal spores and reproduction
1729	Micheli	publication of *Nova plantarum genera*
1852	Vittadini	use of gelatin in attempts to culture the causal agent of muscardine disease, *Beauvaria*
1869	Raulin	chemically defined medium for *Aspergillus niger*
1875	Brefeld	use of gelatin and carragheenan in pure culture methods for fungi
1878	Koch	passage procedure for pure cultivation of pathogenic bacteria
1878	Lister	pure culture of bacteria by dilution isolation
1881	Koch	the streaked plate procedure
1881	Hesse, Fannie	Dr Hesse passes his wife's recommendation of agar as a solidifying agent to Koch
1882	Koch	aetiology of tuberculosis and reference to agar
1883	Koch	the poured plate procedure
1884	Hesse, Walther	publication of experiments on airborne bacteria and culture procedures
1887	Petri	'eine kleine Modifikation'

the microscope and removing the rest of the suspension with sterile absorbent paper. Students of microbiology often remain oblivious to Brefeld's contributions to experimental microbiology, but his protocol for pure culture was innovative and reliable; its major drawback was the difficulty of adopting it for the cultivation of very small organisms like bacteria. Before leaving mycology, the contribution of Raulin – a protégé of Pasteur's at the École Normal in Paris – deserves attention. Raulin's objective was the formulation of a fully chemically defined nutrient medium for the culture of fungi and in particular *Aspergillus niger*. The need for such a medium, he argued, was paramount in order that quantitative studies of fungal growth (substrate utilization, environmental optimization) could be made. Raulin was very much aware of the obligatoriness of pure cultures for such analyses, and he makes it clear in his writing (Raulin 1869) that cultures contaminated with 'foreign vegetation' were abandoned. The subsequent extensive studies of growth yields and substrate conversions in fungi by Raulin's compatriot Terroine (Terroine & Wurmser 1922) relied heavily on this pioneering research.

However, Pasteur's and Koch's investigations of alcoholic, lactic and butyric fermentations, and of anthrax, were made in the absence of pure culture methods. We can assume that the reproducibility of their findings was a consequence of using rather selective media. Pasteur adopted the procedure of 'passage' (serial transference of infective material from one animal to another) for the study of bacterial fermentation: by transferring small volumes of bacterial suspensions from one flask of sterile medium to another, 'pure cultures' in terms of the fermentation produced were obtained, but these were not pure in terms of the organisms present. Both men also studied anthrax by means of the passage method. Pasteur inoculated sterile urine or wort with blood from a diseased sheep and after serial subcultures he assumed that a pure

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

culture of the anthrax bacillus had been obtained. Koch isolated this bacillus from infected mice and followed its growth in serum and aqueous humour, but the method was not designed to produce pure cultures. However, Koch also passaged anthrax through mice and the blood of the twentieth animal was found to contain only anthrax. In the late 1870s such a procedure was believed to be 'the best and surest method of pure cultivation' (Koch 1878). The passage method was fallible, needless to say, and microscopic examination as a criterion of sterility or pure culture was unwise because of the very small sample size in comparison with the size of the infected source. Consequently Lister (1878) pursued the pure culture objective by a different route. His classic paper on the lactic fermentation contained the first reliable procedure for bacterial pure cultivation and its principle was that of serially diluting the source material to extinction and assuming that a drop from the dilution that produced growth contained a single bacterium. Lister developed a precision syringe that delivered drops of 0.01 minim (less than 1 μl) in volume for use in this procedure. Unfortunately Lister's ingenuity still left unresolved problems, perhaps the most important of which was the almost certain loss of numerically minor species in a mixed bacterial population by an early stage of dilution. And so to 1881 and Koch's final resolution of the pure culture dilemma.

Regarding the technical requirements for a pure culture method, Koch came to conclusions identical with those of Brefeld: the substrate should be solid, transparent and sterile. Ultimately Koch decided upon 2.5–5% gelatin solutions to which meat infusion was added ('nutrient gelatin'). Slabs of this medium were made by pouring it hot onto glass sheets and allowing it to cool and set; then, by means of a platinum wire, mixed bacterial populations were streaked over the solid substrate and individual colonies were easily differentiated. Details of this technique were included in the momentous paper of 1881, referred to at the beginning of this paper. However, Robert Koch was present at the International Medical Congress in London in August of that year, and Lister (1881), during the course of his address to the Congress, announced that Koch would exhibit 'his methods of procedure' to a limited number of people at King's College London later that same week. Among that small audience was Pasteur, whose gracious 'c'est un grand progres, Monsieur' must have expressed the admiration of all present. The manner in which gelatin became replaced by the seaweed polysaccharide agar-agar (now referred to simply as agar) as the preferred solidifying agent is related in detail by Hitchens & Leikind (1939). Fannie Hesse (née Eilshemius), being in the habit of using agar in the preparation of fruit and vegetable jellies, recommended its use to her husband Walther for his studies of airborne bacteria. So successful was this new gelling substrate that Hesse passed the information to Koch, probably during a short stay at the Imperial Health Office in Berlin, or by letter. Koch was quick to replace gelatin by agar and used it in his research on the tubercle bacillus, but gave the modification but brief mention in his classic paper on tuberculosis aetiology (Koch 1882). Walther Hesse published an account of his own investigations 2 years later (Hesse 1884). Two additional developments followed soon thereafter, both of which were soon to be taken for granted but which had major impacts on bacteriology. First was Koch's own contribution, the poured-plate method for isolating bacteria (Koch 1883) and second was Petri's introduction of a cultivation dish which ever since has been called after its designer (Petri 1887). Petri's publication is notable for the modesty of its title.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Exploring new dimensions

The isolation and cultivation procedures developed by Koch and his generation led to such a blossoming of microbiology that during the intervening years a spectacular insight into the growth, biochemistry and genetics of most groups of microorganisms has been gained. The great majority of this research has been made with pure cultures: only very exceptionally, however, do monospecies populations develop naturally. The analysis of microbial interactions and the behaviour of microbial communities has been constrained by a number of conceptual and experimental difficulties (Bull 1980; Bull & Slater 1982*a*) including a belief (quite mistaken) that multispecies associations were unstable and a lack of suitable experimental systems. Moreover, an awareness of the significance of mixed substrate systems has emerged only in fairly recent times (Harder & Dijkhuizen 1976; Bull & Brown 1979; Egli *et al.* 1981*a*, *b*) despite the fact that most natural populations of microorganisms assimilate nutrients from mixtures of carbon and other substrates and that the growth media for many commercial processes comprise complex mixtures of energy, carbon, nitrogen and other nutrients. Similarly the endeavour of most twentieth century microbiologists has been the avoidance of heterogeneous (multiphase) culture systems and those in which the environmental conditions fluctuate in a regular or irregular fashion. However, both circumstances are of common occurrence in natural ecosystems and rapidly are becoming features of novel bioreactor designs (e.g. fluidized bed and film reactors, and pressure cycle fermenters).

In the second half of this introductory chapter we shall indicate ways in which microbiological investigations have been stimulated to move on from the Koch tradition. The eleven papers that follow examine in detail various new dimensions that are being added to microbiology research.

Mixed cultures and microbial communities

It is generally accepted that one almost universal feature of natural and of man-created ecosystems is the development of mixed populations, or communities, of microorganisms. The existence of such complexity was increasingly recognized by microbiologists of the early twentieth century, but with notable exceptions, among whom can be counted Winogradsky, Beijerinck and Gause, few were stimulated to analyse community structures and activities. On the one hand, community complexity made their study a daunting prospect, while, on the other, ecology *per se* was not a subject that attracted the widespread attention of microbiologists. Inevitably, also, the debate that raged – and continues – on the merits and demerits of field and laboratory analyses in microbial ecology probably has inhibited such studies and only in relatively recent times has a fully integrated field-laboratory approach started to resolve some of the major problems. This point is illustrated in terms of an estuarine-laboratory study in the paper by Laanbroek & Veldkamp (this symposium).

Much current research is focused on environmentally relevant laboratory models (Bull 1980) for studying community behaviour, be the environment in question an animal gut, an estuarine sediment or whatever. It is now possible to examine mixed microbial populations in such ecosystems over long periods (years rather than hours or weeks), and the once prevailing view of laboratory communities being inherently unstable has been soundly refuted (Bull & Slater 1982*b*). The stimuli for research into microbial communities and interactions have been diverse, but enquiries into how environmental pollution affects indigenous microfloras and how the addition of microorganisms into the environment affects the properties of that environment

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

have been particularly potent. The effects of polychlorinated biphenyl and DDT pollution on marine phytoplankton communities have been studied extensively by Wurster and his colleagues (see Bull & Brown (1979) for review), and systems of this type are vital in effective environmental monitoring where the restrictions of monospecies systems are becoming well appreciated by protection organizations. Similarly, the pitfalls of using pure cultures in biodegradation studies of xenobiotic and naturally occurring chemicals are now recognized and the biodegradative capacity of microbial communities is often far greater, qualitatively or quantitatively or both, than pure cultures of their individual members (Bull 1980). Not surprisingly the focus has been predominantly on the degradation and persistence of pesticides and petroleum and its constituents, a topic that emerges in several of the papers in this volume (those by Dalton & Stirling, Slater & Bull, and Williams). However, the literature on synergistic biodegradation is growing rapidly and examples are being reported for a very wide range of compounds including antibiotics (e.g. benzylpenicillin (Johnsen 1981)).

Another significant stimulus to research on community dynamics has come from attempts to introduce specific microorganisms into environments for purposes of increasing soil fertility and plant growth, microbiological pest and pathogen control, pollution abatement and mineralization of crop residues *in situ*. Each of these practices relies for its success on the establishment, competitiveness and survival of the inoculant populations. The use of *Rhizobium* and *Azotobacter* inoculants for improving the nitrogen nutrition of plants is well known but, for example, for inoculations of rhizobia to be successful they must be able to nodulate in the face of competition from soil bacteria, including other strains of *Rhizobium*. Johnston & Berringer's (1976) study of mixed inoculations of pea roots with effective and ineffective strains of *R. leguminosarum* is an interesting and important example of such research. Similarly the mycorrhizal status of trees is known to be a significant factor in their vigour, and mycorrhizal inoculant schemes are being developed for the revegetation of derelict and poor-quality land and for optimizing crop yields. Marx (1975), among others, has shown that this technology is a feasible one and that the appreciation of symbiont specificity and mycorrhiza establishment are critical when attempting to recover severely disturbed soils of the type resulting from strip mining.

A generally accepted feature of microbial ecosystems is their lack of the long-term community stability that attends macroecosystem climax communities. Such communities are frequently able to maintain themselves even in the face of severe disturbances because of the way in which they have modified the environment. In contrast, microorganisms, although they have a poor ability to create stable conditions within the environments that they colonize, adapt rapidly to environmental disturbances by changing their community structure. The maintenance of a high level of informational heterogeneity is therefore characteristic of microbial communities: considerable adaptive advantage derives from preserving diverse populations with physiological capabilities and tolerances whose ranges exceed those utilized or experienced within a particular habitat at a particular time. A recent, striking illustration of this point is contained in the work of Atlas and his colleagues on bacterial communities in the Gulf of Alaska (Hauxhurst *et al.* 1980, 1981). Microbial adaptation to environmental disturbance also proceeds within individual species via the acquisition of additional genetic information from external sources. Clarke (1980) and Williams (this symposium) review the means whereby such adaptation can occur and they discuss how novel metabolic pathways and tolerances evolve via such mechanisms as plasmid transfer. Naturally mixed cultures are the *sine qua non* for the study of such gene flux in microbial communities, but many critical questions remain unanswered. For

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

example, which of the presently known mechanisms of gene transfer in bacteria are exploited *in situ*? What is the frequency of gene transfer in microbially 'dilute' situations that often exist in water or soil, and how might such a frequency be modified in populations concentrated on surfaces and at interfaces? What are the taxonomic boundaries to gene transfer in bacteria and to what extent does gene transfer occur in populations of other microorganisms? What is the significance of and relation between high species diversity and gene transfer for microbial adaptation? The stability of a genetically engineered organism can be of vital importance in the scaling-up of the use of such organisms for industrial use. The loss of desirable genes, for example coded on a plasmid, can lead to the progressive development of a 'mixed culture' during an industrial process. The technological implications of this can be profound and this topic is explored from the standpoint of the chemical engineer by Ollis (this symposium).

The exploitation, invariably empirical, of mixed microbial cultures in traditional beverage, food, fodder and waste treatment processes has a very long antecedence but in recent years the development of defined mixed cultures has been considered for a range of biotechnological purposes, including single cell protein and organic acid production, methanogenesis, bioconversions, metal extraction and recovery, detoxification and the protection of perishable foods (Bull 1981). The incentive to develop a mixed-culture process for single cell protein from methane and natural gas was largely prompted by the finding that monospecies cultures of methylotrophs grew erratically on such substrates, produced low biomass yields or failed to utilize the mixture of *n*-alkanes in natural gas. Indeed, Mateles and his colleagues have discussed the proposition that mixed cultures may be desirable for single cell protein processes because of their greater ability than pure cultures to withstand accidental contamination (Mateles 1979; Rokem *et al.* 1980). Thus, when *Pseudomonas* strain C was grown on methanol in continuous culture, together with a mixture of heterotrophic soil bacteria (*ca.* 1% total population), the culture was resistant to infection from faecal and pathogenic bacteria: it was concluded that the niche(s) of pathogens was occupied by the other heterotrophs, thereby making the mixed culture less susceptible to invasion. There is also a considerable resurgence of interest in the use of mixed cultures to protect perishable foods. The development of acid conditions to preserve foodstuffs is not a recent discovery but it is 'an ancient method with a future' (Raa 1981). Raa, for example, has recently reviewed the possibilities for the preservation of fish by lactic acid bacteria: addition of lactic acid bacteria and a fermentable sugar to fish causes a rapid decrease in pH and the production of a fish silage of good nutritional value which resists spoilage. In an identical manner oriental foods such as nahm (traditional Thai fermented pork) and fermentation processes such as koji (saccharification of grain starch by *Aspergillus oryzae*), have been protected very successfully against bacterial contamination by lactic acid producers. *Staphylococcus aureus* contamination is a major problem with semi-moist and salted foods such as nahm, while anaerobic foods are susceptible to contamination from clostridia. The deployment of antagonistic *Lactobacillus* and *Pediococcus* species is considered to be very effective against such contamination of tightly packed nahm even under neutral pH conditions (Inoue *et al.* 1981). Before leaving this brief discussion of mixed-culture technology it is timely to point out that 1981 also marks the centenary of the first urban use of methane for street lighting in the city of Exeter (McCarty 1981). The microbiology and biochemistry of anaerobic digestion and methanogenesis is discussed by Mah later in this symposium. One means of unravelling the metabolic interactions within the complex community that is now favoured is the analysis of co-cultures of defined members of the community. The work of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Laube & Martin (1981) is illustrative of this approach. Here cellulose was fermented by monocultures of *Acetivibrio cellulolyticus* and co-cultures of *A. cellulolyticus* with *Methanosarcina barkeri*, *A. cellulolyticus* with *Desulfovibrio* sp. and *A. cellulolyticus* with *Desulfovibrio* sp. with *M. barkeri*: only the three-membered community caused a rapid conversion of cellulose to methane and carbon dioxide, the three species effecting cellulolysis, acetogenesis and H_2 production, and methanogenesis, respectively.

Finally, there is a certain irony in the fact that medical microbiology also has stimulated mixed-culture research in recent years, and Smith (this symposium) reviews the present status of community activities in the establishment of infectious diseases. Chemical transformations of antimicrobial agents by, for example, the indigenous gut microflora can cause significant changes in the infection potential and susceptibility of pathogens (Onderdonk *et al.* 1979). Those and related metabolic toxicological and nutritional situations can be closely simulated in various types of continuous culture apparatus and some recent researches in this area relate to rodent intestinal ecosystems (Veilleux & Rowland 1981), the sheep rumen (Henderson *et al.* 1981) and colon microbial communities of the horse (Davies 1979) and man (Miller & Wolin 1981).

Mixed substrates

In 1942 Monod first described the phenomenon of diauxie in *Escherichia coli* growing in batch culture on certain mixtures of two carbohydrates. The phenomenon was characterized by a double growth cycle consisting of two exponential phases separated by a phase of zero or diminished growth rate. Each cycle corresponded to the exclusive utilization of one of the two carbon sources due to the inhibitory effect of one on the synthesis of the enzyme(s) catabolizing the other (Monod 1949). Following this, the phenomenon of diauxie was reported widely, occurring with diverse microorganisms and substrates, the latter not necessarily being carbohydrates. It became generally accepted that, when faced with a mixture of substrates, microorganisms would grow on one at a time, the 'richer' substrate being chosen first. The repression of synthesis of enzymes specific to the utilization of 'poorer' substrates was viewed as a control mechanism sparing the organism from unnecessary, and hence wasteful, synthesis.

In one sense perhaps this rather satisfying model appealed too much to the microbial biochemist of the 1950s and led to the view that microbial cells, especially bacteria, were tightly regulated to ensure the most efficient use of available substrates. This was an oversimplification for two reasons: (1) batch growth with substrate mixture at 50–100 mM concentrations was in most cases far removed from physiological conditions, and (2) it tended to ignore the fact that what really matters when microbial species compete for limited nutrients usually is not frugal and economical housekeeping but the fastest response to a changing environment and fastest growth in it – even if this sometimes involves wastage of energy. The 1960s saw the recognition of the great driving force behind the combination of adaptability and the rate of growth, a point that was beautifully expressed by B. D. Davis in his plenary address to the Cold Spring Harbor Symposium in 1961: 'I am sure that nature takes account of slight differential growth rates even more minutely than a banker compares the interest rates on bonds. No device that improves the economy of operation of a cell will be neglected, including of course, not only speed of reproduction in a given environment but also adaptability to fluctuating environments . . . the evolutionary race goes to the swift.'

The most important single influence on the re-evaluation of the utilization of mixed substrates by monospecies cultures probably was the introduction of continuous culture techniques.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

When grown under carbon limitation in a chemostat the enzyme content of and metabolism of mixed substrates by microorganisms were often found to be quite different from those encountered in analogous batch cultures. At low dilution rates (i.e. low specific growth rates) diauxic behaviour generally disappeared and the synthesis of enzymes required for metabolizing both substrates became derepressed. Examples of such behaviour were reported from many laboratories (Tempest 1970; Jannasch & Mateles 1974). While this situation, on the face of it, appears to be an inefficient use of an organism's limited energy budget, it does result in its being triggered to respond immediately to transient appearances of a variety of growth substrates, a factor of great importance for successful competition within nutrient limited habitats. Studies of the response of pure cultures to mixed substrates under conditions of continuous cultivation (Harder & Dijkhuizen 1976; Dijkhuizen & Harder 1979*a*, *b*; Egli *et al.* 1982*a*, *b*) are now being extended to detailed analyses of the behaviour of two different organisms subjected to mixed substrate limitations (Gottschal & Kuenen 1980). Therefore, it seemed to us to be most appropriate to start this Discussion Meeting with the simplest of our 'new dimensions' in microbiology, utilization of mixed substrate (Harder & Dijkhuizen), to be followed by the related topic of mixed-substrate co-metabolism (Dalton & Stirling).

The significance of the co-metabolism of substrates is especially high in the total biodegradation or partial chemical transformation of xenobiotics and rather recalcitrant natural materials, and the generation of co-metabolic products can have several consequences (e.g. provision of growth substrates for one or more species, increased recalcitrance, increased toxicity) (Bull 1980). Another pertinent reason for examining mixed-substrate systems is the opportunity that they provide for studying the adaptation of organisms to novel compounds. An early example of such adaptation concerning xenobiotic assimilation is the selection of a mutant of *Pseudomonas putida* able to dehalogenate and hence utilize the herbicide dalapon while growing as a pure culture on the non-chlorinated parent compound, propionate (Senior *et al.* 1976). The carbon-limited condition ensured that a strong selective pressure for dalapon-utilizing mutants was continuously exerted.

Culture and habitat heterogeneity

Microbial ecosystems are, with few exceptions, heterogeneous (1) spatially with respect to the distribution of nutrients and organisms and (2) temporally with respect to fluctuating environmental conditions. Interfaces and surfaces provide unique environments not found in either of the adjoining phases and in which microbial activity may be altered appreciably. Nevertheless, most microbiologists have striven to devise experimental systems that deliberately seek to avoid such heterogeneity. However, interfacial phenomena may be of prime importance in determining microbial behaviour, and Bull (1980) has suggested a number of these that need detailed study: rate effects, in terms of metabolizable substrates; biomass retention, which may endow populations with greater tolerance of environmental perturbations; partitioning of chemicals that might affect their availability as substrates or result in the development of toxic or detoxified environments; genetic exchange within and between different microbial populations. The attachment of microorganisms to surfaces usually constitutes the first step in the causation of animal and plant diseases and related interactions. The responses of microorganisms to chemical and physical gradients have been subject to increasing study in laboratory model systems such as the gradostat, gel-stabilized gradients and microbial films, and in microbial colonies. Such environmental analogues and their utility as research tools are described in this volume by Wimpenny, while Ellwood *et al.* explore the relation between

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

microbial surface structures, adhesion and surface-associated growth in chemostat cultures in which surface structures such as metal foil are deliberately introduced. Light itself can constitute an additional physical gradient, and the superimposition of this on other fluctuating environmental conditions is explored in a later paper (Shilo) with respect to photosynthetic communities existing in various types of aquatic environment.

Microorganism immobilization onto the surface of or within appropriate support materials is an important feature of current bioreactor design (Bull 1981), from which has emerged a range of packed bed, fluidized bed, rotating disc, entrapment and encapsulation systems. The advantages claimed for these systems are several and include enhanced catalytic half lives, enhanced tolerance towards environmental stresses such as toxic materials and non-optimal temperatures, high rates of substrate transformation without organism washout, and improved economics.

References (Bull & Quayle)

Brefeld, O. 1875 Methoden Zur Untersuchung der Pilze. *Verh. phys.-med. Ges. Würzb.* N.F. **8**, 43–62.

Brock, T. D. 1966 *Principles of microbial ecology*. Englewood Cliffs, N.J.: Prentice-Hall.

Bull, A. T. 1980 Biodegradation: some attitudes and strategies of microorganisms and microbiologists. In *Contemporary microbial ecology* (ed. D. C. Ellwood, J. N. Hedger, M. J. Latham, J. M. Lynch & J. H. Slater), pp. 107–136. London: Academic Press.

Bull, A. T. 1981 Strategies in microbial process optimization. In *Global Impacts of Applied Microbiology Sixth International Conference* (ed. S. O. Emejuaiwe, O. Ogumbi & S. O. Sanni), pp. 623–645. London: Academic Press.

Bull, A. T. & Brown, C. M. 1979 Continuous culture applications to microbial biochemistry. *Int. Rev. Biochem.* **21**, 177–226.

Bull, A. T. & Slater, J. H. 1982*a* Historical perspectives. In *Microbial interactions and communities* (ed. A. T. Bull & J. H. Slater). London: Academic Press. (In the press.)

Bull, A. T. & Slater, J. H. 1982*b* Microbial interactions and community structure. In *Microbial interactions and communities* (ed. A. T. Bull & J. H. Slater). London: Academic Press. (In the press.)

Buller, A. H. R. 1915 Micheli and the discovery of reproduction in fungi. *Trans. R. Soc. Can.* **9**, 1–25.

Bulloch, W. 1938 *The history of bacteriology*. London: Oxford University Press.

Clarke, P. H. 1980 The Leeuwenhoek Lecture, 1979. Experiments in microbial evolution: new enzymes, new metabolic activities. *Proc. R. Soc. Lond.* B **207**, 385–404.

Davies, M. E. 1979 Studies on the microbial flora of the large intestine of the horse by continuous culture in an artificial colon. *Vet. Sci. Commun.* **3**, 39–44.

Davis, B. D. 1961 Cellular regulatory mechanisms. *Cold Spring Harb. Symp. quant. Biol.* **26**, 1–10.

Dijkhuizen, L. & Harder, W. 1979*a* Regulation of autotrophic and heterotrophic metabolism in *Pseudomonas oxalaticus*: growth on mixtures of acetate and formate in continuous culture. *Arch. Microbiol.* **123**, 47–53.

Dijkhuizen, L. & Harder, W. 1979*b* Regulation of autotrophic and heterotrophic metabolism in *Pseudomonas oxalaticus*: growth on mixtures of oxalate and formate in continuous culture. *Arch. Microbiol.* **123**, 55–63.

Egli, T., Käppeli, O. & Fiechter, A. 1982*a* Regulatory flexibility of methylotrophic yeasts in chemostat cultures: simultaneous assimilation of glucose and methanol at a fixed dilution rate. *Arch. Microbiol.* **131**, 1–7.

Egli, T., Käppeli, O. & Fiechter, A. 1982*b* Mixed substrate growth of methylotrophic yeasts in chemostat culture: influence of the dilution rate on the utilization of a mixture of glucose and methanol. *Arch. Microbiol.* **131**, 8–13.

Gottschal, J. C. & Kuenen, J. G. 1981 Physiological and ecological significance of facultative chemolithotrophy and mixotrophy in chemolithotrophic bacteria. In *Microbial growth on* C1 *compounds* (*Proceedings of the Third International Symposium, Sheffield*, 1980) (ed. H. Dalton), pp. 92–104. London: Heyden.

Harder, W. & Dijkhuizen, L. 1976 Mixed substrate utilization. In *Continuous culture*, vol. 6 (*Applications and new fields*) (ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans & J. Melling), pp. 297–314. Chichester: Ellis Horwood.

Hauxhurst, J. D., Krichevsky, M. I. & Atlas, R. M. 1980 Numerical taxonomy of bacteria from the Gulf of Alaska. *J. gen. Microbiol.* **120**, 131–148.

Hauxhurst, J. D., Kaneko, T. & Atlas, R. M. 1981 Characteristics of bacterial communities in the Gulf of Alaska. *Microb. Ecol.* **7**, 167–182.

Henderson, C., Stewart, C. S. & Nekrep, F. V. 1981 The effect of monensin on pure and mixed cultures of rumen bacteria. *J. appl. Bact.* **51**, 159–169.

Hesse, W. 1884 Über quantitative Bestimmung in der Luft enhaltenen Mikroorganismen. *Mitt. Kaiserl. Gesundht, Berl.* **2**, 182–207.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Hitchens, A. P. & Leikind, M. C. 1939 The introduction of agar-agar into bacteriology. *J. Bact.* **37**, 485–493.
Inoue, Y., Takano, M. & Shibasaki, I. 1981 Antagonistic action of lactic acid bacteria from nahm toward food-deteriorating bacteria. In *Microbial utilization of renewable resources*, vol. 1 (ed. H. Taguchi), pp. 108–115. Osaka: International Centre for Cooperative Research in Microbial Engineering.
Jannasch, H. W. & Mateles, R. I. 1974 Experimental bacterial ecology studied in continuous culture. *Adv. Microb. Physiol.* **11**, 165–212.
Johnsen, J. 1981 Synergistic degradation of benzyl penicillin by a *Pseudomonas* strain and a *Fusarium* strain. *Curr. Microbiol.* **6**, 27–30.
Johnston, A. W. & Berringer, J. E. 1976 Mixed inoculations with effective and ineffective strains of *Rhizobium leguminosarum*. *J. appl. Bact.* **40**, 375–380.
Koch, R. 1878 *Untersuchungen über die Aetiologie der Wundinfections – Krankheiten*. Leipzig.
Koch, R. 1881 Zur Untersuchung von pathogenen organismen. *Mitt. Kaiserl. Gesundht, Berl.* **1**, 1–48.
Koch, R. 1882 Die Aetiologie der Tuberkulose. *Berl. klin. Wschr.*, no. 15, pp. 221–230.
Koch, R. 1883 Über die neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft and Wasser. *Aerztliches Vereinsblatt Dt.*, no. 237.
Laube, V. M. & Martin, S. M. 1981 Conversion of cellulose to methane and carbon dioxide by triculture of *Acetivibrio cellulolyticus*, *Desulfovibrio* sp. and *Methanosarcina barkeri*. *Appl. environ. Microbiol.* **42**, 413–420.
Lister, J. 1878 On the lactic fermentation and its bearings on pathology. *Trans. path. Soc., Lond.* **29**, 425–467.
Lister, J. 1881 On the relation of minute organisms to unhealthy processes arising in wounds and to inflammation in general. *Trans. Int. Med. Congr.* (ed. Sir William MacCormac), vol. 1, pp. 311–319.
Marx, D. J. 1975 Mycorrhizae and establishment of trees on strip-mine land. *Ohio J. Sci.* **75**, 288–297.
Mateles, R. I. 1979 Biotechnology in SCP production: is pure culture operation desirable? In *Global Impacts of Applied Microbiology Fifth International Conference* (ed. P. Mantangkasombut), pp. 315–319. Bangkok: GIAM-V Secretariat.
McCarty, P. L. 1981 History of anaerobic digestion. In *Anaerobic digestion* (ed. W. Baader), pp. 1–12. Amsterdam: Elsevier/North Holland.
Miller, T. L. & Wolin, M. J. 1981 Fermentation by the human large intestine microbial community in an *in vitro* semi-continuous culture system. *Appl. environ. Microbiol.* **42**, 400–407.
Monod, J. 1942 Recherches sur la croissance des cultures bacteriennes. Paris: Hermann et Cie.
Monod, J. 1949 The growth of bacterial cultures. *A. Rev. Microbiol.* **3**, 371–394.
Onderdonk, A. B., Kasper, D. L., Mansheim, B. J., Louie, T. J., Gorbach, S. L. & Bartlett, J. G. 1979 Experimental animal models for anaerobic infections. *Rev. infect. Dis.* **1**, 291–301.
Petri, R. J. 1887 Eine Kleine modification des Koch'schen Plattenverfahrens. *Zentbl. Bakt. ParasitKde* **1**, 279–280.
Raa, J. 1981 Biochemistry of microbial fish spoilage and preservation by lactic acid bacteria and added acid. In *Global Impacts of Applied Microbiology Sixth International Conference* (ed. S. O. Emejuaiwe, O. Ogumbi & S. O. Sanni), pp. 3–16. London: Academic Press.
Raulin, J. 1869 Études chimiques sur la végétation. *Annls Sci. nat.* (5) **11**, 93–99.
Rokem, J. S., Goldberg, I. & Mateles, R. I. 1980 Growth of mixed cultures of bacteria on methanol. *J. gen. Microbiol.* **116**, 225–232.
Senior, E., Bull, A. T. & Slater, J. H. 1976 Enzyme evolution in a microbial community growing on the herbicide Dalapon. *Nature, Lond.* **263**, 476–479.
Stephenson, M. 1949 *Bacterial metabolism*, 3rd edn. London: Longmans, Green & Co.
Tempest, D. W. 1970 The place of continuous culture in microbiological research. *Adv. microb. Physiol.* **4**, 223–250.
Terroine, E. F. & Wurmser, R. 1922 L'énergie de croissance. I. Le développement de l''Aspergillus niger'. *Bull. soc. chim. Biol.* **4**, 518–567.
Veilleux, B. G. & Rowland, I. 1981 Simulation of the rat intestinal ecosystem using a two-stage continuous culture system. *J. gen. Microbiol.* **122**, 103–115.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES