ISOLATION AND CHARACTERIZATION OF GLIDING FILAMENTOUS PHOTOTROPHIC BACTERIA FROM THE ZARA HOT SPRINGS

By

Rania M. Deranieh

June 1990

Under the Supervision of

Dr. Fuad Hashwa

Submitted in partial Fulfillment of the
Requirements for the Degree of Master of Sciences,
in Biological Sciences, Faculty of Graduate Studies,
University of Jordan.

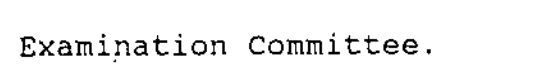
Amman, Jordan

ISOLATION AND CHARACTERIZATION OF GLIDING FILAMENTOUS PHOTOTROPHIC BACTERIA FROM ZARA HOT SPRINGS

By Rania M. Deranieh

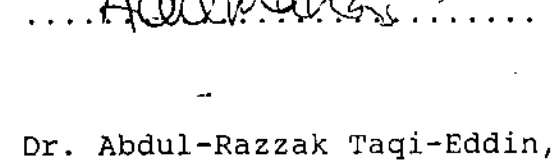
This thesis was prepared under the supervision of Dr. Fuad Hashwa and has been approved by all members of the Examination Committee.

Dr. Fuad Hashwa, Chairman of Committee, Associate Professor.

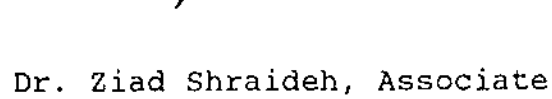


Dr. Adel Mahasneh, Professor.

Dr. Abdul-Razzak Taqi-Eddin, Assistant Professor.



Dr. Ziad Shraideh, Associate Professor.



Date of Defense: June 3rd, 1990

ABSTRACT

Six pure cultures of thermophilic gliding filamentous phototrophic bacteria have been successfully isolated from the microbial mats of the Zara hot springs - Jordan. These organisms have been found growing at temperatures up to 63°C forming an essential component of the microbial mats growing in these springs. They belong to a unique group of phototrophic bacteria, the Chloroflexaceae, unique in both their characteristics and their taxonomic status. Up to date, only one species belonging to this group, Chloroflexus aurantiacus, has been isolated in pure culture. Morphological studies revealed that all our isolates consist of filaments of an indefinite length. The width ranges from 0.6 to 1.2 μm. All the isolates appear to have chlorosomes as well as a high content of inclusion bodies. They grow best as photoheterotrophs under semiaerobic conditions. The optimum temperature for growth is 50 - 55°C, the pH optimum is 6.5 - 8.5. The majority of the isolates appeared to be good producers of amylolytic, proteolytic and lipolytic enzymes. Pigment analysis carried out by both spectrophotometry and thin - layer chromatography indicated the presence of both bacteriochlorophylls a and c as well as carotenoids as the major photosynthetic pigments of these organisms. Analysis of cell membrane fatty acids by gas - chromatography indicated that our isolates may be separated into two distinct groups with the differences between them being enough to separate them into two different species.

ACKNOWLEDGEMENTS

I would like to express my heartful appreciation and gratitude to Dr. Fuad Hashwa for his never ending support, assistance and guidance and for always being there when needed.

My sincere thanks are due to members of the committee, Prof. Adel Mahasneh, Dr. Abdul Razzak Taqi-Eddin, and especially Dr. Ziad Shraideh for his generous and great help with the photomicrography part of the work.

My special thanks are also due to Dr. B. Tindall and especially to Dr. R.M. Kroppenstedt of the German Collection of Microorganisms and Cell Cultures (DSM), for his valuable help with the fatty acid analysis of the isolates and also for his fruitful discussions and valuable suggestions.

I would also like to thank Mrs. Muna Hindiyeh of the Royal Scientific Society (RSS) for her help with the Microbial Identification System (MIS). Thanks are also due to Mrs. S. Janakat for her help with the electron microscopy, and to Z. Shasha'a for typing the manuscript.

My deep appreciation extends to all my colleagues especially to Mr. A. Sunna' and to Mr. N. El-Banna, and to all those in the Department of Biological Sciences.

I am deeply indebted to all my teachers who, in one way or another, helped me widen my scope and increase my knowledge in many aspects.

Last but not least, I wish to express my deepest gratitude and my eternal indebtedness to my family, especially my mother, and to my friends for their endless support and encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

The photosynthetic prokaryotes which belong to the subclass Gracilicutes (Stanier et al., 1981), comprise a wide and heterogeneous group of microorganisms that are found in various kinds of habitats including extremes of salinity, pH and temperature. Belonging to this group of organisms is the relatively recently established family, the Chloroflexaceae. This family was established by Trüper in 1976 in order to accommodate Chloroflexus aurantiacus , a thermophilic gliding filamentous photosynthetic bacterium and other such organisms that presumably would be discovered later on.

C. aurantiacus , the only species in its genus, was isolated from alkaline hot springs by Pierson and Castenholz in 1971. Most of the information found in literature concerning the family Chloroflexaceae pertains to this organism since it is the only member in this family that has been successfully isolated in pure culture. Unfortunately, C. aurantiacus, has not been studied extensively although now, it is an organism of interest to a variety of scientists including microbiologists, biochemists, and more specifically to photobiologists and phylogenists interested in early microbial evolution.

The importance of this organism stems from the fact that it may represent a living proof of ancient life forms on

Earth. Evidence has been given for the existence of similar organisms some 3,500 million years ago as indicated by the microfossils of chert and stromatolites (Doemel & Brock, 1974 ; Awramik, 1981 ; 1984) . The modern analogue of those stromatolites, the microbial mats that are predominantly found in hot springs, still demonstrate the presence of similar organisms (e.g. Chloroflexus sp.) and in the same laminated arrangement as found years ago.

From an evolutionary point of view these organisms are interesting since they have still managed to exist through millions of years although limited to habitats of extreme environments, those thought to have prevailed in old times. This long term existence may possibly entail a high genetic stability and persistence of these organisms in being able to maintain themselves through time and under various environmental conditions. Additionally, the phylogenetic aspects of these organisms are also worthwhile exploiting. 16S rRNA studies have revealed the absence of correlation between Chloroflexus and the other green photosynthetic bacteria which were once thought to be the closest relatives. Chloroflexus has also been proven to be distantly related to cyanobacteria (Gibson et al. 1985; Reichenbach et al., 1986; Oyaizu et al., 1987). According to Gibson et al., (1985), the Chloroflexus group "has the phylogenetic depth of a phylum". Apparently, these filamentous photosynthetic bacteria stand on their own amongst other groups.

The importance of Chloroflexus may also be regarded from other standpoints. Being a thermophilic bacterium by nature, makes it an attractive candidate as a potential source of thermostable compounds that may have some biotechnological applications.

Chloroflexus seems to be quite unique in terms of physiology, biochemistry, and cytology. Therefore, further investigations about this organism, its characteristics and its types and varieties distributed all over the globe would undoubtedly cast some light upon these little studied organisms.

In Jordan, the Zara thermal springs, found at a proximity to the eastern Dead Sea shore, provide a potential habitat for Chloroflexus - like organisms, therefore it was considered as a suitable site to carry out this research, the first of its kind here in Jordan. This project was undertaken in an effort to survey and characterize some of the types of gliding filamentous photosynthetic bacteria by isolating them from the microbial mats found in the thermal springs.

The project aims at the following main points :-

- Description and anatomy of microbial mats found in the Zara hot springs.

- Isolation and purification of gliding filamentous phototrophic bacteria from the layers of the microbial mats.
- Light and electron microscopical examination of the purified isolates.
- Physiological and nutritional studies of the isolated cultures.
- Optimization of growth conditions.
- Spectrophotometric and chromatographic analyses of the photosynthetic pigments.
- Fatty acid analysis of the cell membranes.

II. LITRERATURE REVIEW

The mere existence and the actual roles of microorganisms found in "odd" places as the extreme environments often intrigues the curiosity of many researchers. The ability of certain organisms to grow and withstand certain environmental pressures while others cannot, clearly denotes the uniqueness of such organisms, especially in terms of ecological and evolutionary aspects.

Hot springs, representing such an extreme environment, have gained quite a special interest. These natural habitats have probably existed throughout most of the time in which organisms have been evolving on Earth, and may thus provide a record for the ancestory of life through the microfossils which are often found associated with such habitats.

Hot springs, found in almost every continent of the world, are regarded as independent geothermally heated systems with an intrinsic supply of chemical nutrients (Mitchell, 1974). According to Castenholz (1969), hot springs have been classified into :-

(1) Volcanic - Na, Cl, HCO_3^- springs which are neutral to highly alkaline waters, associated with recent volcanic

activity and representing the bulk of the alkaline hot springs of the earth.

(2) Volcanic - acid SO_4^{-2} waters which are closely associated with volcanic activity and fumaroles. In these types of springs, the pH-value may reach as low as 1.0 due to the formation of sulfuric acid (resulting from the oxidation of sulfides that are usually abundant in such types).

(3) Calcareous, travertine - depositing springs. These types are widely scattered all over the world. They are generally near neutrality, have a high concentration of Ca, Mg, and HCO , and are supersaturated with carbon dioxide.

(4) Meteoric, low salinity thermal waters that are characterized by their association with diastrophism rather than volcanism.

(5) Thermal brines, these waters are thought to be of a conate origin i.e. they originated by the entrapment of water in sediment, and often have a high concentration of methane.

II.A. Some chemical and physical aspects of hot springs:-

Certain common characteristics of hot springs are determined by the properties of thermal outflows. Thermal waters

generally come from surface water that percolates down to the vicinity of hot magma, is heated, and then driven to the surface. The hot water dissolves minerals as it moves upwards, and therefore emerges with a mineral content that is characteristically quite high and sometimes exclusive to such environments (Castenholz, 1969 ; Mitchell, 1974).

Many ions and minerals found in these thermal waters may be either absent or very rare in the surface waters of streams and lakes. Such elements include S, As, Mn, Mo and several others, some of which may serve as micronutrients for specific organisms while at the same time being highly toxic to many others. The high leaching and carrying capacity of thermal waters often results in a high concentration of total dissolved solids (TDS): about 2000 mg of TDS per liter, in contrast to other surface waters which have a much lower concentration of about 150 mg TDS per liter.

Other properties that are almost universally characteristic of hot spring waters are the low concentrations of dissolved organic compounds and of oxygen, the shallowness and the clarity of the water (Castenholz, 1969).

II.B. Biological aspects of hot springs :-

The extreme nature of the hot springs environment creates a restriction to the types of organisms that are able to survive and grow in these habitats.

Brock (1978), gave data in support of the concept "that increased temperatures simplify population structure". Using hot springs as an example, he demonstrated that eukaryotic organisms are confined to temperatures below 62°C which is the upper limit for the growth of fungi (Brock, 1978), while on the other hand, prokaryotic microorganisms appear to be abundant at higher temperatures and some bacteria have even been found at temperatures exceeding the boiling point of water (Brock, 1986). So, each hot spring represents a sequence of communities that decrease in cellular complexity towards the upper limit of the thermal gradient represented by the source water of the spring (Brock, 1970, ; Tansey & Brock, 1972 ; Mitchell, 1974).

II.C. Microbial mats : -

The most obvious and distinguished community that is found in almost all neutral hot springs is the microbial mat system which represents a community by itself. Hot spring microbial mats are usually found at temperatures below 73°C (Brock & Brock, 1966). They exhibit maximum thickness in the temperature range of 50-55°C reaching 1-3 cm in extent

(Bauld & Brock, 1973). They are basically formed by the association of two groups of prokaryotic phototrophs, the first group consists of cyanobacterial populations often limited to one or two types, while the second group consists of gliding filamentous phototrophic bacteria, basically Chloroflexus (or Heliothrix in limited cases).

The two groups are arranged in the form of layers held together by abundant extracellular gelatinous materials ; each layer being dominated by the group of microorganisms that is best suited for the conditions prevailing within that specific microenvironment.

Other types of bacteria may be randomly distributed within the layers but are often concentrated in the lower mat surfaces where decomposition processes often take place.

Most of the mat structures that have been mentioned in literature showed a similar repetitive vertical sequence of microorganisms building the mat (Castenholz, 1969, 1984 ; Bauld & Brock, 1973 ; Jørgensen et al. 1983 ; Ward et al., 1984). Cyanobacteria tend to dominate the topmost layer of the mat thus conferring a yellow - greenish colour depending on light intensity and on the type of cyanobacterial population(s) prevailing in the mat. The underlayer , an orange , orange - red to flesh coloured gelatinous mat is usually built up of gliding filamentous anoxygenic

phototrophic bacteria which almost invariably consists of representatives of the genus Chloroflexus (Pierson & Castenholz, 1974). A similar organism, Heliothrix, that resembles Chloroflexus in some aspects has been reported to grow not as an undermat but as a top mat with a layer of oxygenic cyanobacteria beneath (Pierson et al., 1984 ; Pierson et al., 1985).

Castenholz (1984) and Giovannoni et al., (1987), have reported that hot springs with a high sulfide content of 30 - 130 μM often develop anoxygenic mats composed entirely of anoxygenic phototrophic bacteria dominated by a Chloroflexus - like bacterium which plays an important role as the primary producer of that community growing as a sulfide dependent photoautotroph.

Thus, the abundance and the versatility of those unique filamentous phototrophic bacteria attracted the attention of Pierson and Castenholz (1971), who laid out the description of the first organism known to belong to this group of bacteria, Chloroflexus. Chloroflexus exhibits unique characteristics demonstrated by neither members of the Chlorobiaceae or the Rhodospirillaceae. These characteristics were regarded as sufficiently significant to warrant the establishment of a new separate family of green non sulfur bacteria, the Chloroflexaceae (Trüper, 1976).

II.D. The Chloroflexaceae:-

This family was established by Trüper in 1976, in order to accommodate Chloroflexus and other organisms that presumably would be discovered later on. It was then defined as: phototrophic bacteria containing chlorobium vesicles and bacteriochlorophyll c, d, or e besides bacteriochlorophyll a; the cells have a gliding motility, the cell walls are flexible, and the growth is filamentous. The type genus is Chloroflexus and the type species is Chloroflexus aurantiacus (Pierson & Castenholz, 1974 a).

Besides the characteristics mentioned in the definition, members of the Chloroflexaceae tend to show a preference for photoorganoheterotrophic growth (Madigan et al., 1974), a capacity to grow well under aerobic conditions in the dark (i.e. to generate energy by respiration) (Pierson & Castenholz, 1974 a), storage of poly - β - hydroxybutyric acid and a carotenoid composition that differs from other phototrophic bacteria.

To date, this family consists of four distinct genera : Chloroflexus (Pierson & Castenholz, 1974 a), Heliothrix (Pierson et al., 1985), Oscillochloris (Gorlenko & Pivovarova, 1977), and Chloronema (Dubinina & Gorlenko, 1975), the first two of which are thermophiles that have

been isolated from hot springs, while the other two have been isolated from mesophilic freshwater. Table 1, lists the differential characteristics of those four genera belonging to the family Chloroflexaceae.

So far, only Chloroflexus aurantiacus has been isolated in pure culture while neither description of the other three genera is based on cultured material.

II.E. Chloroflexus :-

The genus Chloroflexus (Greek chloros, green, and Latin, flexus, bending) consists of flexible multicellular filamentous green bacteria. The filaments are of an indefinite length, the cells 0.5 to ca. 1.0 um in diameter, 2 to ca. 6 um in length.

The organism is gram negative, it possesses no flagella and is motile by gliding. Chloroflexus grows primarily as a photoheterotroph and secondarily as a photoautotroph. Its photosynthetic pigments are distributed between the cytoplasmic membrane and the chlorosomes which underlie it.

II.E.1 Classification of Chloroflexus:-

Chloroflexus has been allocated to the anoxygenic

Table 1. Differential characteristics of the genera of the Chloroflexaceae (Pierson & Castenholz, 1974; Dubinina & Gorlenko, 1975; Gorlenko & Pivovarova, 1977; Pierson *et al, 1985).*

CHARACTERISTIC	*Chloroflexus*	*Heliothrix*	*Oscillochloris*	*Chloronema*
Optimum temperature for growth (°C)	20-25 or 50-60	40-55	10-20 or 20-35	3-15
Gas vesicles	-	-	+	+
Gliding motility	+	+	+	+
Diameter of filaments (μm)	0.5-1.0	1.0-1.5	1.0-1.5 or 4.5-5.5	1.5-2.5
Chlorosomes	+	-	+	+
Bacteriochlorophyll :				
a	+	+	+	nt
c or d	+	-	+	+
Facultatively anaerobic	+	+	+	+
Colour	Orange - green	Orange	Green	Green
Sheath	+	-	-	+
Documented species	*C. aurantiacus*	*H. oregonensis*	*O. chrysea*	*C. giganteum* *C. spiroideum*

Symbols : -, negative ; +, positive ; nt, not tested

phototrophic bacteria (Trûper & Pfennig, 1981 ; Imhoff, 1988; Pfennig, 1988). It is capable of performing photosynthesis with a bacteriochlorophyll under anaerobic conditions using a variety of organic compounds as the photosynthetic electron donors.

In spite of the minor affinities that Chloroflexus has to the other anoxygenic phototrophs, it has been separated formally from both the Chlorobiaceae and the Rhodospirillaceae and has been assigned to the family Chloroflexaceae, the characteristics of which have been mentioned earlier.

According to the latest edition of Bergey's manual (Pfennig, 1988) Chloroflexus along with the three genera : Heliothrix, Chloronema and Oscillochloris, have all been grouped under "Multicellular Filamentous Green Bacteria", as a subacategory of green bacteria in spite of the fact that the family name Chloroflexaceae still stands valid.

In earlier works, Castenholz (1969) referred to Chloroflexus as "members of the heterotrophic achlorophyllous flexibacteria". He then referred to them as "Phototrophic flexibacteria" (Castenholz, 1973) although only superficial appearance, dimensions and gliding behaviour related them at that point to the non-photosynthetic flexibacteria as characterized by Lewin (1969) .

Other workers, such as Reichenbach and Dworkin (1981), regarded gliding motility as an important taxonomic feature. Thus they divided all gliding bacteria, a phylogenetically heterogeneous group, into three clusters with Chloroflexus being the main representative of the first group. The second group consisted of cyanobacteria and non-phototrophic organisms while the third group consisted of almost all unicellular gliding bacteria.

II.E.2 The discovery of Chloroflexus:-

The credit for the discovery of Chloroflexus goes back to Beverly Pierson and Richard Castenholz who, in 1971, described some characteristics of two types of phototrophic filamentous gliding bacteria that occurred abundantly in alkaline hot springs and that were then designated as F-1 and F-2.

F-1, which was later on given the name Heliothrix oregonensis (Pierson et al., 1985) has been found in a limited number of Oregon hot springs (U.S.A), forming dense orange surface masses at temperatures up to 55 C (Pierson & Castenholz, 1971). However, since this organism could not be obtained in pure culture, most of the work and investigations have diverted towards the second type F-2, which was

consequently given the name Chloroflexus aurantiacus (Pierson & Castenholz, 1974 a). The holotype of Chloroflexus (J-10-f1) was isolated from hot spring inoculum (pH 7.5, 55°C) collected in the canyon at Sokokura, Japan 25 January, 1970 (Pierson & Castenholz, 1974 a). All strains that were subsequently isolated were also thermophilic with a temperature range of 45-73°C. One mesophilic strain, "Chloroflexus aurantiacus var. mesophilus" (Pivovarova & Gorlenko, 1977) was isolated from freshwater lake Beres-tyanse (Gorlenko, 1975). However, the later differs from the thermophilic strain only in terms of growth temperature since it has a range of 10-40°C.

Since then, and up till now, only one species of Chloroflexus has been reported, and that is the type species Chloroflexus aurantiacus (Pierson & Castenholz, 1974 a).

II.E.3. Habitats of Chloroflexus: -

Chloroflexus populations are most widely distributed in hot springs of neutral to alkaline pH, at temperatures ranging from 45°C to 73°C. They tend to form gel-like, flesh-coloured layers that generally underlie a thin layer (ca. 1 mm) of cyanobacteria forming what is referred to as the microbial mat (Pierson & Castenholz, 1974 a). However, mats consisting purely of Chloroflexus (i. e . without

cyanobacteria) have been reported (Castenholz, 1973). Such mats are usually found in sulfur hot springs where the cyanobacteria are inhibited by the high sulfide content.

Besides hot springs there was one incidence where Chloroflexus was isolated from a freshwater lake (20-25 °C) (Gorlenko & Pivovarova, 1977) where it formed a mat in association with cyanobacteria at the bottom surface of the lake.

Chloroflexus-like organisms have also been found in a hypersaline stratified microbial mat. This was reported in a study that involved the use of transmission electron microscopy as the sole diagnostic tool used to study microbial communities in situ (Stolz, 1984). Otherwise no further evidence has been given for the presence of similar organisms in other habitats.

II.E.4 Chloroflexus in situ :-

The close association between Chloroflexus and cyanobacteria in the laminated microbial mats is of an interest because it sheds some light on the role of Chloroflexus in its natural habitats.

Being the dominant organism in the mat, and again, having the ability to form stromatolites would undoubtedly

indicate its involvement in maintaining both the structural as well as the functional integrity of the mat.

Brock (1978), carried out several experiments and gave direct evidence to support the notion that Chloroflexus is indeed the structural component of the mat. One of these in situ experiments, (Brock, 1978), involved the reduction of the light intensity reaching an area of a typical microbial mat in a hot spring by 98%. Twenty four hours later, the test revealed the absence of the cyanobacterial populations since they could not efficiently utilize low light intensities, while the Chloroflexus populations flourished thus keeping the structure of the mat intact.

Furthermore, Brock (1969) showed that the initial colonization of a new substratum by Synechococcus (a cyanobacterium) to form a microbial mat, requires the presence of Chloroflexus which forms a filamentous matrix that supports the structure and that remains throughout the growth of the mat.

Besides being the basic structural element, Chloroflexus also accounts for the growth of such mats. This has been demonstrated through the accumulation of almost pure populations of Chloroflexus just beneath the surface. These phototrophic populations are characterized as being the healthiest and the most active as they were able to in

corporate ^{14}C - bicarbonate far more better than the populations found in lower parts of the mat (Brock, 1978). They are also characterized by their high productivity, thus contributing largely to the primary production and hence the growth of the mat.

Growing best as a photoheterotroph within the mat, Chloroflexus may have two possible sources of organic matter for photoassimilation : excreted photosynthate of cyanobacteria, or fermentation products such as acetate, propionate, butyrate, ethanol and lactate. The later product may also be used in dark metabolism (Ward et al., 1984 ; Anderson et al., 1987). Interestingly, the organism may change its mode of growth from one form to another in response to the environmental factors prevailing within a specific microenvironment. It may even shift its position within the mat in order to reach appropriate conditions to keep a beneficial status. For example, evidence has been presented by Doemel and Brock (1974, 1977) suggesting that Chloroflexus migrates vertically upwards at night or when light is reduced or eliminated artificially by filters or covers, moving on top of the cyanobacterial component, presumably to a more oxygenated region where more active respiratory processes could occur (Madigan et al., 1974) and so, growth could be resumed.

II.E.5. Physiological aspects of Chloroflexus :-

II.E.5.a. Nutrition :-

From the studies carried out on the nutrition of Chloroflexus so far (Pierson & Castenholz, 1974 a; Madigan et al., 1974), it appears that this phototrophic organism may be one of the most nutritionally versatile organisms known.

Madigan et al., (1974), conducted nutritional studies on a number of strains of Chloroflexus and concluded that this organism can, under the proper conditions, display metabolic diversity being able to grow aerobically as a light independent heterotroph, and anaerobically as a photoautotroph or a photoheterotroph (Appendices I and II).

Supported by other lab experiments it has been demonstrated that Chloroflexus grows primarily as a photoheterotroph and is capable of utilizing a wide variety of simple organic compounds as carbon and energy sources. These included organic acids, short chain fatty acids, amino acids, hexoses and some alcohols as well as yeast extract and casamino acids. Uptake experiments carried out on bacterial mats using radio actively labeled organic compounds have emphasized such findings . It also demonstrated the

ability of Chloroflexus to photoassimilate some of the organic compounds produced within the mat either in the form of photosynthates or as fermentation products (Bauld & Brock, 1974 ; Anderson et al., 1987).

However, when growing in sulfide springs where organic matter is minimal, Chloroflexus grows then as a photoautotroph and uses sulfide as the source of electrons with elemental sulfur being the major oxidation product in addition to thiosulfate or tetrathionate (Brock, 1978).

Regarding the nitrogen source, Chloroflexus is apparently not a nitrogen - fixer as proved by the acetylene - reduction technique and therefore depends on the availability of ammonium ions and several amino acids in its growth medium.

II.E.5.b Temperature and pH :-

The Chloroflexus strains mentioned in the original description and that were isolated from hot springs have an optimum temperature for growth of 52-60 °C with an upper limit of 65-70 C and a lower limit of 30-35 °C (Pierson & Castenholz, 1974 a).

Most of the strains subsequently isolated exhibit a similar range except for the mesophilic variety " C .

aurantiacus var. mesophilus" (Pivovarova & Gorlenko, 1977) which showed a temperature range for growth of 10 - 40 °C with an optimum of 20-25 °C (Gorlenko, 1975).

So far, Chloroflexus has never been found in acid hot springs but has been confined to environments of a neutral to alkaline pH with an optimum of 7.6 - 8.4.

II.E.5.c. Growth:-

In its natural environment Chloroflexus grows in the form of sheets typically forming an orange, photoheterotrophic mat underneath a top layer of cyanobacteria in non-sulfide hot springs (Giovannoni et al., 1987). While in the lab, Chloroflexus grows in liquid medium as flocs, clumps, small aggregates or even as a homogeneous suspension depending on the strain and on the age of the culture (Brock, 1978) ; on agar, it grows as a typical gliding bacterium, forming long swirls and flexuous strands (Brock, 1978).

As mentioned earlier, the organism grows best as an anaerobic photoheterotroph but is relatively a slow grower ; the most rapid growth obtained with the original isolate was (0.3 doublings / h at 55 °C) (Pierson & Castenholz, 1974 b).

II.E.5.d Light and oxygen : -

Another characteristic of Chloroflexus is its ability to withstand wide fluctuations in light and oxygen gradients.

According to the study carried out by Madigan and Brock (1977), Chloroflexus is able to photosynthesize using a wide variety of light intensities ranging from 1,800 Lux (160 ft. candles) up to 90,000 Lux (8000 ft. candles). Therefore, it is able to maintain active populations throughout the various depths of the mat in contrast to the cyanobacterial populations which are usually confined to the top layers (0.5 - 1.0 mm) since they can only use high light intensities.

The Chloroflexus populations found in the upper portion of the mat are usually orange in colour due to the high content of carotenoids. These pigments consist of echinenone and myxobactone (Castenholz & Pierson, 1981), while those populations found in the lower portion of the mat usually attain a dark green colouration due to the high content of bacteriochlorophylls.

As for oxygen, Chloroflexus is capable of withstanding wide fluctuations in oxygen concentrations, a characteristic that is shared with the Rhodospirillaceae.

In the mat, Chloroflexus is subjected to conditions ranging from complete anaerobiosis to full oxygen saturation as a result of algal photosynthesis. However, in the later condition, bacteriochlorophyll synthesis may somewhat be repressed (Doemel & Brock, 1976) and even the synthesis of chlorosomes (Brock, 1978). So, it is at night when anaerobic conditions prevail that oxygen - sensitive pigment synthesis takes place (Castenholz, 1984). Pierson and Castenholz (1974), reported that net increase of chlorophylls in the culture ceased with full aeration both in the dark and light.

II.E.6. Structural aspects of Chloroflexus : -

II.E.6.a. Morphology :-

Chloroflexus is a filamentous organism consisting of a trichome of cells that are attached together. The filaments are of an indefinite length, but generally 30 to 300 μm, the cells are 2 to ca. 6 μm in length and 0.5 to ca. 1.0 μm in diameter depending on the strain and on the phase of growth.

Granular inclusions are frequently observed when using transmission electron microscopy, they have been identified as poly-β-hydroxybutyric acid granules, polyphosphate granules , and possibly glycogen bodies , in addition to

polyglucose which may be found in cells under certain conditions (Sirevåg & Castenholz, 1979).

The DNA area is centrally located and the cell interior is relatively simple with no invaginations of the cell membrane except for mesosomes which are found throughout the cells as well as at the sites of septum formation. Chlorosomes, formerly known as chlorobium vesicles, are found in phototrophically grown cells where they are firmly attached to the inner side of the cytoplasmic membrane, but are not derived from it (Blankenship & Fuller, 1986).

The protoplast of Chloroflexus is surrounded by a typical unit membrane 8-9 nm thick (Pierson & Castenholz, 1974 a) ; the cell wall consists of peptidoglycan with some characteristics of peptidoglycan from gram-positive bacteria (Jürgens et al., 1987). Although Chloroflexus is a gram-negative bacterium, no lipopolysaccharide (LPS) compounds comparable to those of most gram-negative bacteria have been found. This was reported in a study involving two strains of Chloroflexus aurantiacus (Meissner et al., 1988). A thin sheath surrounding the trichome may sometimes be present. No flagella are present ; the mode of locomotion is by gliding.

II.E.6.b The photosynthetic apparatus :-

The photosynthetic apparatus of Chloroflexus consists of two cytologically distinct structures ; the chlorosome, which contains the main light-harvesting antenna pigment, and the cytoplasmic membrane in which the reaction center is located (Fig. 1).

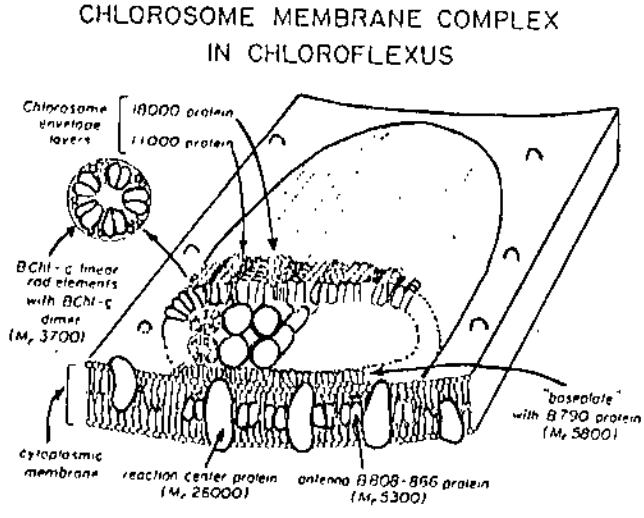


Fig. 1. Topographical model of the chlorosome cytoplasmic membrane complex (Feick & Fuller, 1984).

a) The chlorosome:-

The chlorosomes of Chloroflexus are similar in many ways to those found in the Chlorobiaceae except for their smaller size and the different membrane attachment mechanism (Sprague et al., 1981). They are ellipsoidal in structure, 100 x 30 nm in size (Blankenship & Fuller, 1986) and are surrounded by an envelope which consists of a 3-5 nm thick single layered membrane (Staehelin et al., 1978). Bacteriochlorophyll c, the major antenna pigment in Chloroflexus, is entirely contained within the chlorosomes, along with carotenoids (chiefly β and γ) in addition to a small amount of bacteriochlorophyll a (Blankenship & Fuller, 1986).

Besides the pigments, SDS-PAGE analysis of purified chlorosomes carried out be Feick & Fuller (1984), revealed the presence of four polypeptides, two of which are closely associated and are located on the surface of the chlorosome. The third polypeptide is found inside the chlorosome associated with bacteriochlorophyll c. The fourth, a minor polypeptide is of an extreme interest since it may be associated with a new spectral species of bacteriochlorophyll a, that absorbs at 790 nm in vivo (Schmidt, 1980; Sprague et al., 1981). It also serves as a conduit for energy transfer from the chlorosome antenna to the reaction center in the cytoplasmic membrane.

b) The cytoplasmic membrane

Pure preparations of the cytoplasmic membrane of Chloroflexus have bacteriochlorophyll a as their principle pigment and contain essentially no bacteriochlorophyll c.

The reaction centers located within the membrane appear to be very different from those of the Chlorobiaceae but very similar to those found in the Rhodospirillaceae (Pierson & Thornber, 1983). They consist of a major polypeptide with an apparent molecular weight of 26,000 (Feick & Fuller, 1984), a bacteriochlorophyll a dimer and a two menaquinone acceptor system (Bruce et al., 1982). In addition to the reaction centers the cell membrane also harbours the respiratory enzymes.

II.E.6.c. Pigments of Chloroflexus :-

The pigments of Chloroflexus have been identified by spectrophotometry in various solvents as bacteriochlorophyll a, bacteriochlorophyll c , and carotenoids (Pierson & Castenholz, 1974 b).

Sonically disrupted cells of Chloroflexus show two absorbance maxima at 802 nm and 865 nm both of which are at

tributed to bacteriochlorophyll a ; a single maximum at 740 nm indicating bacteriochlorophyll c and several other maxima at 400 - 500 nm all of which are attributed to various carotenoids. While in crude methanolic extracts bacteriochlorophyll a is indicated by a single peak at 770 nm , bacteriochlorophyll c at 667 nm , and carotenoids at the range of 400 - 500 nm (Appendix III).

The carotenoids of Chloroflexus are conspicuous in the cells both in culture and in nature. They consist largely of monocyclic γ- carotene (22%) and the bicyclic β-carotene (28%) (Halfen et al. , 1980) in addition to glycosides of γ-carotene (28%), echinenone and myxobactone. However, Chloroflexus completely lacks carotenoids of the chlorobactene series, the main type in the green sulfur bacteria.

As for the bacteriochlorophylls, bacteriochlorophyll a and bacteriochlorophyll c were the only types reported to be found in Chloroflexus (Pierson & Castenholz, 1974 b). Gloe and Risch (1978), however, were able to demonstrate that bacteriochlorophyll c obtained from Chloroflexus is different from that obtained from other members of the Chlorobiaceae by possessing a stearyl alcohol tail, and thus is referred to as bacteriochlorophyll cs. In addition, a baseplate bacteriochlorophyll which absorbs at 790 nm in vivo has been reported by Schmidt (1980). This bacteriochlorophyll is located in the chlorosome adjacent to the point

of attachment to the cell membrane (Fig. 1).

The funtion of this chlorosome specific B 790 bacteriochlorophyll a became clear when fluorescence spectroscopic measurements of intact Chloroflexus cells showed that this pigment component actively facilitated energy migration from chlorosomes to the cytoplasmic membrane (Betti et al., 1982).

II.E.6.d Lipids of Chloroflexus :-

Three strains of Chloroflexus have been studied in terms of their lipid content, all of which appeared to be essentially identical (Kenyon & Gray, 1974). They all possess both monogalagtosyl - and digalagtosyl - diglyceride lipids in addition to phosphatidyl glycerol and phosphatidylinositol, the later for which Chloroflexus is the only phototrophic bacterium known to contain as a major phospholipid (Keynon & Gray, 1974).

Simple wax esters (C_{28} - C_{38}) , unusual in bacteria and never in phototrophic bacteria, have been detected soley in Chloroflexus, accounting for 2.5 - 3.0% of the cell dry weight (Knudsen et al., 1982) ; the major wax species being the fully saturated C_{36} (Knudsen et al., 1982).

Regarding the fatty acid constituents, it appears that long straight chain fatty acids with a high degree of saturation (saturated & monounsaturated) predominate in Chloroflexus aurantiacus (Kenyon & Gray, 1974; Meissner et al., 1988). Significant amounts of C_{17} and C_{18}-C_{20} fatty acids which have not been detected in other green bacteria (e.g. Chlorobium) are characteristically present in Chloroflexus in addition to four fatty alcohols (C_{16}-C_{19}).

As for lipopolysaccharides, none of their typical constituents, including the 3-hydroxy fatty acids which are characteristic lipid A constituents, have been found in Chloroflexus (Knudsen et al., 1982; Meissner et al., 1988).

III. GENERAL DESCRIPTION OF THE ZARA AREA

Zara hot springs, the springs from which the samples have been collected for this research, are located in the central part of Jordan some 100 - 1000 m to the east of the Dead Sea shore (ca. 31° 36′ latitutde, 35° 37′ longitude) (Fig. 2). The springs lie at an altitude of 100 - 300 m b.s.l., they flow westerly and then discharge along the slopes overlooking the Dead Sea forming numerous waterfalls. This area, mentioned frequently in old literature and by the Greeks, has been referred to as Therma Kalirrhoes (beautiful springs) because of their position looking down to the Dead Sea (Rimawi & Salameh, 1988).

The area of the springs experiences a Mediterranean climate with hot dry summer and cool winter with moderate humidity. The vegetation is quite pronounced in that area where long spiky grasses predominate especially in summer when some areas may become inaccessible due to the density of the growth of these grasses. Shrubs, some trees and even orchids have been found growing somewhere close to the springs.

According to Salameh and Khudeir (1985) ; Rimawi and Salameh (1988), the Zara hot springs issue from lower Cretaceous Sandstone and older units. The elevated tempera

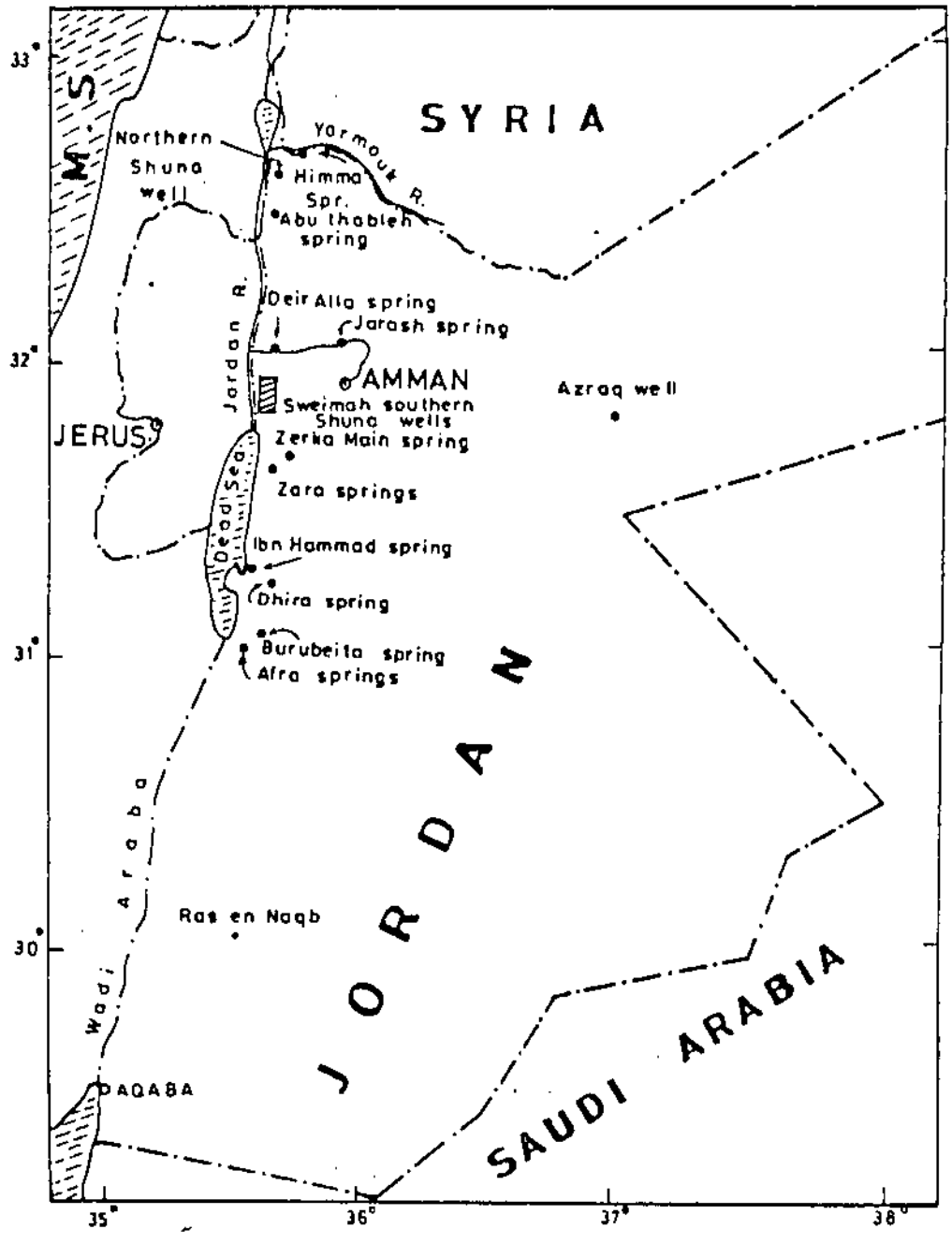


Fig. 2. Map showing the location of the Zara hot springs from which the samples were collected.

ture of the springs - ranging from ambient temperature up to a maximum of 63 °C - is due to the accumulation of thermal energy. This is due to the presence of a thermal isolating layer in the form of unsaturated sandstone and\or shales which raises the temperature of the water accumulating below this layer (the reservoir of the spring water) to 73 - 82°C (Rimawi & Salameh, 1988). The temperature of the different springs tends to be constant over the year with very minor fluctuations (Salameh, 1986).

IV. MATERIALS AND METHODS

IV.A. Environmental measurements :-

Field trips to the Zara area and sample collections were made every 3-4 months (a trip per season) specifically to the hot spring No. 41 which has the highest temperature of all other springs measured to date in that area. The trips were made in order to collect samples of the microbial mats for the purpose of isolating gliding filamentous photosynthetic bacteria and at the same time to observe any seasonal changes in the composition and in the arrangement of the populations forming the microbial mats.

The experimental site covered an area of the spring starting at the source and ending at about 50 m away. In addition to collecting samples, field measurements including temperature and pH of the spring water were also recorded.

The temperature was measured in the field by inserting a mercury thermometer in the water for at least two minutes. The pH was determined in the lab as follows : screw - cap glass tubes were completely filled with spring water and were tightly closed while being totally submerged in the water . The tubes were then heated in the lab to the

original temperature and the pH was determined using a pH-meter.

IV.B. Sampling : -

Representative mats of three different types were chosen for sampling. Two pieces (3 x 3 cm) of each mat were cut and removed with the aid of alcohol pre-sterilized instruments consisting of a scissors, a forceps and a sharp spatula. One piece was placed in a glass jar 2/3 filled with native spring water and the other in a Petri dish. All containers were tightly closed, labeled and transported back to the lab for further treatment in the same day.

IV.C. Description of the mats : -

The jars were usually kept at room temperature under natural sunlight for further observations.

The samples in the Petri dishes were used for detailed description of the microbial mats and for the isolation of gliding filamentous phototrophic bacteria. From each mat sample, a little piece was removed, rinsed with sterile distilled water to remove soil particles and other debris, blotted dry and then sectioned with a sharp razor blade.

The sections were then photographed using a Zeiss stereomicroscope equipped with a Zeiss MC 63 photomicrographic camera. The layers of each section were then separated and their biological components were observed under the phase contrast microscope. The layers consisting basically of filamentous bacteria were then separated from the mat samples and were used as inocula for the isolation process as will be described later on.

IV.D. Isolation and purification : -

IV.D.1 Media :-

Medium D, originally developed by R.P. Sheridan (1966) and referred to as Castenholz medium (1969), is the basic nutrient medium that was used throughout this project for the isolation and enrichment of the purified cultures.

Medium D in addition to glycylglycine and NH_4Cl (Table 2), was prepared as a 10-fold concentrated stock that was stored unautoclaved, in the darkness at 4°C for no more than three weeks. Upon use, the medium was diluted with distilled water, additions were made (if required) and the pH was adjusted to 7.8 with 2M NaOH, agar (1.5%) was added if necessary and the medium was then autoclaved. After cooling, the pH would go down to 7.4, a solution of sterile

Table 2. Media used for cultivation of the isolates.

Medium D		Micronutrient solution	
Distilled water	1000 ml	Distilled water	1000 ml
Nitriloacetic acid	0.1 g	H_2SO_4 (conc.)	0.5 ml
Micronutrient solution	0.5 ml	$MnSO_4.H_2O$	2.28 g
$FeCl_3$ solution (0.29 g/l)	1.0 ml	$ZnSO_4.7\ H_2O$	0.5 g
$CaSO_4.2\ H_2O$	0.06 g	H_3BO_3	0.5 g
$MgSO_4.7\ H_2O$	0.1 g	$CuSO_4.5\ H_2O$	0.025 g
NaCl	0.008g	$Na_2MoO_4.2\ H_2O$	0.025 g
KNO_3	0.1 g	$CoCl_2.6\ H_2O$	0.045 g
$NaNO_3$	0.7 g		
Na_2HPO_4	0.11 g		
Additions :-			
Glycylglycine	0.8 g		
NH_4Cl	0.2 g		
Yeast extract (Difco)	2.0 g		
$NaHCO_3$	1.5 g		

$NaHCO_3$ that has been prepared separately and adjusted to 7.4 with 2 M NaOH is added to the medium to give a final concentration of 0.15%.

This medium, now referred to as DS medium (medium D + supplements) is the one used throughout this project unless otherwise stated).

IV.D.2. Isolation :-

Several small (2-5 mm^2) pieces of the collected hot spring mats were blotted dry to remove excess water and were then placed onto prewarmed DS-agar plates . The plates were then incubated in an inverted position at 50 - 55 °C for several days under tungsten light (40 Watt opaque light bulbs) placed about 30 cm away from the window of the incubator. The plates were then checked under a dissecting microscope for outgrowths spreading out of the mat samples. Whenever a tiny light orange coloured wisp consisting of filamentous bacteria was seen, it was carefully pulled away with the tip of a pin to a new spot on the plate. The process was repeated several times until pure cultures were obtained. Following that, an agar block with a good amount of growth was cut, inverted and spotted several times onto a new plate of DS medium to which 0.2% yeast extract (Difco) has been

added. The plates were sealed with masking tape (that has been pricked at the sides) in order to prevent the plates from drying out quickly, in addition, the incubator was always supplied with a beaker of water to maintain an ample humid atmosphere.

To transfer the purified cultures into liquid medium, a few agar blocks with dense growth on them were placed in ca. 2 ml liquid medium, homogenized and incubated for 10-14 days, at 55°C under semiaerobic conditions and in the presence of light.

IV.E. Enrichment and cultivation : -

Cultures were commonly maintained in liquid photoheterotrophic medium (medium DS supplemented with 0.2% yeast extract), (this medium will also be referred to as "culture medium" throughout the rest of the text). The cultures were grown semiaerobically in screw cap tubes or flasks at 50-55°C under tungsten light and were always kept in a static position since shaking seemed to hinder their growth.

Continuous transfer of the cultures into fresh medium (every 3-4 days for tubes and every 5-6 days for flasks) was essential to keep the cultures growing and in a good condi-

tion, otherwise the cultures would just die and fade away.

IV.F. Maintenance of the cultures : -

IV.F.1. Short-term maintenance procedures :-

Due to the difficulties that were encountered in maintaining the cultures and in the need of continuously transferring the cultures into fresh medium, several approaches have been tried in an effort to maintain the viability of the cultures for the longest time possible.

Liquid culture medium, culture medium + 8% gelatin, agar-semisolidified medium (0.5% agar) and agar-solidified medium (1.5% agar) have all been inoculated and incubated until a sufficient amount of growth was obtained. The cultures were then kept at 50°C, at room temperature and at 4°C. Viability of the cultures at - 20°C was also tested in vials containing equal volumes of liquid medium and of 20% glycerol. Viability of all cultures was then checked after one, two, and four weeks.

IV.F.2 Long-term maintenance procedures :-

Lyophilization :-

Cells at their exponential phase of growth were harvested by centrifugation, suspended in 1% casamino acids and centrifuged again at 1500x g for 5 min. The supernatant was removed and the pellets were dispensed into sterile lyophilization tubes. The cells were freeze-dried for 5-6 h. The tubes were then sealed under vacuum and stored at 4°C.

IV.G. Selection of the most suitable conditions for the growth of the cultures :-

The most suitable conditions for the growth of the cultures were determined by incubation under various combinations of conditions including light, aeration and source of carbon (autotrophic or heterotrophic).

In terms of light, the cultures were incubated under darkened and under lightened conditions. Furthermore, to determine the relative light intensity that is most suitable for the cultures, 5, 40 and 100 Watt light bulbs have been used separately; these corresponded to 1.5, 6.72, and 16.8 W/cm^3, respectively. In terms of aeration, the cultures were incubated under aerobic, semiaerobic, and anaerobic conditions. As for the source of carbon (i.e. the preference for autotrophic or heterotrophic growth) , the

cultures were inoculated into each of the following media:

DS medium, DS medium supplemented with 0.05% $Na_2S.9H_2O$ and DS medium supplemented with 0.2% yeast extract.

Temperature and pH optima and ranges were also determined for all six isolates.

IV.H. Growth rate determination :-

For each isolate, 10 ml of a 24-48 h preculture were inoculated into 150 ml of freshly prepared photoheterotrophic medium in 250 ml screw cap flasks and incubated under the proper conditions. The growth of the cultures was then followed by optical density measurements at 600 nm against a control of uninoculated medium using the Perkin-Elmer spectrophotometer Model 200.

The pH of each sample was also measured in order to monitor changes in the acidity accompanying the growth of the cultures.

IV.I. Growth on single carbon sources :-

The cultures were tested for their ability to grow in

the presence of one organic carbon source both aerobically and anaerobically, in the presence or in the absence of light.

The medium used in this experiment consisted basically of the mineral medium, medium D, 0.02% NH_4Cl, 0.15% $NaHCO_3^-$ and a mixture of ten vitamins expressed in their final medium concentration: vitamin B_{12} (200 ng/ml), thiamine.HCl (60 ng/ml), nicotinic acid (100 ng/ml), para-amino benzoic acid (1 ng/ml), biotin (200 ng/ml), calcium pantothenate (100 ng/ml), pyridoxal.HCl (20 ng/ml), inositol (1 µg/ml), riboflavin (20 ng/ml), folic acid (200 ng/ml). Each organic substrate was then added to a final concentration of 0.25% after it has been neutralized and filter-sterilized. For growth under aerobic conditions, cotton plugged tubes were used, while for anaerobic growth conditions, screw cap tubes were inoculated then sparged thoroughly with nitrogen gas using a sterile bubbling apparatus attached to a sterilized filter unit, the air space was filled with nitrogen gas and then the tubes were closed tightly.

One set of tubes was placed in the presence of light and the other under darkened conditions at 50°C. About a week later the tubes were checked for any apparent growth and the results were scored directly.

IV.J. Screening for enzyme production :-

The ability of the isolates to degrade starch, carboxymethyl-cellulose, skimmed milk and Tween 80 was tested according to the following : agar-solidified DS medium plates each supplemented with a single substrate were prepared.
A few drops of each culture were then placed in glass cylinders that were partially inserted in the agar, a control of uninoculated medium was also added. 24 h later, the plates were checked for positive or negative results.

For gelatin breakdown, tubes containing DS medium supplemented with 0.2% yeast extract and 8% gelatin were inoculated, 3-4 days later liquifaction of gelatin was checked.

Catalase production was detected by the addition of a few drops of 10% H_2O_2 to 1-2 ml of actively growing cultures.

IV.K. Antimicrobial activity :-

The ability of the isolates to produce antimicrobial substances against representative groups of microorganisms (Table 3.) was tested using the agar diffusion assay (cylinder method). Aliquots of both actively growing cul-

tures and of supernatant were added to cylinders partially inserted in nutrient agar or in malt extract agar plates pre-seeded with the test organisms. The plates were checked 48 h later for the development of clear zones around the cylinders as an indicative of the presence of antimicrobial substances.

Table 3. Test microorganisms used for detection of antimicrobial activity :

Gram-negative-bacteria		Gram-positive-bacteria	
Escherichia coli	JCM 5	*Staphylococcus aureus*	JCM
Salmonella typhimurium	JCM	*Bacillus cereus*	JCM 44
Klebsiella sp.	JCM 140	*B. subtilis*	JCM 55
Pseudomonas aeruginosa	JCM 178		
Yeasts :			
Candida albicans	JCM 445		
Saccharomyces cerevisiae	JCM 400		

* All cultures were obtained from the Jordanian Collection of Microorganisms (JCM).

IV.L. Photomicrography :-

Cells of all six isolates were prepared for photomicrography by placing a drop of each culture on a slide that has been thoroughly cleaned and precoated with a thin layer of 0.2% washed agar. Photomicrographs were then taken using a phase contrast microscope (Zeiss standard microscope 16) equipped with a Zeiss MC 63 photomicrographic camera.

IV.M. Preparation of cells for electron microscopy :-

Whole cell preparations were made using filaments taken from actively growing liquid cultures. One drop of bacterial culture was placed on a copper grid that has been covered with a bioloform film. 3-5 min. later, after the filaments were allowed to settle on the grid, excess fluid was removed and the bacteria were negatively stained for 1.0 min with 1% phosphotungstic acid (PTA) adjusted to pH 7.0. Excess stain was removed with a filter paper and the grids were allowed to dry at room temperature. Later on, the specimens were examined in a Zeiss EM 10 B electron microscope.

IV.N. Pigment analysis :-

For the purpose of determining the pigment composition of the isolated pure cultures, the following approaches were used:

IV.N.1. Spectrophotometry :-

Cells of the pure cultures, grown semiaerobically and anaerobically for comparative purposes were harvested by centrifugation at 4500x g for 10 min and then suspended in the appropriate media as follows : -

IV.N.1.a.Intact cells

For the determination of *in vivo* absorption spectra, intact cells were suspended in an aqueous 60% sucrose solution (w/v) that was thoroughly mixed giving a homogeneous suspension.

IV.N.1.b.Organic solvent extracts

Cells were suspended either in absolute methanol(Pierson & Castenholz, 1971), or in 90% acetone. Extractions were carried out at 4°C in the dark (in aluminum foil covered tubes) for 24 h (methanolic extracts) and for 2

h (acetone extracts). The suspensions were then centrifuged and the extract decanted into 1 cm path quartz cuvettes under darkened conditions.

All absorbancies and spectra of both in vivo and organic solvent extracts were determined in the range of 400 nm - 900 nm using the Perkin - Elmer - Hitachi double beam UV-visible recording spectrophotometer Model 200.

IV.N.2. Chromatography :-

In order to obtain large amounts of cells, cultures were grown in 250 ml screw-capped flasks containing 100 - 150 ml enrichment medium. The cells were routinely harvested by centrifugation at 15000x g and the pellets were collected, pooled and stored frozen at - 20°C until needed.

Two procedures were followed for the preparation of the pigment extracts. Thin layer chromatography was performed on silica gel plates F (ICN Biomedicals) after they have been activated for 30 min at 120°C. All solvents used were of a reagent grade and all preparations and manipulations of the extracts were done at room temperature in dim light with aluminum foil wrapped glassware to provide minimal exposure to light.

(1) The first procedure was modified from the method of

Montesinos et al., (1983) who performed it on members of the Chlorobiaceae. 1.0 g wet weight of each isolate was suspended in 5 ml of 90% acetone. Extraction(s) of pigments was/were carried out at 4°C for 24 h. Afterwards, the extracts were centrifuged for 10 min, the pellets were removed, and the extracts (supernatants) were saponified with 20% (wt/vol) KOH-methanol (5% final concentration) and then evaporated by flushing with nitrogen gas. Pigments were then transferred to diethylether in a separatory funnel after the addition of 10% NaCl. The ether layer containing the pigments was washed twice with distilled water and was then centrifuged at 4,500x g for 10 min to remove the remaining water. The resulting ether extract containing the pigments was bubbled with nitrogen gas after the addition of a few crystals of Na_2SO_4. The concentrated extracts were used directly for thin layer chromatographic analysis. Separation was performed using a solvent mixture of benzene-petroleum ether-acetone (10 : 3 : 2 vol/vol).

(2) The second procedure was followed according to Gloe et al., (1975) who also, used this procedure for members of the Chlorobiaceae. 1.0 g wet weight of each isolate was treated with 100 ml of acetone, magnetically stirred in the dark for about 30 min and then centrifuged at 10,000 x g for 10 min. The same step was repeated using a mix -

ture of 135 parts of acetone and 65 parts of carbon tetrachloride. Both extracts were combined and then divided into two equal portions. The first portion was treated two times with about 100 ml 0.5 N hydrochloric acid to obtain the magnesium-free bacteriopheophytins. As a result, an acidic acetone-water phase and a carbon tetrachloride phase were formed, the later of which contained the pigments. After separation of the two phases, the carbon tetrachloride phase was washed three times with distilled water. Distilled water was also added to the second portion of the extracts (which had not been treated with HCl previously). The carbon tetracholride phases obtained from both portions were each evaporated after the addition of Na_2SO_4 by magnetic stirring. The concentrated extracts were then used for thin layer chromatographic analysis. Separation was achieved using the solvent system of carbon tetrachloride: acetone (90 : 10 vol/vol).

The separated pigments of each of the six isolates were then scrapped off, eluted and dissolved in acetone after which they were each subjected to spectrophotometric analysis at a spectral range of 400 - 900 nm.

IV.O. Fatty acid analysis :-

Based on the method developed by Sasser (Kroppenstedt, 1988), in cooperation with Hewlett - Packard, the membrane fatty acid composition of our cultures was determined using the Microbial Identification System (MIS) at the Royal Scientific Society in Jordan.

The cells, cultured under reproducible conditions were harvested by centrifugation and transferred to clean dry screw cap tubes. The cells were lysed by the addition of a strong methanolic base combined with heat and pressure and the fatty acids were liberated from the celluar liquids in the form of fatty acid sodium salts. The fatty acids were then converted into their methyl ester forms and then extracted from the aqueous phase into an organic phase. Free fatty acids and any residual reagents were washed off with a mild base. The washed organic extracts were then applied to the proper sample bottles after which they were identified both quantitatively and qualitatively by gas chromatographic analysis.

V. RESULTS

V.A. Study area - environmental measurements :-

All of the field research was carried out on Zara spring No. 41, the source of which has the highest temperature (63 °C) of all other springs measured to date in the area and in Jordan.

The temperature and the pH were characterized by being relatively constant throughout the year with a variation of +/- 1 °C. The temperature ranged from 63 °C down to ambient temperature at the end of the stream. The pH values ranged from 6.3 at the source up to 7.1 at the terminal study site (about 50 m away from the source). The depth of the water varied from 4 - 25 cm while the width of the stream ranged from 0.5-1.5 m. The characteristic H_2S smell was very weak throughout the spring except for one site where the smell was relatively strong, this site was inaccessible and therefore was not used for sampling. Figures 3 & 4 show a general view of the study area.

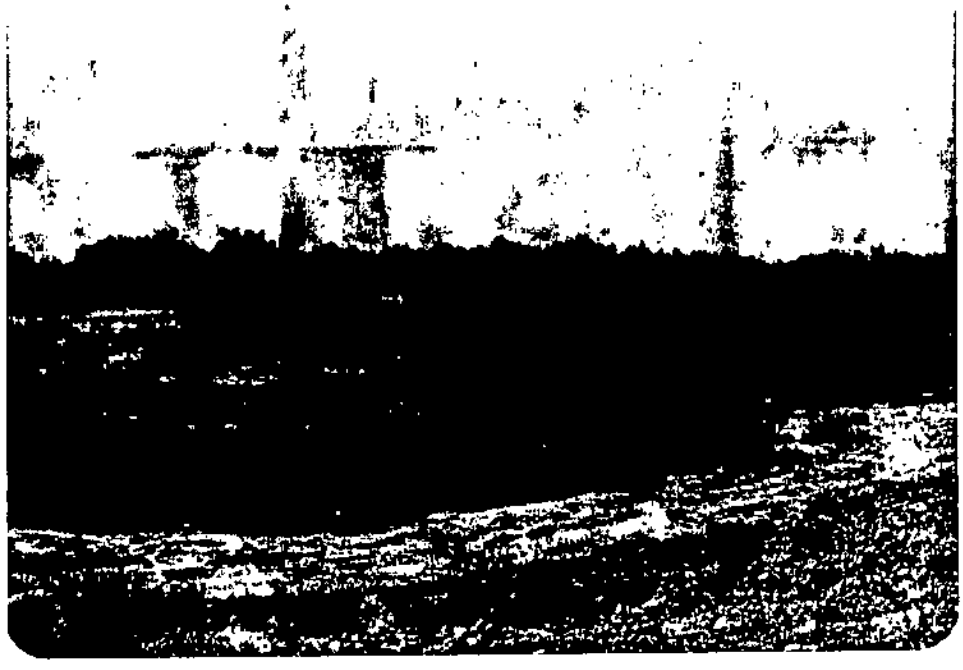


Fig. 3. A panoramic view of the study area.

Fig. 4. Effluent of spring No. 41 from which the samples were collected.

V.B. Description and anatomy of the microbial mats :-

During the study period, three basic types of microbial mats could be observed along the stream. They were all of the laminated form (i.e. consisted of several consecutive layers), ranged in thickness from 5-15 mm, and showed maximum development during the spring season. Samples of each type were usually collected, dissected, examined microscopically and described.

(1) The first type, the floating type (Fig. 6.a.) was usually abundant at the source area where the water flows very slowly and where the temperature reaches a maximum of 63 - 64 °C. This mat was about 5 mm thick and consisted basically of two prominant layers (Fig. 6.b.). The upper layer, green in colour and about 0.5 mm thick, consisted almost exclusively of a cyanobacterial population (Oscillatoria in autumn and winter (Fig. 6.c.), and Synechococcus in spring and summer (Fig. 6.d.). The second layer, which was bright orange in colour, consisted almost solely of filamentous bacteria all year round (Fig. 6.e). Interestingly, in late winter, a third layer usually developed in between the two prominant layers. This greenish layer was found to consist of a mixture of Oscillatoria and Synechococcus over-layed by a newly formed layer of Synechococcus .

Fig. 5. The source of spring No. 41.

2) The second type, the submerged type was collected from locations with a temperature of ca. 54 °C. These mats were always found attached to rocks completely covered with rapidly flowing waters (Fig. 7.a.). The surface layer of this type of mat was dark green and consisted basically of the cyanobacterium Spirulina (Fig 7.b.). The second flesh-coloured layer, consisted of filamentous bacteria and was directly attached to the rock substratum.

(3) The third type, the attached type was the most prominant form in the stream. These mats were often found attached to the banks of the stream, to par-

tially uncovered rocks or even to protruding objects such as dead tree branches occasionally found in the stream (Figs. 8 & 9). Samples of these mats were usually collected from locations with temperatures ranging from 54-59°C. They were 7.5-10.0 mm thick and consisted of several distinctive layers (Fig. 10.a). The surface layer ca. 1 mm thick was dark green in colour and was dominated by Spirulina (Fig.10.b). The second layer, 2-3 mm thick contained Synechococcus and Synechocystis in addition to filamentous bacteria and some crystals. Immediately below, was the distinctive orange layer formed solely of filamentous bacteria (Fig. 10.c). The following whitish layer which had a sponge - like consistency, seemed to be a rudimentary layer since it contained crystals, cell remenants (i.e. empty sheaths, deteriorated cells, ... etc.) and lots of sand particles. A second cyanobacterial layer appeared just beneath the white layer and was dominated by Oscillatoria and Pseudoanabena. This layer was then followed by several layers having the same consecutive arrangement of the preceeding ones except that their contents were quite deteriorated and mixed with lots of debris. In winter, however, the mats showed a similar arrangement of layers except that the position of the cyanobacterial populations was reversed. Oscillatoria and Pseudoanabena

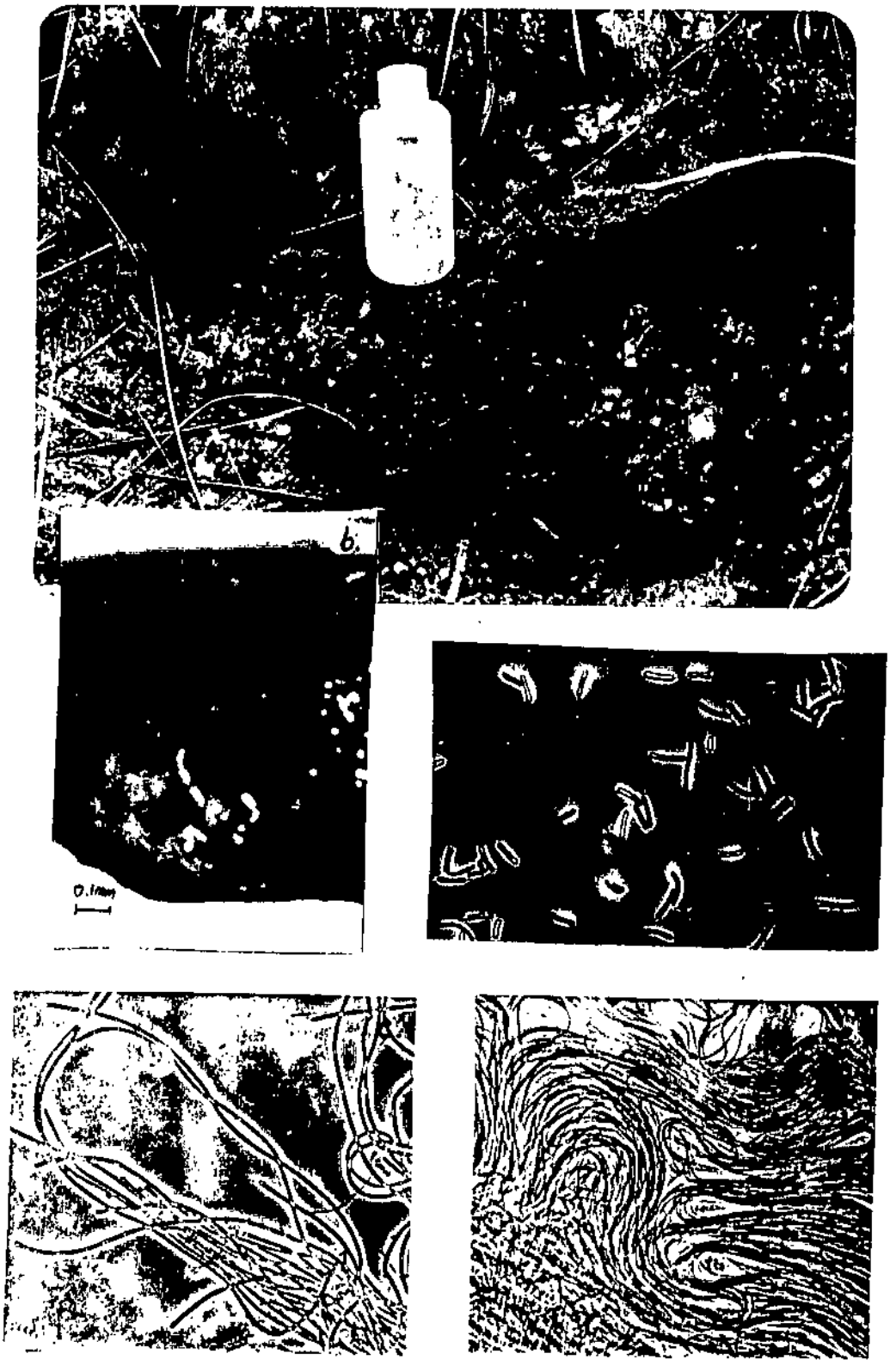
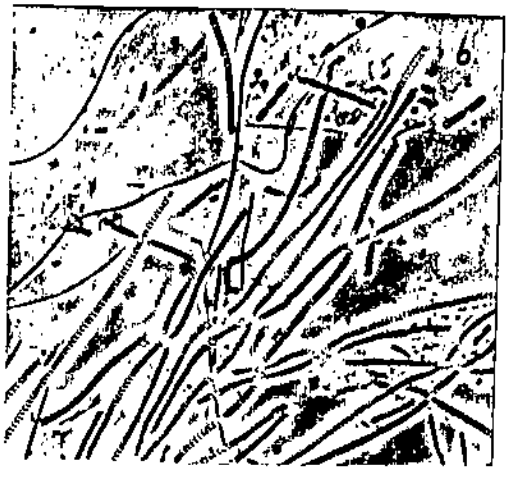


Fig. 6. a) A microbial mat of the floating type.
b) Vertical section of the mat.
c) Oscillatoria.
d) Synechococcus.
e) Filamentous bacteria.

Fig. 7. a) Microbial mats of the submerged type
b) Spirulina, the major cyanobacterium found in the submerged mats.

Fig. 8. Microbial mats attached to the banks of the stream.

Fig 9. Microbial mats attached to and growing on dead tree branches.



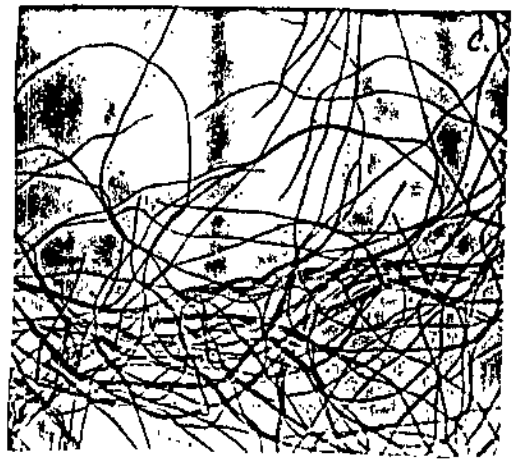


Fig. 10. a) Vertical section of an attached mat showing the different consecutive layers.
b) Spirulina .
c) Filamentous bacteria.

dominated the upper layer while Spirulina dominated the second cyanobacterial layer. Thus clearly reflecting the seasonal variation of the microbial populations forming the mats.

V.C. Isolation of the microorganisms : -

Over sixty initial inocula (small pieces of microbial mats) have been used throughout the study period in the attempts made to isolate thermophilic gliding filamentous phototrophic bacteria. The isolation and purification of these organisms took a very long time during which many difficulties have been encountered. For example, the filamentous and slimy nature of these organisms often made it very difficult to get rid of the contaminating microorganisms often found adhering to the filaments. This difficulty was overcome by continuously searching (under the dissecting microscope) for newly grown wisps and then pulling them away with the aid of a pin from the rest of the colonial mass. By repeatedly doing so, contaminants were gradually diluted out until eliminated. Another approach that was used involved incubating the cultures under various conditions including elevated temperatures (up to 68 °C), anaerobic conditions, media with minimal amounts of organic carbon..etc.,in an effort to eliminate any contaminants that cannot withstand such harsh conditions. Another difficulty that

was encountered during the isolation and purification process was the very slow and poor growth of the isolates on DS-agar plates,so that hardly enough cells could be obtained for subculturing before the plates would have dried out.

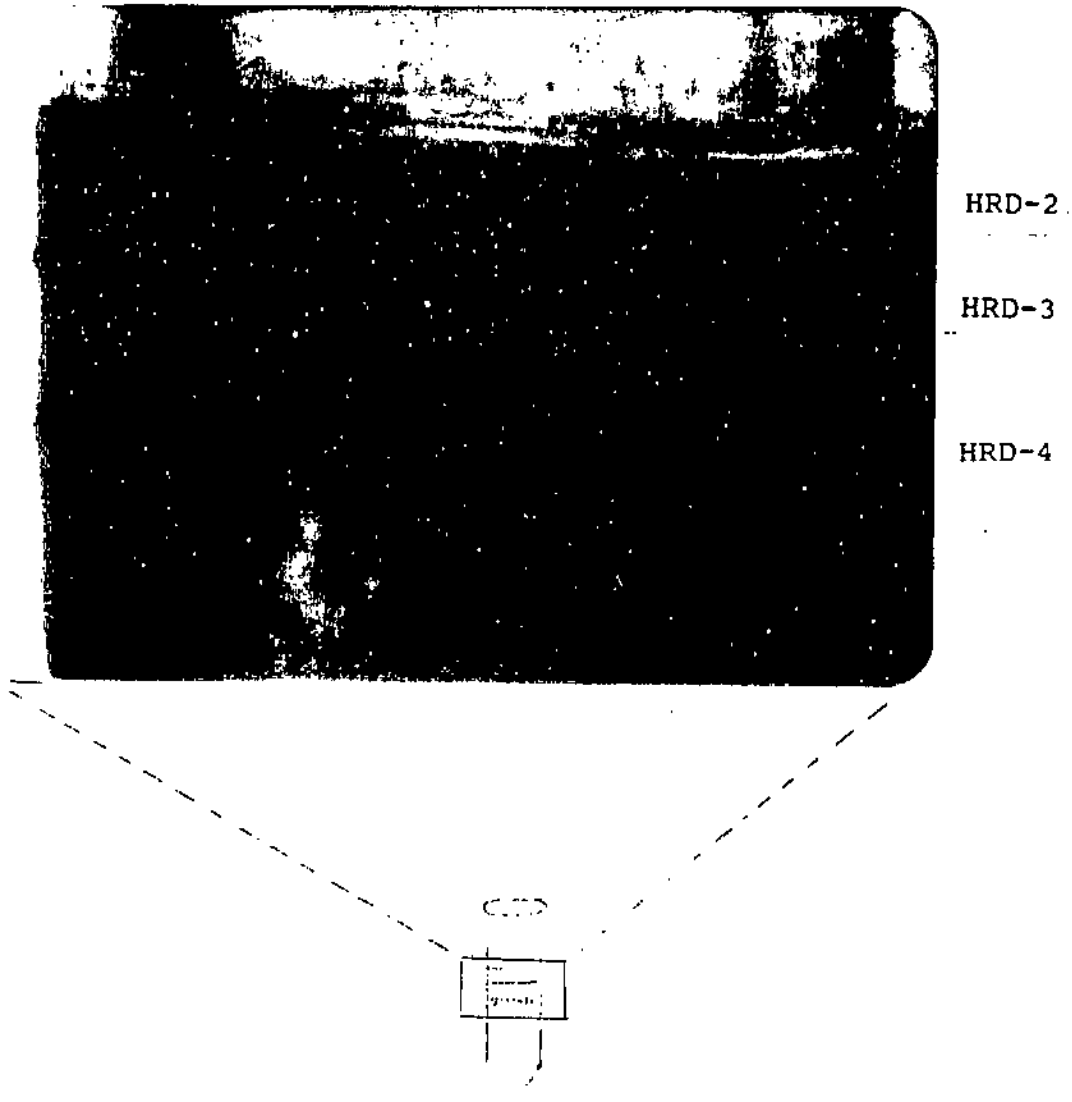


Fig. 11. A tube of solid agar medium showing the separate layers of growth.

The streaking method seemed to be inefficient in promoting more growth by separating the filaments since apparently, a clump or a mass of filaments was always needed to initiate the growth of new filaments on the surface of agar plates. Maintaining the viability of the cultures was one of the most difficult situations since the best conditions for their growth were not yet elucidated and had to await further studies. These studies in turn had to await the development of a procedure that would provide more cells and better growth. This task was finally achieved by transferring the purified isolates into DS-agar plates supplemented with 0.2% yeast extract. By that time, a total of eleven pure cultures were obtained on solid culture medium. However, the transfer from solid to liquid medium lead to the loss of some cultures while others managed to grow and develop. The remaining isolates were then transferred to cotton plugged agar tubes using the agar shake tube method and were then placed in the anaerobic jar. About ten days later, it was found that some cultures have grown forming microcolonies arranged in a single horizontal layer in the upper part of the solid medium. Other cultures grew forming two or three distinctive separate layers (Fig.11). The agar "sausage" was then removed from the tube under aseptic conditions, each layer was cut separately, homogenized and transferred into liquid medium. So, the final resultant of the isolation and purification processes was six pure isolates. They were designated as HRD-1, HRD-2, HRD-3, HRD-4,

HRD-5, and HRD-6. Thus, all subsequent descriptions and characterization mentioned throughout this work are based on these six isolates.

During the course of the work, and during each experiment, the purity of the cultures was continuously checked in order to eliminate any possible interferences or doubts.

V.D. Maintenance of cultures : -

For short term maintenance the best approach proved to be the storage of the cultures at 4 °C in tubes of culture medium (DS broth + 0.2% yeast extract) to which 8% gelatin has been added - a method developed through this work. As such, the cultures remained viable for 4 - 6 weeks.

On agar medium, the results obtained were not constant and some cultures tended to lyse with time ; liquid culture medium and semisolid agar medium proved to be inappropriate for storage for more than one week regardless of the temperature.

None of the isolates tolerated freezing at - 20 °C whether stored in plain culture medium or with the addition of glycerol.

As for long term maintenance, the isolates are currently stored at 4°C in their lyophilized form.

V.E. Morphological studies : -

V.E.1. Appearance in culture :-

All the isolates behaved in a similar fashion when cultured whether on solid or in liquid media.

On the surface of agar plates, the isolates formed swirls and coils typical of gliding bacteria, the colonies appeared light orange in colour but when harvested they appeared brownish - orange ; the only exception in this matter is the isolate HRD-5 which usually appeared much darker than the rest of the isolates. Microscopy of intact colonies revealed the neat arrangement of the filaments (Fig.12), they appeared as stacks or bundles of closely packed filaments from which a bundle would often emerge to form a neighbouring colony. The colonies were shiny and had a slimy consistency. In agar overlays, the cultures formed colonies with lobe-like structures (Fig. 13) with each lobe connected to another with a thin stalk. In agar-shake cultures, filaments formed orange colonies in the top few millimeters and it was rarely that colonies were seen in deeper parts. The colour of liquid cultures was a dull dark orange

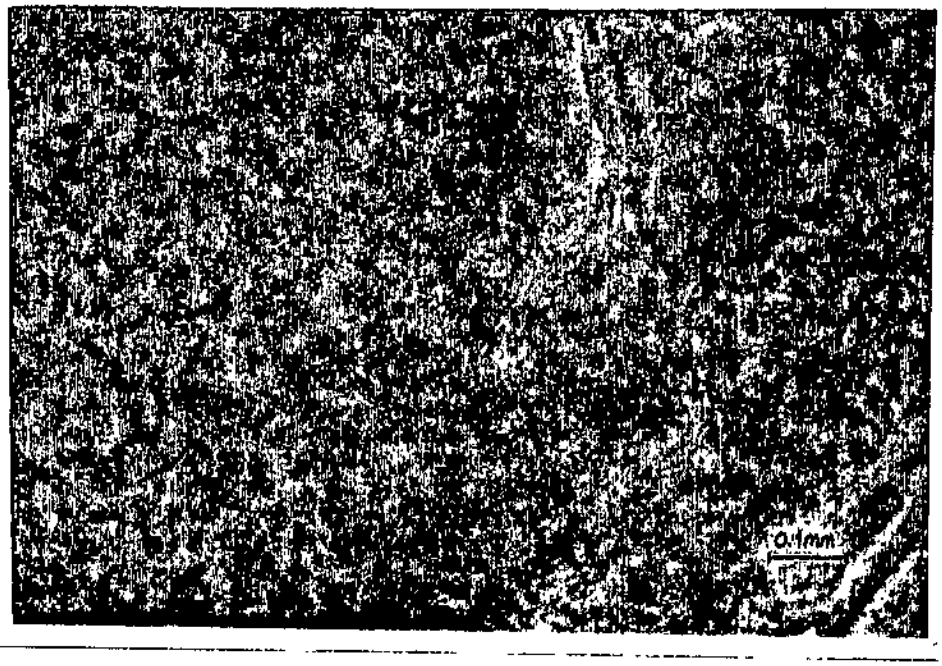


Fig. 12. A close-up revealing the arrangement of filaments within the colonies on agar-surface.

Fig. 13. The appearance of colonies in agar over-lays.

and showed a tint of green sheen when put close to a light source. the color was always the same whether grown under aerobic, anaerobic or semiaerobic conditions. For the first few days, the cultures would grow forming smooth homogeneous suspensions with a silky appearance. Later on, the cells would start to clump with each other, precipitate and then, complete lysis would occur leaving a clear yellow-orange suspension.

Concomitant changes in the morphology of the filaments during the growth have been followed microscopically. The cultures start as thin filaments with an indefinite length; this form is attained all through the lag and exponential phases of growth. Once the cultures enter into the declining or death phase, the filaments start to fragment and become thicker. Bulges or bulb-like structures two to three times the original width of the filament start to form irregularly along the length of the filament. Following that, the filaments start to disintegrate releasing rod shaped cells from within the sheath.

V.E.2 Light microscopic observations :-

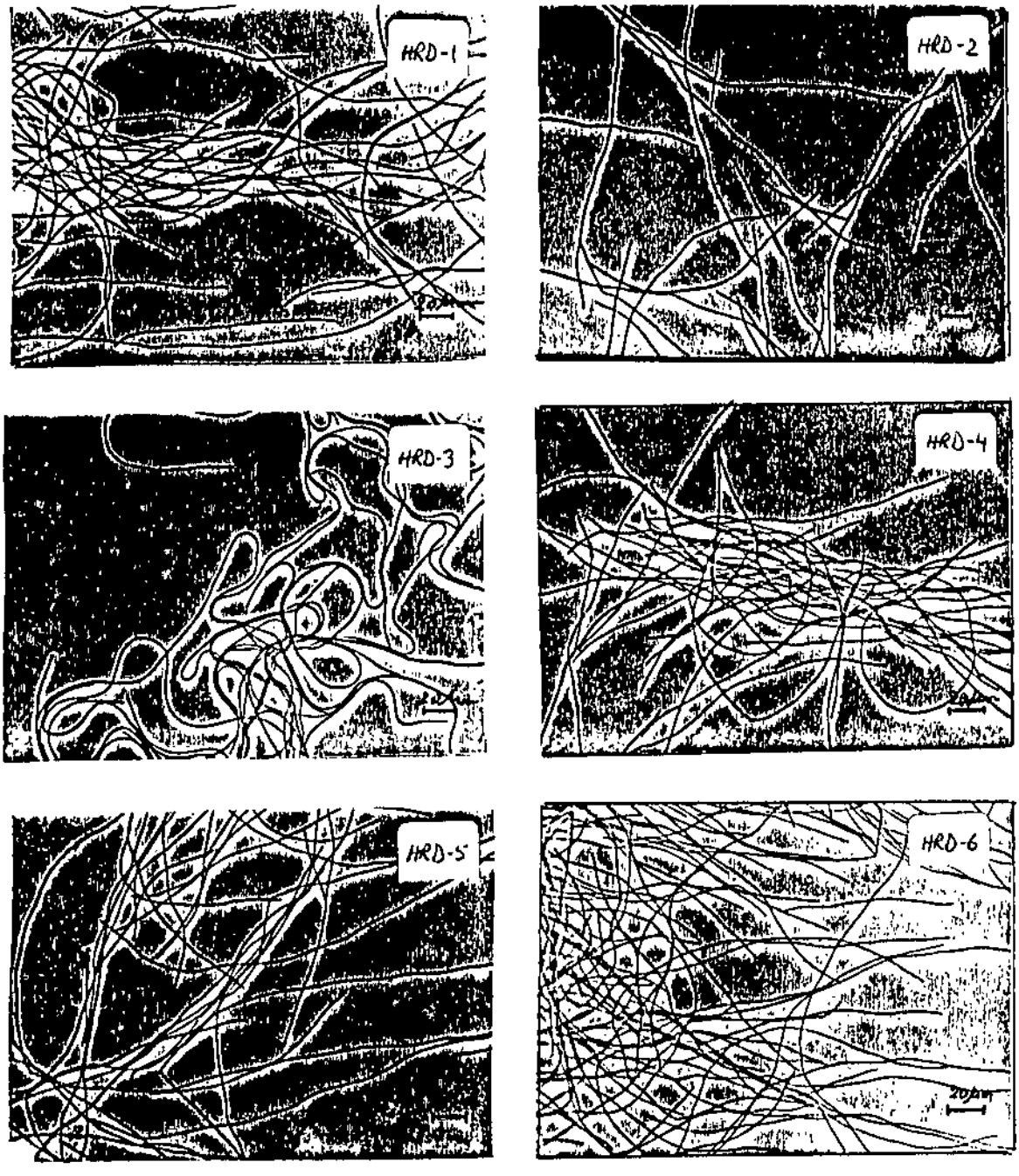
In all six isolates, the bacteria had a filamentous shape with a length of more than 300 μm (Plate I). The width of the filaments varied according to the isolate and according

Table 4. Differential characteristics of the six bacterial isolates (isolated from the Zara hot springs in Jordan)

Characteristic	Isolate					
	HRD-1	HRD-2	HRD-3	HRD-4	HRD-5	HRD-6
Length of filaments (μm)	>300	>300	>300	>300	>300	>300
Diameter of filaments (μm)	0.8 - 1.0	0.6 - 0.7	1.0 - 1.2	1.0 - 1.2	0.8 - 1.0	0.6 - 0.7
Septations	+	+	+	+	+	+
Sheath	+	+	+	+	+	+
Gas vesicles	-	-	-	-	-	-
Inclusions	++	+	+	+	++	+
Chlorosomes	+	+	+	+	+	+
Lengthwise striations	+	-	-	-	+	-
Capsule (slime layer)	+	-	-	-	-	-
Colour	Orange	Orange	Orange	Orange	Orange	Orange
Gliding motility	+	+	+	+	+	+
Gram-stain	-	-	-	-	-	-

Plate I

Light micrographs of the six cultures of gliding filamentous phototrophic bacteria that were isolated from the Zara hot springs



to the phase of growth. In general, the isolates were divided into three groups in terms of filament width at their early stages of growth: isolates HRD-2 and HRD-6, 0.6 - 0.7 µm ; isolates HRD-1 and HRD-5, 0.8 - 1.0 µm ; isolates HRD-3 and HRD-4, 1.0 - 1.2 µm (Table 4). All of the filaments exhibited a flexing behaviour under the microscope. The filaments were undifferentiated, not branched and they all appeared to have a thin sheath surrounding the trichome which was often seen as short translucent segments at the end or inside the filaments. Dark and light coloured inclusions were often seen along the length of the filaments. Neither flagella nor gas vesicles were present. The filaments gave a negative gram-stain, however, when aged cultures were used, parts of the disintegrating filaments showed a gram-positive reaction as well as did the cells that have slipped out of the sheaths.

V.E.3 Electron microscopic observations :-

Electron microscopic examinations of whole preparations of negatively stained cells revealed that the filaments consisted of cells uniseriately arranged in multicellular filaments. The length of individual cells varied from 1.25 µm up to 20 µm, thus a wide variety of sizes could be found within the same filament. However, the dominant size seen in all the isolates was in the range of 6 - 8 µm. Such

variations are clearly seen in isolate HRD-3 (Fig. 14). Numerous electron dense inclusions or bodies were observed scattered all over in the cytoplasm, these were most clearly demonstrated in the isolates HRD-5 and HRD-1, which had structures similar to bi-concaved discs (Figs. 15 & 16). These two isolates also showed lengthwise striations along the length of the filaments. Many different inclusions, most probably denoting storage material, were apparent in all isolates. Additionally, dark diffuse regions were observed in the center of each cell probably indicating the nucleoid region of that cell. Chlorosomes were most clearly seen in the isolate HRD-4 appearing as white oblong structures (similar to short rods) ; they were especially clear in a partially lysed cell (Fig. 17). There were no intracytoplasmic unit membrane structures such as the vesicular, tubular, or lamellar membranous structures typical of either the purple phototrophic bacteria or the cyanobacteria. The layers of the cell membrane could not be clearly distinguished due to low resolution. However, two envelopes could be seen, one surrounding each cell, and immediately next to it another membrane that surrounds the trichome as a whole. An intact outer layer appearing like a capsule or a slimy layer was seen in isolate HRD-1 (Fig. 18). The interesting phenomenon of bulging and bulb-formation appearing in aged cultures is exemplified in Figure 19.

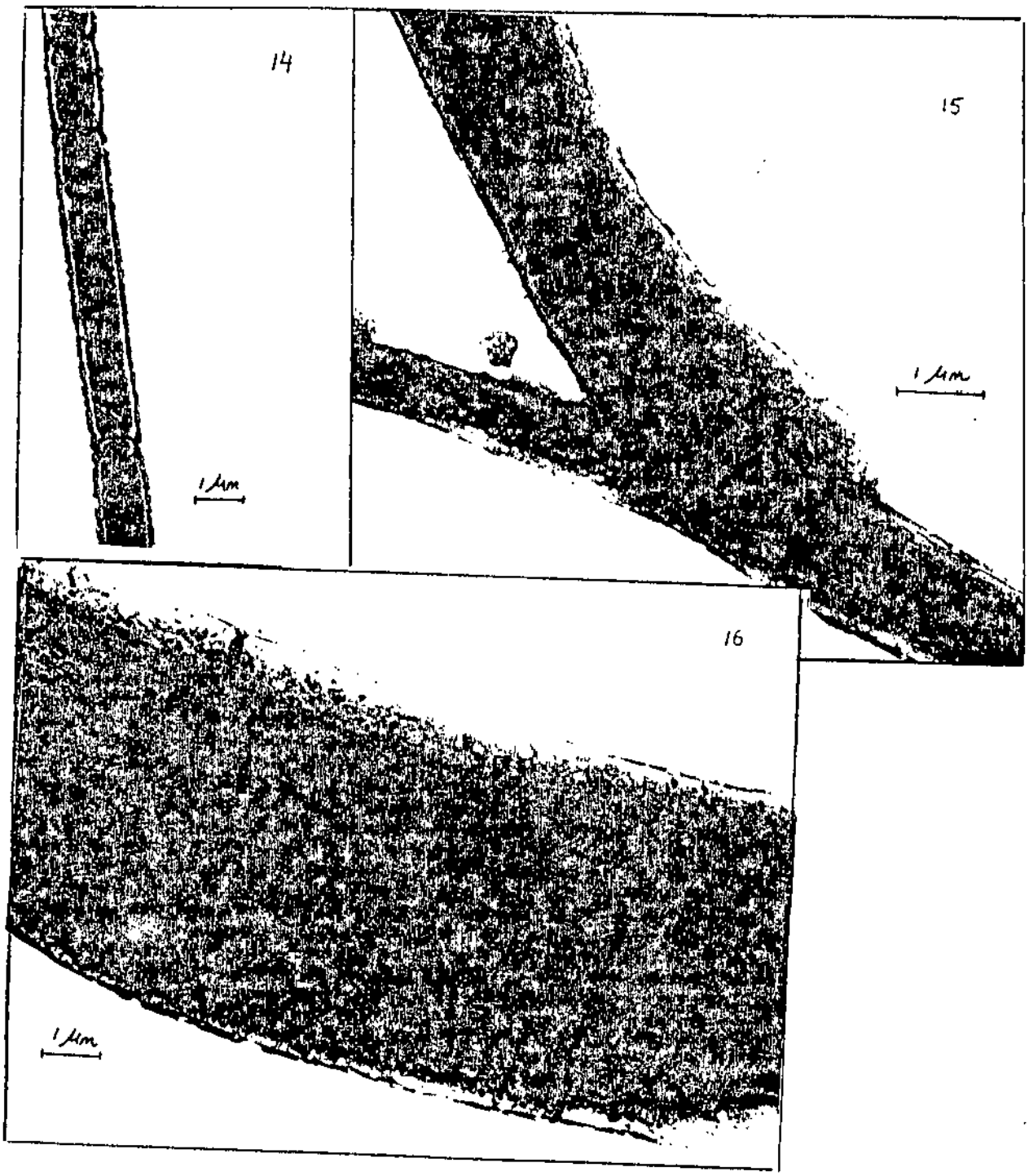


Fig. 14. Electron micrograph of isolate HRD-3 showing various sizes of individual cells within the same filament.

Figs. 15 & 16. Electron micrographs of isolate HRD-1 & HRD-5, respectively, showing an abundance of inclusion bodies & lengthwise striations.

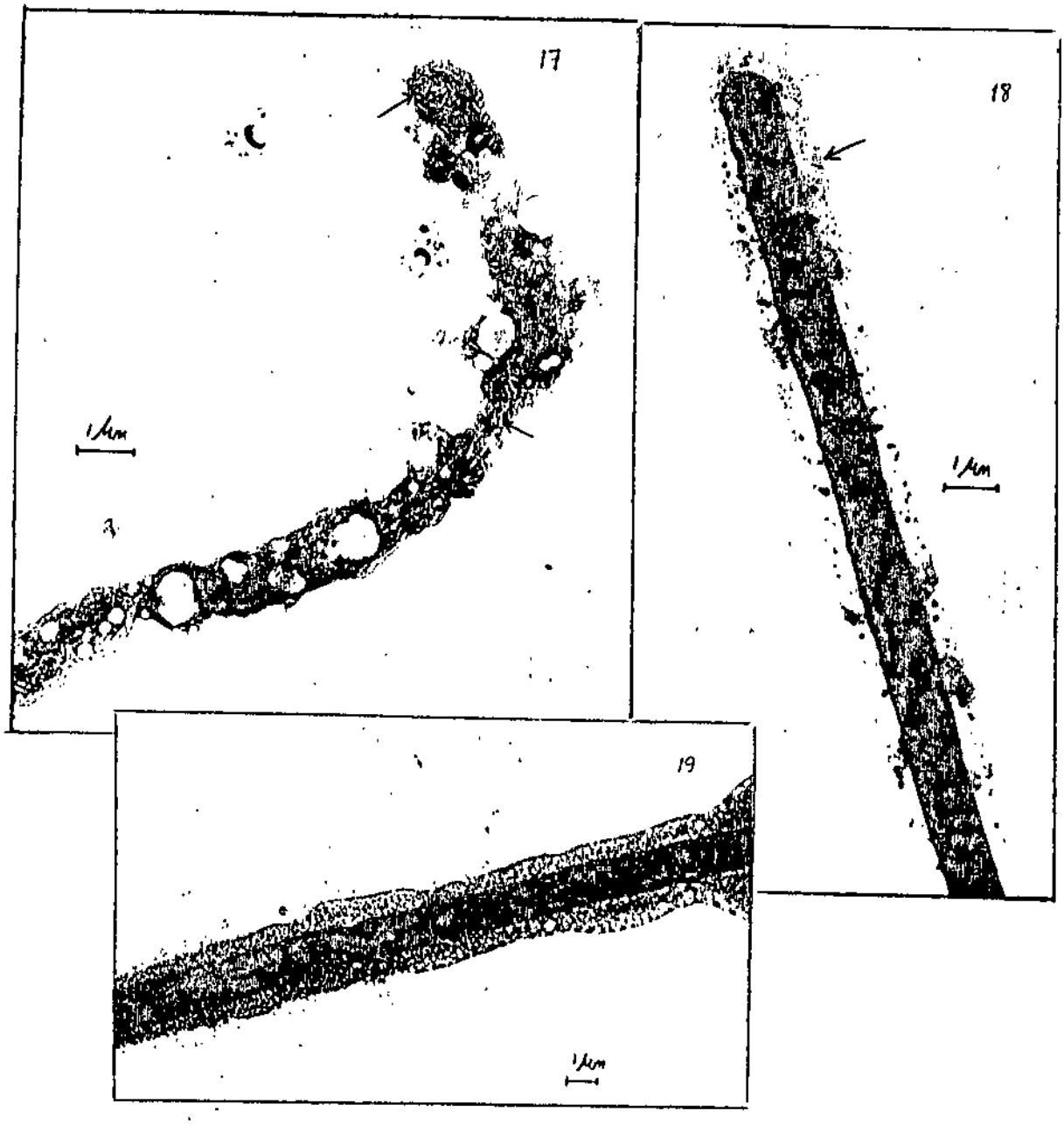


Fig. 17. Electron micrograph of isolate HRD-4 showing the rod shaped chlorosomes.

Fig. 18. Electron micrograph of isolate HRD-1 showing the capsule or slime layer surrounding the filament.

Fig. 19. Electron micrograph of a bulging cell from an aged culture of isolate HRD-1.

V.F. Growth characteristics : -

All isolates grew best as photoheterotrophs under semi-aerobic conditions with medium light intensity (6.72 W/cm^3) and with 0.2% yeast extract serving as a source of organic carbon. These results were based according to the following: -

- Poor and slow growth of the isolates was obtained whenever they were grown under autotrophic conditions, with or without $Na_2S.9H_2O$ in the presence or in the absence of light.

- No measurable growth occurred in the dark whatsoever whether using organic or inorganic media when incubated under aerobic, semiaerobic or anaerobic conditions. However when cultures grown in DS-medium supplemented with 0.2% yeast extract were transferred to light conditions, growth was readily attainable within a few days.

- Light intensity was essential in obtaining good growth. When high light intensities were used (16.8 W/cm^3), the filaments usually adhered to the far side of the growth vessel and then collapsed and died. When low intensities (1.5 W/cm^3) were used, growth was very slow and weak. However, with medium light intensities (6.72 W/cm^3), growth was most

intense and in the form of a smooth homogeneous suspension.

As for aeration, incubating the cultures under aerobic (without shaking), semiaerobic and anaerobic conditions all resulted in measurable amounts of growth. However, under semiaerobic conditions both growth rates and cell yields (Biomass) were higher. Another point worth mentioning is that shaking of the cultures (in the rotary shaker) always seemed to hinder their growth. A few small pellicles would be formed in the first few days after which they would just lyse or deteriorate and no more growth is obtained. The optimum temperature for the growth of the isolates was 50 - 55°C. The upper growth limit was between 68°C and 70°C ; the lower limit was approximately 35 - 37°C. The optimum pH ranged from 6.5 - 8.5. No measurable growth was obtained below pH 5.0 or above pH 10.0 (Fig. 20). The cell yield of the isolates grown under photoheterotrophic conditions was relatively high. It was about 1 - 1.2 g wet weight / 100 ml medium and about 0.1 - 0.12 g dry weight / 100 ml medium.

Table 5 summarizes the results obtained by studying the growth characteristics of the isolates.

V.G. Growth rate : -

The growth curve was determined for each isolate by fol-

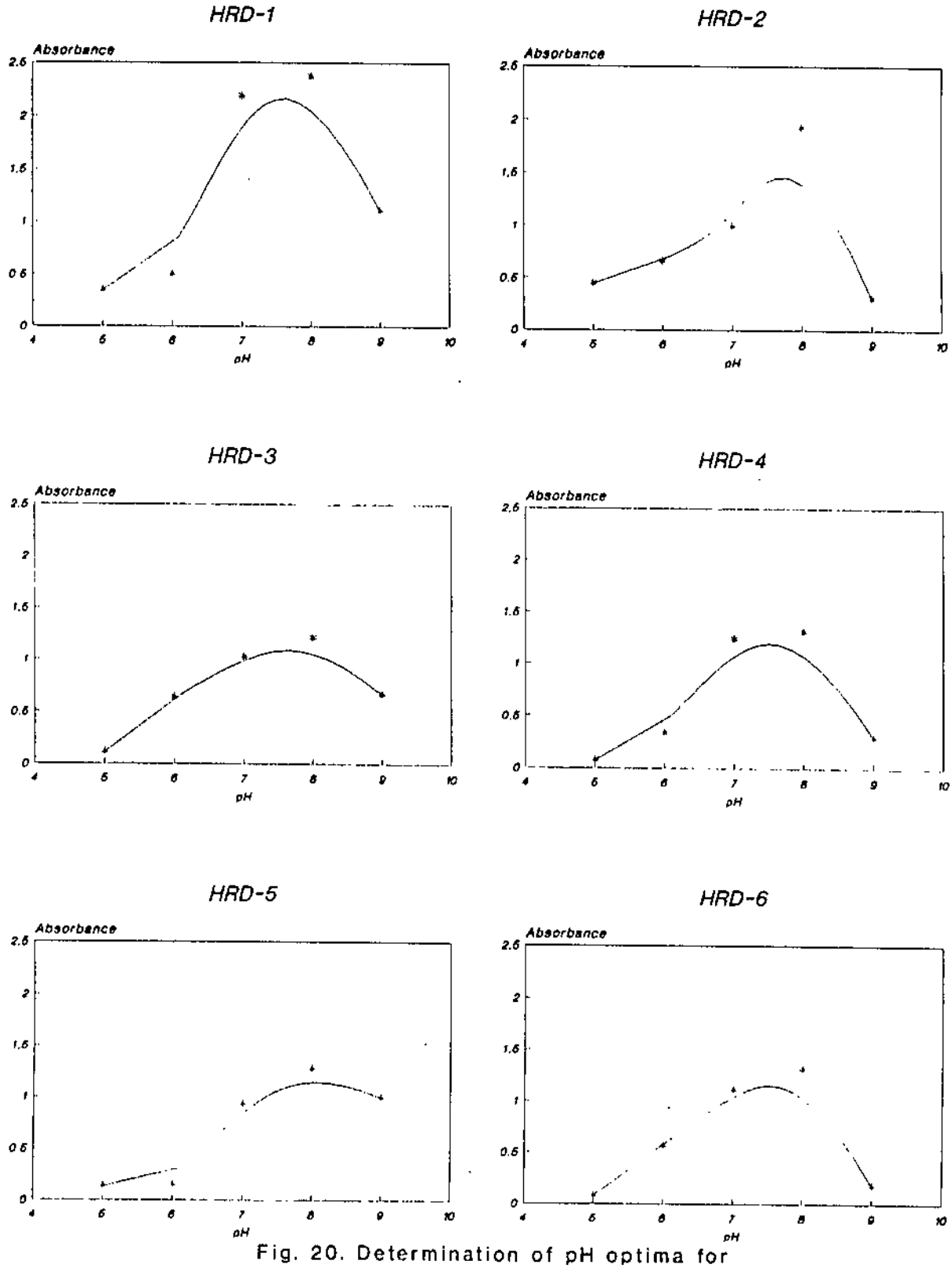


Fig. 20. Determination of pH optima for the growth of the isolates.

Table 5. Some growth characteristics of the six bacterial isolates

Characteristic	Isolate					
	HRD-1	HRD-2	HRD-3	HRD-4	HRD-5	HRD-6
Optimum Temperature for growth (°C)	50 - 55	50 - 55	50 - 55	50 - 55	50 - 55	50 - 55
Upper temperature limit (°C)	68 - 70	68 - 70	68 - 70	68 - 70	68 - 70	68 - 70
Lower temperature limit (°C)	35 - 37	35 - 37	35 - 37	35 - 37	35 - 37	35 - 37
Optimum pH for growth	7 - 8	7 - 8	7 - 8	7 - 8	7 - 9	7 - 8
Cell yield (g/100 ml) :						
Wet weight	1.0	1.1	1.5	1.2	1.2	1.1
Dry weight	0.1	0.11	0.15	0.12	0.12	0.11

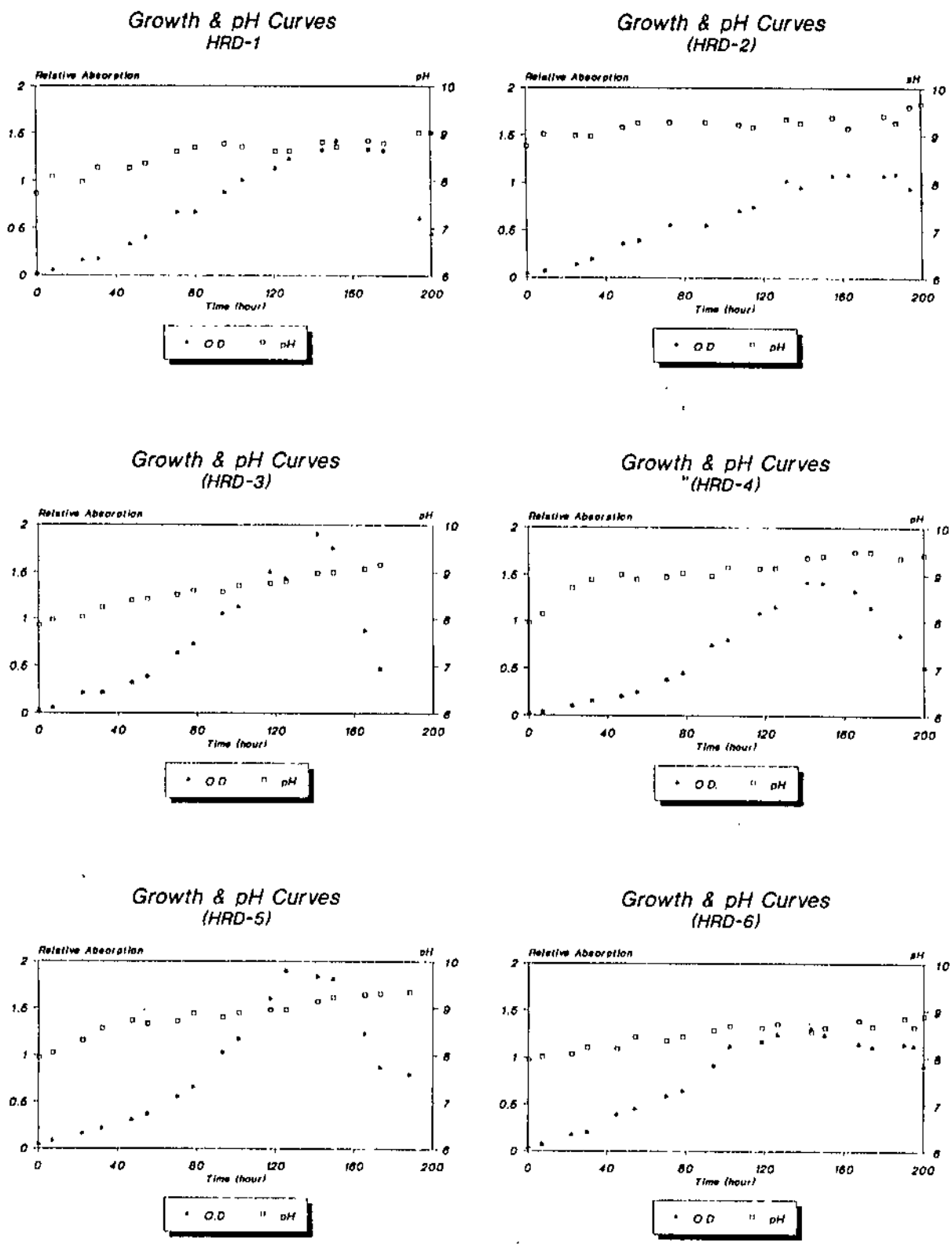


Fig. 21. Growth & pH curves.

lowing the changes in optical density at 600 nm against a control of uninoculated medium (Fig. 21). Starting at 0:00 time with an initial optical density of 0.02 - 0.03, the growth was followed every 8 - 10 h. All the isolates exhibited and relatively short lag phase of about eight hours after which the exponential phase of growth started but at a steady and gradual rate, this phase took about 80 - 120 h. The stationary phase was short in comparison to the exponential phase except for cultures HRD-2 and HRD-6 in which this phase extended for about 60 h.

The pH values were measured concomitantly to detect the changes in acidity or alkalinity accompanying the growth of the cultures. It is clear that the pH was increasing gradually during the growth of the cultures but within a narrow range from ca. 7.5 to ca. 9.5 (Fig. 21). This increase in pH was most obvious during both the exponential phase and by the time the culture has started to decline.

V.H. Growth on carbon sources : -

V.H.1 Growth on single substrates under aerobic conditions:-

Table 6, lists the results of nutritional experiments using defined media carried out under aerobic conditions.

Table 6. Utilization of organic compounds under aerobic conditions

Isolate	HRD-1		HRD-2		HRD-3		HRD-4		HRD-5		HRD-6	
Substrate	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1. Medium DS + Yeast extract	++	-	++	-	++	-	++	-	++	-	++	-
2. Glutamate	-	+	-	-	-	+	-	+	-	+	-	+
3. Aspartate	+	-	++	-	-	-	++	-	+	-	-	-
4. Glycylglycine	-	-	-	-	+	-	+	-	+	+	+	+
5. Acetate	-	-	-	-	-	-	-	-	+	-	-	-
6. Pyruvate	++	+	++	+	++	+	++	+	++	++	++	+
7. Lactate	-	-	-	-	-	-	-	-	++	+	-	-
8. Succinate	-	-	-	-	-	-	-	-	-	-	-	-
9. Malate	+	-	+	-	+	-	+	-	+	-	+	-
10. Butyrate	+	-	-	-	+	-	+	-	-	-	+	-

Table 6. (contd.)

Substrate	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
11. Citrate	-	+	+	+	+	+	-	+	-	-	-	+
12. Ribose	-	-	-	-	-	-	-	-	-	-	-	-
13. Glucose	++	++	++	++	++	++	++	++	++	++	++	+
14. Galactose	-	-	+	+	-	-	-	-	++	+	-	-
15. Ethanol	+	-	+	+	+	-	+	-	+	-	+	-
16. Glycerol	++	+	++	+	-	+	++	+	++	+	++	+
17. Mannitol	-	-	-	-	-	-	-	-	-	-	-	-
18. Yeast extract	-	-	-	-	+	-	+	-	+	+	+	+
19. Casamino acids	++	-	+	++	+	-	+	-	-	++	+	-
20. HCO_3^-	-	-	-	-	-	-	-	-	-	-	-	-

using defined media carried out under aerobic conditions. The test substrates have been selected as representatives of some tricarboxylic acid intermediates, short chain alcohols, amino acids and sugars. The data shows clear nutritional differences among the isolates. No two isolates were identical whether in terms of substrates utilized or in terms of cellular yield. In general, under light conditions, most of the isolates were able to utilize about 45% of the total substrates tested while under dark conditions, about 25% of the substrates were utilized. Pyruvate, glycerol and glucose supported the growth of all six isolates under both light and dark conditions except for isolate HRD-3 which did not grow in the presence of glycerol under light. With pyruvate and glycerol, the cell-yield was higher under light conditions as observed by the turbidity of the cultures while with glucose, almost identical yields were obtained under both conditions. Some substrates were utilized only in the presence of light such as aspartate, malate, butyrate and ethanol while other substrates, such as glutamate, supported the growth of all isolates only under dark conditions. Succinate, ribose, and mannitol were not utilized by any of the isolates. Lactate, on the other hand, supported the growth of only one isolate : HRD-5, while galactose supported the growth of only two isolates : HRD-2 and HRD-5. The results obtained with HCO_3^- as the test substrate, clearly demonstrate the inability of all the isolates to grow in the absence of an organic source of carbon.

V.H.2. Growth on single substrates under anaerobic conditions:-

Table 7, shows the results of substrate utilization experiments under anaerobic conditions. In this part of the experiment, even more variations occurred between the isolates in terms of their abilities to use different substrates. Generally, however, more substrates were utilized under light than under dark conditions, in addition to the fact that growth in the presence of light was always better and gave a higher yield. All the isolates were able to utilize both pyruvate and glucose under both conditions of light and dark except for isolate HRD-6 which did not grow in the dark with glucose serving as the substrate. Some substrates such as aspartate, supported the growth of all the isolates under light conditions only. Glycerol on the other hand, was the only substrate that could support the growth of all the isolates in the complete absence of light. Succinate, ribose, mannitol, in addition to glutamate were all poorly utilized or even not at all as was the case under aerobic conditions. The inability of the isolates to grow with HCO_3^- serving as the test substrate, again demonstrates the importance of the presence of an organic carbon source for their growth.

A point worth mentioning concerning both parts of the experiment is that neither yeast extract nor glycylglycine or

Table 7. Utilization of organic compounds under anaerobic conditions

Isolate	HRD-1		HRD-2		HRD-3		HRD-4		HRD-5		HRD-6	
Substrate	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1. Medium DS + Yeast extract	+	–	+	–	+	–	+	–	+	–	+	–
2. Glutamate	–	+	–	–	–	+	–	+	–	–	–	–
3. Aspartate	+	+	++	–	+	–	++	–	+	–	+	–
4. Glycylglycine	–	–	–	–	–	+	+	+	+	+	+	+
5. Acetate	–	+	+	–	–	–	–	–	+	–	–	–
6. Pyruvate	+	+	++	++	++	++	++	+	++	++	++	++
7. Lactate	++	–	++	–	–	–	–	–	+	+	–	–
8. Succinate	–	–	–	–	–	–	+	–	+	–	–	–
9. Malate	+	+	+	+	+	–	+	–	–	–	+	–
10. Butyrate	–	–	–	+	+	–	+	–	–	–	+	–

Table 7. (contd.)

Substrate	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
11. Citrate	+	+	–	+	+	+	+	–	–	–	+	–
12. Ribose	+	–	+	–	–	–	–	–	–	–	–	–
13. Glucose	–	–	++	–	–	–	++	–	++	–	++	–
14. Galactose	+	+	+	+	++	+	–	–	++	+	++	+
15. Ethanol	+	–	+	+	–	–	++	+	+	–	+	–
16. Glycerol	–	+	++	+	–	+	–	+	++	+	++	+
17. Mannitol	+	–	+	–	+	–	–	–	–	–	–	–
18. Yeast extract	–	–	–	+	–	+	++	+	++	–	++	–
19. Casamino acids	++	+	+	–	+	–	+	–	+	–	+	–
20. HCO_3	–	–	–	–	–	–	–	–	–	–	–	–

HCO_3^- when used separately, promoted sufficient growth with good yields as when compared to the control (DS-medium + 0.2% yeast extract) inoculated at the same time and with the same amount of cells.

V.I Screening for enzyme production:-

Table 8, summarizes the results obtained for all the isolates while screening for enzyme production. Fig. 22 shows the results obtained for the production of amylase. Compared to the control of uninoculated medium (central well), it is clear that the isolates are good producers except for isolate HRD-6 which is a relatively weak one. Fig. 23 shows the breakdown of casein. The isolate responsible for the largest zone is isolate HRD-3. The rest of the isolates show a milder activity as compared to the control of uninoculated medium (central well). Carboxymethyl cellulose (CMC) was also broken down by all isolates as indicated by the clear zones that appeared 24 h later. Tween 80 was most efficiently broken down by isolate HRD-3 as indicated by the large amounts of precipitate that were seen. The other isolates were also active in this respect but not to the same extent. Only four out of the six isolates were capable of liquifying gelatin: HRD-1, HRD-3, HRD-4, and HRD-5. As for catalase production, all the isolates gave negative results.

Table 8. Screening for enzyme production

Test	Isolate					
	HRD-1	HRD-2	HRD-3	HRD-4	HRD-5	HRD-6
Starch hydrolysis	+	+	+	+	+	+
Casein hydrolysis	+	+	++	+	+	+
CMC hydrolysis	+	+	+	+	+	+
Gelatin hydrolysis	+	-	+	+	+	-
Production of catalase	-	-	-	-	-	-

* +, positive ; -, negative ; +, weakly positive

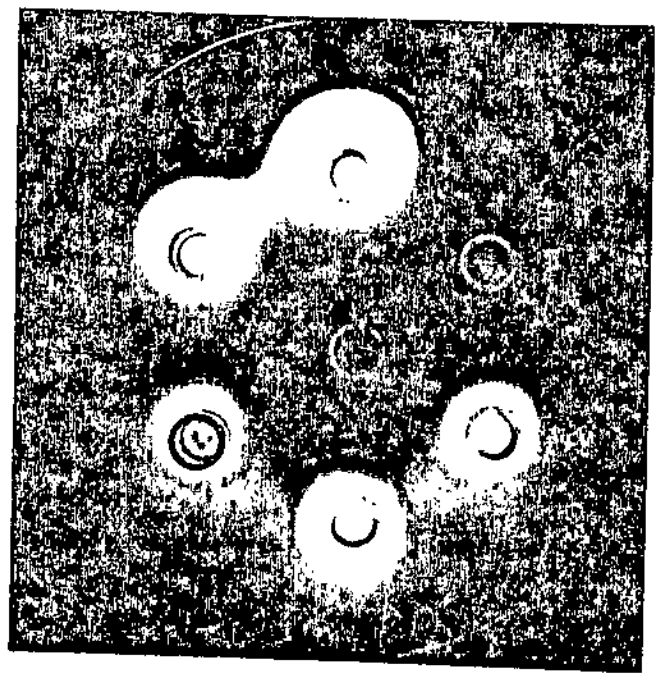


Fig. 22. Degradation of starch.

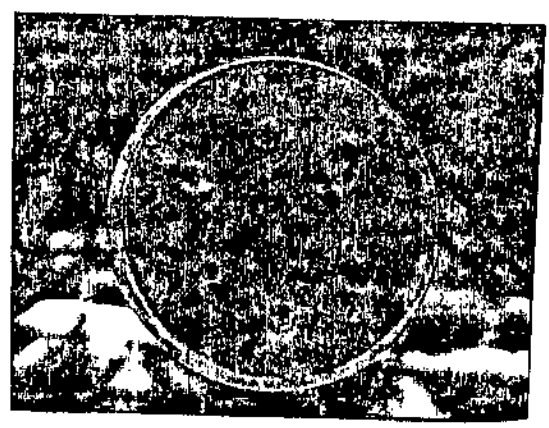


Fig. 23. Degradation of casein.

As for catalase production, all the isolates gave negative results.

V.J. Screening for antimicrobial activity : -

Using the agar diffusion assay (cylinder method), aliquots of both actively growing cultures and of supernatant showed no signs for the presence of antimicrobial activity against Gram-positive and Gram-negative bacteria or yeasts. However, E. coli was mildly inhibited by most isolates.

V.K. Pigment analysis :-

V.K.1. Spectrophotometry :-

The absorption spectra obtained for the different isolates showed a similar pattern except for minor shifts in the range of some peaks. Although the quantitative differences in the pigments were not taken into consideration in this experiment, it was always made sure to use the same initial biomass (0.1 g wet weight) for each isolate whether for in vivo studies or for extraction. In spite of this measure, it was found out during the experiment that isolate

HRD-6 had to always be diluted about ten times to give a concentration of pigments that is equivalent to the rest of the isolates.

Comparing the scans of the anaerobically and the semi-aerobically grown cells of all six isolates revealed no differences in the position or in the height of the peaks.

V.K.1.a *In vivo* absorption spectra :-

Absorption sepctra of intact cells suspended in 60% sucrose (w/v) showed several maxima (Fig. 24). The principle maximum was at 743 nm for isolate HRD-1 ; at 745 nm for isolates HRD-2 and HRD-5 ; at 748 nm for isolate HRD-6 ; and at 750 nm for isolates HRD-3 and HRD-4. This range (743-750 nm) indicates the presence of bacteriochlorophyll *c* (Oelze, 1985). The two other absorption maxima at 868-873 nm and at 802-805 nm indicates the presence of bacteriochlorophyll *a* (Pierson & Castenholz, 1974 a, b ; Thornber *et al.*, 1978). The group of absorption maxima observed at the range of 400-500 nm indicates the presence of carotenoid pigments (Pierson & Castenholz, 1974 b). Table 9, summarizes the spectral characteristics of intact cells suspended in 60% sucrose.

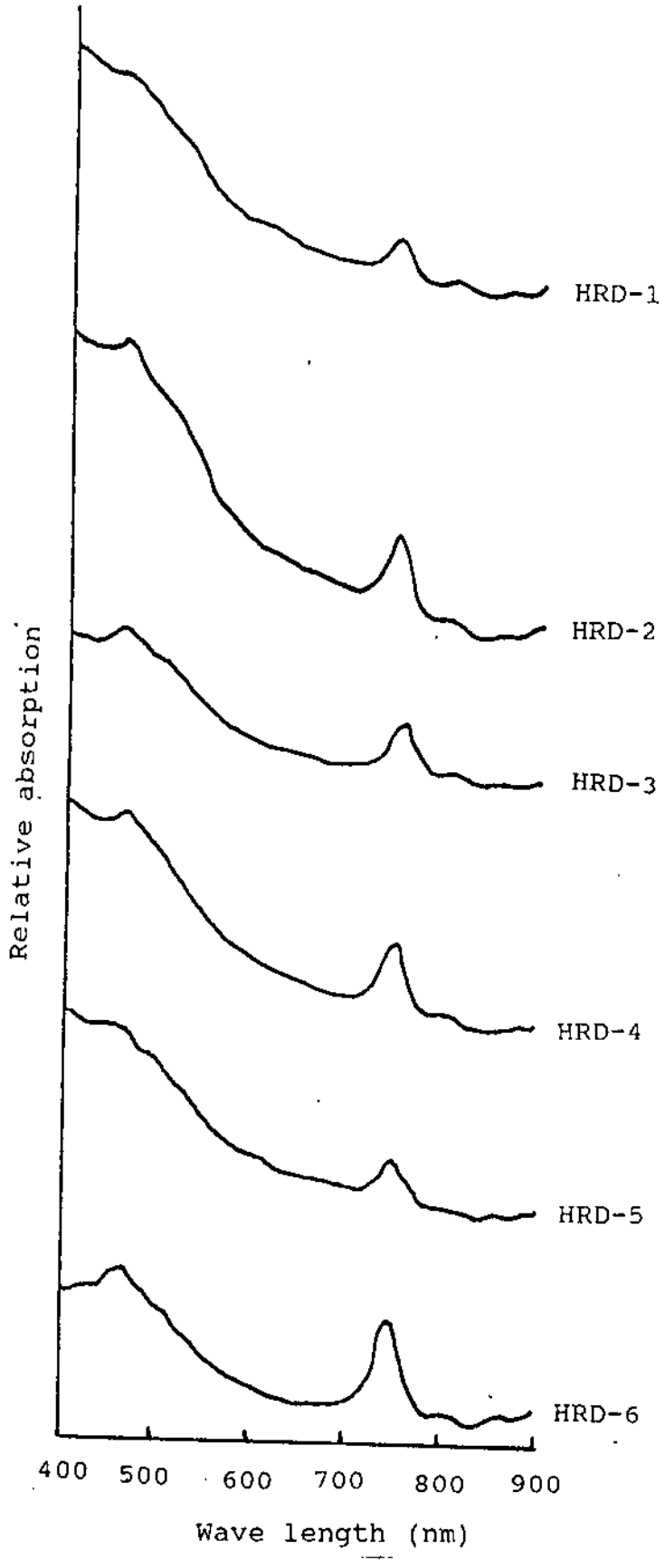


Fig. 24. Absorption spectra of intact cells suspended in 60% sucrose.

Table 9. Spectral characteristics of intact cells suspended in 60% sucrose

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	870 (P)	802 (P)	743 (P)	503 (S)	449-461 (S)	412 (S)
HRD-2	868 (P)	805 (P)	745 (P)	510 (S)	465 (P)	438 (S)
HRD-3	870 (P)	805 (P)	750 (P)	510 (S)	466 (P)	-
HRD-4	873 (P)	802 (P)	750 (P)	510 (S)	466 (P)	-
HRD-5	871 (P)	802 (P)	745 (P)	611 (S)	500 (S)	466 (P)
HRD-6	870 (P)	803 (P)	748 (P)	502 (S)	471 (S)	463 (P)

V.K.1.b. Organic solvent extracts : -

Total pigment extracts in acetone and in methanol confirmed the presence of both bacteriochlorophylls a and c in all six isolates. In acetone extracts (Fig. 25 ; Table 10), bacteriochlorophyll a is indicated by a single absorption maximum at 770-775 nm while bacteriochlorophyll c is indicated by the principle absorption maximum at either 662 nm as in isolates HRD-3 and HRD-4 ; at 665 nm as in isolates HRD-5 and HRD-6 ; or at 666 nm as in isolates HRD-1 and HRD-2. Additionally, two or three small peaks and/ or shoulders in the spectrum range of 572-630 nm are observed in the spectra of all our isolates (Fig. 25). Nothing similar to these bands have been mentioned previously in literature. The peaks and shoulders appearing in the spectral range of 400-500 nm indicate the presence of carotenoid pigments. One major peak appears in the range of 432-436 nm. Three other bands appear at 484-488 nm, at 460-463 nm, and at 410-420 nm. As mentioned earlier, no attempts were made to identify any of the carotenoids due to the absence of similar studies with which we could compare our results.

In methanol extracts (Fig. 26), a single broad peak appearing in the range of 766-776 nm in all six isolates denotes the presence of bacteriochlorophyll a while the major peak at 667-670 nm implicates the presence of bacteriochlorophyll c (Pierson & Castenholz, 1971). A small

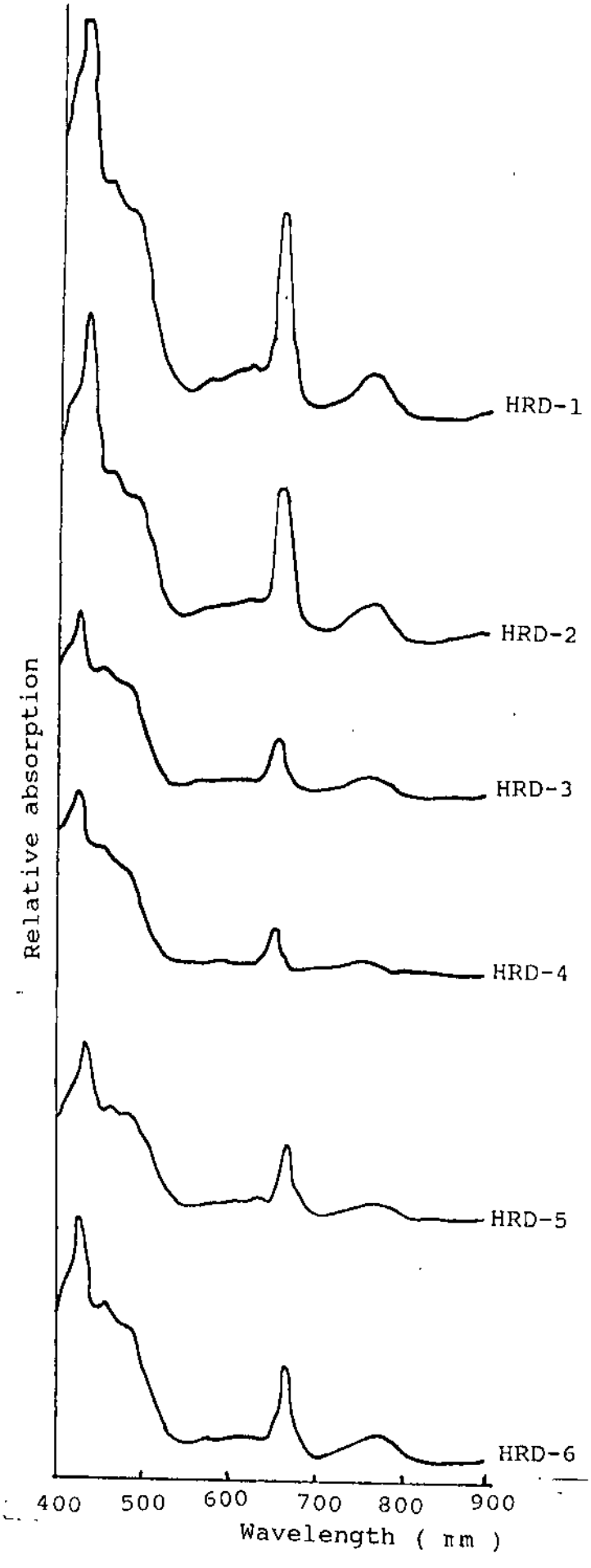


Fig. 25. Absorption spectra of acetone extracts of whole cells.

Table 10. Spectral characteristics of acetone extracts of whole cells

Isolate	Wavelength (nm) of major features in spectrum								
HRD-1	772 (P)	666 (P)	630 (P)	618 (S)	582 (S)	488 (S)	462 (P)	434 (P)	-
HRD-2	770-772 (P)	666 (P)	630 (P)	616 (S)	580 (S)	485 (P)	463 (P)	436 (P)	420 (S)
HRD-3	770 (P)	662 (P)	626 (P)	572-598 (S)	484 (S)	460 (P)	433 (P)	410 (S)	-
HRD-4	770 (P)	662 (P)	630 (P)	612 (S)	575 (S)	484 (S)	460 (P)	432 (P)	410-412 (S)
HRD-5	771-775 (P)	665 (P)	628 (P)	615 (S)	581 (S)	484 (S)	462 (P)	435 (P)	415 (S)
HRD-6	772 (P)	665 (P)	628 (P)	582 (S)	488 (S)	462 (P)	436 (P)	418 (S)	-

* S, Shoulder in spectrum ; P, Peak in spectrum

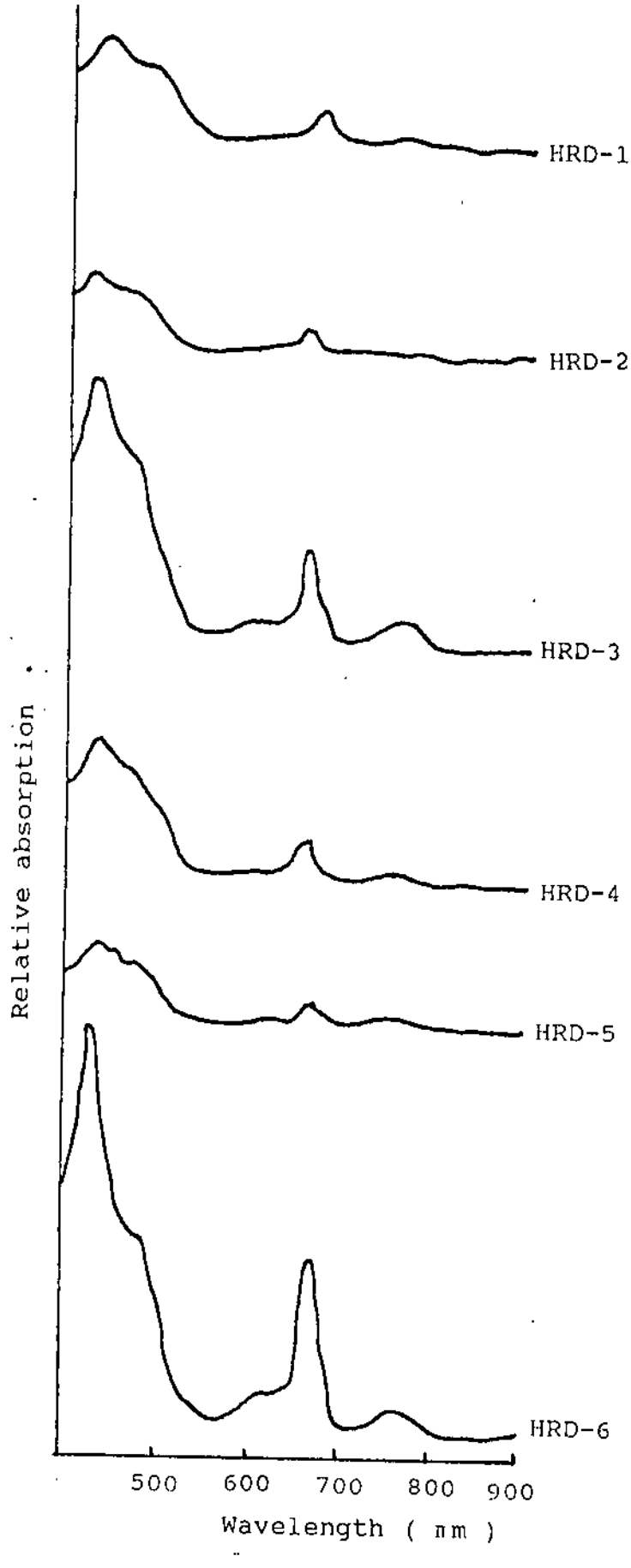


Fig. 26. Absorption spectra of methanol extracts of whole cells.

Table 11. Spectral characteristics of methanol extracts of whole cells

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	766-776 (P)	669 (P)	626 (P)	488 (S)	438 (P)	-
HRD-2	769 (P)	670 (P)	610-633 (P)	480 (S)	435-440 (P)	-
HRD-3	768 (P)	669 (P)	614-621 (P)	480 (S)	436 (P)	-
HRD-4	770 (P)	667 (P)	618 (P)	481 (S)	436 (P)	-
HRD-5	766-776 (P)	669 (P)	612-622 (P)	481 (S)	453 (S)	437 (P)
HRD-6	768-774 (P)	669 (P)	622 (P)	484 (P)	438 (P)	-

* S, Shoulder in spectrum ; P, Peak in spectrum

peak also appears in the range of 610-633 nm, this peak probably accounts for a compound that may be found in a very low concentration and probably corresponds to the 2-3 peaks and/or shoulders seen in the spectra of acetone extracts. The accumulated peaks and shoulders found in the range of 400-500 nm again, indicate the presence of carotenoid pigments. Within that range, all the isolates shared the presence of two peaks, the first at 480-488 nm and the second at 435-440 nm. An additional shoulder at 453 nm appears only in isolate HRD-5. A similar shoulder was neither seen in any of the other five isolates nor in the reference diagram of Chloroflexus (Appendix III). Table 11, summarizes the spectral characteristics of methanol extracts of whole cells.

V.K.2 Chromatography :-

Pigment extracts were prepared according to two procedures. The first procedure which was modified from the method of Montesinos et al., (1983) was used in an attempt to separate the pigments by thin layer chromatography using a solvent mixture of benzene : petroleum ether : acetone (10:3:2, vol/vol).

The carotenoids were efficiently separated into three distinct coloured spots : light yellow, orange and dark yel-

low with R_f - values of 0.55, 0.71, and 0.83, respectively. However the bacteriochlorophylls appeared as a green continuous band with no separation whatsoever. Therefore, this method was considered inadequate for the determination of the bacteriochlorophyll content of the isolates.

The second alternative procedure, modified from the method of Gloe et al., (1975) proved to be more adequate and efficient for separation of the pigments. It involved the preparation of two types of pigment extracts, I and II ; the first containing the bacteriochlorophylls as they are, while in extract II, the bacteriochlorophylls were converted by acidification into their bacteriopheophytinized form. The pigments in the two extracts were then separated by thin layer chromatography using a solvent mixture of carbon tetrachloride : acetone (90 : 10, v/v). Chromatography of extract I disclosed four bands, two accounting for carotenoids and two green bands while chromatography of extract II disclosed four coloured bands in addition to the carotenoid bands (Fig. 27). Therefore, we decided to discard extract I and instead to use extract II throughout the rest of the experiment since it appeared to give better separation of the bacteriochlorophyll part of the pigments.

The R_f-values of the separated pigments (extract II) were measured for each isolate and are listed in Table 12. Each coloured spot of each of the six isolates was then scrapped

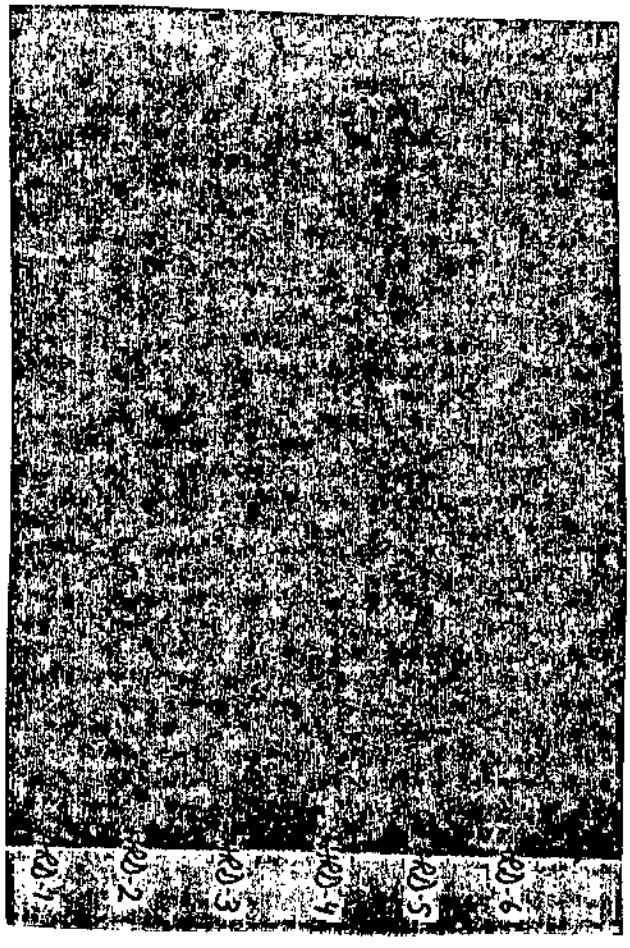


Fig. 27. TLC showing the seperated pigments of all six isolates

Table 12. R_f -values of the separated pigments

Colour of spot	R_f -values					
	HRD-1	HRD-2	HRD-3	HRD-4	HRD-5	HRD-6
1. Brown- green	0.080	0.075	0.080	0.080	-	0.080
2. Light green	0.161	0.150	0.155	0.150	0.155	0.161
3. Dark green	0.204	0.193	0.193	0.193	0.193	0.198
4. Maroon	0.376	0.344	0.344	0.349	0.360	0.365
5. Orange	0.591	0.580	0.580	0.586	0.591	0.591
6. Yellow	0.752	0.774	0.784	0.817	0.784	0.763

off, eluted and dissolved in acetone. Spectrophotometric scans from 400-900 nm were made for each of the eluted spots.

Tables 13-18, summarize the absorption maxima of the separated pigments of all six isolates in order to show any variations in the positions and in the absorption maxima. The light green and the dark green spots (Tables 14 & 15) respectively, have both been identified as bacteriopheophytin c since their absorption spectra and maxima corresponded to those of bacteriopheophytin c obtained from Chloroflexus aurantiacus, strain J-10-fl (Pierson & Castenholz, 1974 b) (Appendix IV).

The brown-green spots obtained from the isolates HRD-1, HRD-2, HRD-3, and HRD-6, showed only two absorption maxima corresponding to the two major peaks of bacteriopheophytin c, the first at 666-667 nm and the second at 410-411 nm. The absence of the rest of the smaller peaks is probably due to the very low concentration in which this pigment was obtained in comparison with both the light green and the dark green spots that were obtained in relatively high concentrations. Thus this brown-green spot cannot be conclusively defined as bacteriopheophytin c. In isolate HRD-4 however, several absorption maxima were obtained, all of which correspond to those of bacteriopheophytin c except for one extra peak which appeared at 756 nm. This peak has neither

Table 13. Spectral characteristics of the brown-green spot.

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	-	667-668 (P)	-	-	-	410-411 (P)
HRD-2	-	667 (P)	-	-	-	410 (P)
HRD-3	-	667 (P)	-	-	-	411 (P)
HRD-4	756 (P)	667 (P)	604-610 (P)	548 (P)	515-519 (P)	411 (P)
HRD-5	-	-	-	-	-	-
HRD-6	-	667 (P)	-	-	-	411 (P)

* P , peak in spectrum.

Table 14. Spectral characteristics of the light green spot.

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	667 (P)	606-610 (P)	548 (P)	512-517 (P)	-	410 (P)
HRD-2	667 (P)	607-610 (P)	548 (P)	516 (P)	-	411 (P)
HRD-3	666 (P)	606-610 (P)	546 (P)	513-515 (P)	-	412 (P)
HRD-4	667 (P)	609 (P)	548 (P)	516 (P)	482 (S)	411 (P)
HRD-5	667 (P)	606 (P)	547 (P)	516 (P)	-	410 (P)
HRD-6	667 (P)	610 (P)	547 (P)	515 (P)	-	411 (P)

* S , shoulder in spectrum ; P , peak in spectrum

Table 15. Spectral characteristics of the dark-green spot

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	667 (P)	608-611 (P)	548 (P)	516 (P)	-	411 (P)
HRD-2	667 (P)	610 (P)	548 (P)	516 (P)	480 (S)	411 (P)
HRD-3	666 (P)	605-606 (P)	547 (P)	515 (P)	-	411 (P)
HRD-4	667 (P)	609-611 (P)	548 (P)	516 (P)	482 (S)	411 (P)
HRD-5	667 (P)	607-613 (P)	548 (P)	517 (P)	480 (S)	412 (P)
HRD-6	667 (P)	610 (P)	548 (P)	516 (P)	480 (S)	411 (P)

* S , shoulder in spectrum ; P , peak in spectrum.

Table 16. Spectral characteristics of the maroon spot

Isolate	Wavelength (nm) of major features in spectrum					
HRD-1	746-747 (P)	677 (P)	617 (P)	524 (P)	-	410 (S)
HRD-2	748 (P)	669 (P)	616 (S)	523 (P)	488 (P)	410 (S)
HRD-3	747 (P)	670 (P)	616 (S)	522 (P)	-	411 (S)
HRD-4	748 (P)	680 (P)	615 (S)	524 (P)	-	410 (S)
HRD-5	747 (P)	673 (P)	615 (S)	523 (P)	486 (P)	410 (S)
HRD-6	743-748 (P)	680 (P)	-	520-526 (P)	-	411 (S)

* S , shoulder in spectrum ; P , peak in spectrum.

Table 17. Spectral characteristics of the orange spot

Isolate	Wavelength (nm) of major features in spectrum	
HRD-1	668 (P)	462-467 (P)
HRD-2	668 (P)	454-458 (P)
HRD-3	668 (P)	411 (P)
HRD-4	-	466 (P)
HRD-5	669 (P)	451 (P)
HRD-6	-	459 (P)

* P , peak in spectrum.

Table 18. Spectral characteristics of the yellow spot

Isolate	Wavelength (nm) of major features in spectrum	
HRD-1	-	452 (P)
HRD-2	-	452 (P)
HRD-3	-	445 (P)
HRD-4	479 (S)	455 (P)
HRD-5	-	446 (P)
HRD-6	-	451 (P)

* S , shoulder in spectrum ; P , peak in spectrum.

been observed in any of the other isolates nor in the reference Chloroflexus strain.

The maroon band (Table 16) was identified as bacteriopheophytin a owing to the similar pattern of absorption spectra and similar absorption maxima of the bacteriopheophytin a isolated from Chloroflexus aurantiacus, strain J-10-fl (Pierson & Castenholz, 1974 b) (Appendix IV). However, one major difference has been observed in all our six isolates and that is the presence of a clearly distinct shoulder at 410-411 nm while nothing as such is observed in the spectrum of the reference strain.

V.L. Fatty acid analysis :-

Using the Microbial Identification System (MIS) at the Royal Scientific Society (RSS) and with the help of Dr. Kroppenstedt (German Collection of Microorganisms (DSM)) the cell membrane fatty acid constituents of all six isolates were identified both quantitatively and qualitatively. The analysis was carried out by gas chromatography after the fatty acids have been freed from their cellular components and converted into their methyl ester forms.

The MIS Computer print-out sheets of fatty acid pattern of all six isolates are in Appendix V. The major fatty acids found in the cell-membranes consisted of palmitic acid

(16:0), oleic acid (18:1 cis 9), stearic acid (18:0), and the unknown fatty acid (18:3 cis 6,12,14) (Table 19). According to Dr. Kroppenstedt (Personal communication), the unknown fatty acid (18:3 cis 6,12,14) has been characteristically found only in Chloroflexus aurantiacus strains. This notion has been furtherly verified by the information obtained from Dr. B. Tindall (German Collection of Microorganisms (DSM)) regarding the fatty acid analysis of one of the reference strains. The analysis showed that this fatty acid was indeed found constituting only 0.83% of the total fatty acids while in our isolates this acid constituted up to more than 10% of the total fatty acids (Table 19).

The data obtained from the fatty acid analysis by the MIS System could not be used for identification of the isolates since at the time of the preparation of this work the photosynthetic bacteria were not yet filed in the Library of the MIS computer and therefore the isolates could not be computer compared with Chloroflexus aurantiacus or any of the other suspected relatives.

On the other hand, the data obtained were used in an attempt to find a relatedness between the six isolates. Fig. 28 shows that the isolates could be separated into two major clusters the first cluster consisting of the isolates HRD-1, HRD-5 and HRD-2 and the second group consisting of the isolates HRD-3, HRD-4, and HRD-6. The differences between the

two clusters were sufficient to separate them into two species as determined by the euclidian distance (Kroppenstedt, personal communication).

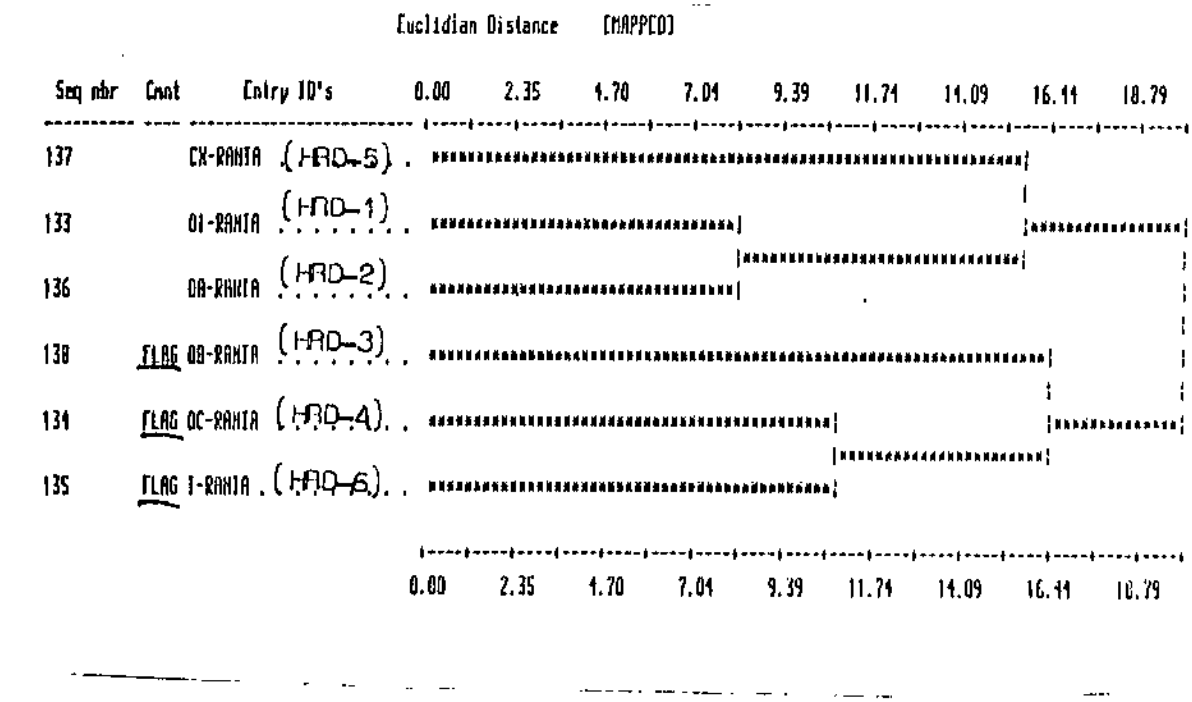


Fig. 28. Dendrogram showing the relatedness of the six isolates to each other as determined by their cell-membrane fatty acid content.

Table 19. Major fatty acids found in the cell membranes

Fatty acid	%						
	HRD-1	HRD-2	HRD-3	HRD-4	HRD-5	HRD-6	*C. aurantiacus*
16:0 (palmitic acid)	16.81	18.54	14.58	16.48	17.81	19.52	18.25
18:0 (stearic acid)	24.64	23.76	29.71	27.28	27.81	28.68	41.51
18:1 cis 9 (oleic acid)	33.12	32.63	29.23	31.74	33.61	32.06	11.07
18:3 cis 6,12,14 (?)	7.20	9.06	10.70	7.01	8.93	7.34	0.83
20:1 cis 11	4.97	3.71	4.19	6.53	5.13	5.07	3.49

* Information obtained from Dr. B. Tindall (Germany) .

VI. DISCUSSION

Through the past decade, some microbial ecologists have been focusing their attention on extreme environments (Mitchell, 1974 ; Brock, 1978 ; Sharp & Munster, 1983 ; Castenholz, 1984). Such environments undoubtedly provide a potential source for new microorganisms with properties that may have wide biotechnological applications. They provide new insights into the interactions between the microoorganisms themselves and their environments, into the physiological adaptations to extreme conditions and into possible biochemical interactions not commonly encountered in other microorganisms.

However, information about such organisms is usually lacking ; this may pause the risk of the organism in question of being neglected and then abandoned possibly missing a great deal of information about it. The reason for this lack of information may be due to the presence of such extreme habitats in remote areas that may not always be accessible. Another reason may be the difficulties encountered in isolating the organisms from their original habitats or even the difficulty of maintaining them under laboratory conditions.

An example of such organisms are the filamentous photo-

trophic bacteria found in hot springs. Up to date, only two species belonging to two different genera have been described, Chloroflexus aurantiacus (Pierson & Castenholz, 1974 a) and Heliothrix oregonensis (Pierson et al., 1985), the later is routinely maintained in the lab as a co-culture with the heterotrophic bacterium Isosphaera pallida, as a result, information based on pure cultures is missing. C. aurantiacus on the other hand, has been isolated in pure form. All of the description regarding this organism is based on the few strains that have been isolated either from the United States or from Japan (Pierson & Castenholz, 1974 a). Therefore, more information is needed about these organisms found in other hot springs distributed all over the globe.

In this work, the Zara thermal springs of Jordan were considered as a potential and suitable habitat for such organisms. These springs were therefore chosen to carry out this research aiming at the isolation and characterization of some of the gliding filamentous phototrophic bacteria from the microbial mats found in these springs in order to point out some of their characteristics and some methods that could be employed to facilitate their isolation and maintenance. Thus, presenting some contribution in an effort to widen the scope of information regarding the Chloroflexaceae.

During the course of the study period (1988-1989), the microbial mats were routinely observed for changes in structure, thickness, and for changes in the microbial populations forming these mats. Seasonal variation was most prominantly reflected in the surface layer of the mats (consisting of cyanobacteria). The cyanobacterial types dominating this layer differed from one season to another most probably in response to varying light intensities in summer and in winter. The association of both Oscillatoria and Synechococcus with hot spring microbial mats has often been mentioned in literature (Bauld & Brock, 1973 ; Brock, 1978). Spirulina, on the other hand, has rarely if ever, been mentioned to be found in hot springs although in Zara it comprises the major cyanobacterium and is found at various temperatures ranging from ambient temperature up to 50-55°C. The gliding filamentous phototrophic bacteria which form an essential part of the mats were always found occupying the same position beneath the cyanobacterial layer no matter which season, thus receiving an almost constant supply of organic matter provided by the layer above and at the same time receiving lower light intensities. Thus, the marked localization of the organisms indicates that their growth in nature is primarily photoheterotrophic.

As far as isolation of the organisms is concerned, isolating duplicates from any one source was avoided by using different pieces of mats obtained from different loca-

tions in order to get different types if possible. During the isolation trials, it was always noticed that inocula taken from the upper (3-4 mm) of the various mats often resulted in a dense growth of gliding filamentous bacteria in contrast to inocula taken from the lower portions of the mats which hardly resulted in any growth of such bacteria. Thus indicating that the healthiest cell populations reside in the upper parts of the mat and furthermore emphasizing the phototrophic nature of these organisms. This confirms the findings of Bauld and Brock (1973), who emphasized this finding by showing that maximum photosynthetic CO_2 fixation occured at that level.

The difficulties that were encountered during the isolation and purification of our cultures clearly indicate that the conventional methods used (e.g. streaking or spreading techniques) are not suitable for their isolation which is probably due to certain growth requirements and to the nature of these organisms. The tube method that was used for the separation of the cultures in this work has not been used for this purpose previously. When the method was first tried on our cultures, some of them grew in the form of one layer while the others formed two or three layers. This separation of layers may be probably correlated with the location or the level that the isolate usually occupies within the mat. It may even reflect the tendency of the cells to grow in a horizontal fashion forming separate

layers on top of each other. This mode of growth may also reflect the sensitivity of the cultures towards oxygen with those growing in the lower parts being the most sensitive. This arrangement could not be attributed to light intensities since all layers were receiving the same intensities (the source of light was positioned horizontal to the tubes).

As for the maintenance of these organisms in the lab, according to literature, the only methods used for maintaining pure cultures of Chloroflexus are freezing in liquid nitrogen and lyophilization. Otherwise, the cultures are either continuously transferred into fresh culture medium every 1-2 weeks or are maintained in co-culture with Synechococcus lividus (Castenholz & Pierson, 1981). In our lab however, we were able to maintain the viability of our cultures for 4-6 weeks by a simple and handy method without the need of continuous transfers. This was made possible by preserving the cultures at 4 °C in tubes of culture medium (DS-broth + 0.2% yeast extract) to which 8% gelatin is added - a method developed through this work. Apparently, it seems that the efficiency of this method is due to the presence of gelatin which possibly acts as a protective agent, since by placing the organisms under the same conditions without the addition of gelatin, the cultures do not survive for more than one week.

According to light and electron microscopical studies,

our isolates exhibit a morphology similar to that described for members of the genus Chloroflexus. The length and width of the filaments, the possession of chlorosomes and inclusion bodies, the septations and arrangement of cell membranes, are all in accordance with the findings of Pierson and Castenholz (1974 a). However there are some few features that we have encountered in our isolates but that have not been reported before. An example of these features are the structures that we have referred to earlier as "similar to biconcaved discs". Longitudinal sections shown by Pierson and Castenholz (1974 a) show what may be similar structures but are larger and are attached to the cell membrane, they have referred to them as mesosomes, while those that are found in our isolates are smaller and appear to be scattered all over the cytoplasm with no apparent attachments to the cell membrane. Another feature that has been observed in only two isolates, HRD-1 and HRD-5, is the presence of striations along the longitudinal axis of the filaments. A similar observation has only been made for the mesophilic strain C. aurantiacus var. mesophilus by Pivovarova and Gorlenko (1977) who reported the presence of a well defined fibrillar structure of the mucous sheath surrounding the filaments of this organism.

The presence of slime in Chloroflexus has not been reported previously, however, our isolates do possess this characteristic and is most properly exemplified in isolate

HRD-1 (Fig. 18), in the rest of the isolates the slime appears as a loose irregular outer layer. The slime probably plays an important role in these organisms by holding the filaments together in their natural habitat within the mat in addition to facilitating their gliding motility over the surfaces or over each other.

The mode of growth of the filaments is unclear although there is a great possibility that it may occur by septum formation through the invagination of the cytoplasmic membrane but on the other hand it may not be so, taking into consideration the inconsistency in the length of individual cells (within the filaments) which ranges from 1.25 μm up to about 20 μm in contrast to what has been mentioned in literature indicating that the usual length is 2-6 μm (Pfennig, 1988).

The series of events that usually took place whenever our cultures started to age in liquid medium are quite interesting and have not been reported previously. The cultures usually start with smooth rigid filaments of an indefinite length and of a uniform thickness after which the filaments gradually become shorter and irregular. This fragmentation of the filaments is probably due to weakening or deterioration of the cell wall or of the surrounding sheath and as a result causing the breakage of the filaments. This stage is then followed by bulb formation (i.e. the swelling of certain parts of the filaments along their length reaching a

width twice or thrice the original width of a filament). A similar observation has been reported by Reichenbach and Golecki (1975) in their description of the fine structure of Herpetosiphon, a non-phototrophic gliding filamentous bacterium. They reported the presence of bulb-like structures only in aging cultures and regarded them as a degeneration phenomenon (Reichenbach & Golecki, 1975). However, the ultra-structure of these bulbs differ markedly from those found in our isolates. In Herpetosiphon, the bulbs are formed by swelling of the cytoplasm while in our case, and as is clearly seen in the electron micrograph (Fig. 19) the bulbs are formed by swelling of the area between the cell membrane and the assumed sheath, this is made clear by the outer boundary line which may represent the sheath. The reason for this swelling may be due to the accumulation of substances in that area infiltrating from the surrounding medium as a result of possible change in the permeability of the sheath or even due to leakage from inside the cells. Following this stage of bulb formation, deteriorated filaments are seen in addition to thin rod shaped cells of various lengths. These cells are probably released from the filaments after rupturing of the bulbs. It is worth mentioning at this point that once the culture has reached the stage of bulb-formation, it could not be used for subculturing, because no growth would occur subsequently whatsoever. Therefore, this stage signifies the beginning of the deterioration of the culture. So, if any subculturing is to

be made, it should be prior to this stage, that is, it should be made when the culture appears as a smooth homogeneous suspension and when the cells are still long and exhibit a uniform thickness.

Optimization of growth conditions revealed that all our isolates grew best as photoheterotrophs under semiaerobic rather than under anaerobic conditions as is the case with the majority of the photosynthetic bacteria (Pfennig, 1967). The experiment in which actively growing cultures were incubated in the dark for about one week and then transferred back into light conditions clearly demonstrated the necessity of light for the growth and reproduction of the cultures. The experiment also showed that although no growth (increase in cellular biomass) was obtained in the dark, the cells remained viable and in a good condition as they were able to resume their growth once they were transferred back to the light. The dependence on organic compounds for better growth was demonstrated through the nutritional studies carried out on all six isolates.

Nutritional experiments carried out in our lab indicated that each of the six isolates required at least one organic carbon source in order to sustain its growth and reproduction. This became clear since none of the isolates was able to grow with HCO_3^- serving as the sole source of carbon. Thus confirming the heterotrophic nature of these organisms.

The fact that our isolates show a low tendency to grow in the dark (especially under aerobic conditions) does not agree with the results obtained by Madigan et al., (1974), who carried out the same experiment using four strains of Chloroflexus. Their study showed that their organisms grew readily in the dark under aerobic conditions using a wide variety of organic compounds. Their organisms showed no growth at all under dark anaerobic conditions, while our isolates managed to grow under such conditions using either pyruvate or glycerol as substrates. These differences in nutritional modes and abilities of these similar organisms probably reflect different conditions prevailing in the environment from which the organisms have originally been isolated. In this case the differences may partly correspond to the nature and type of cyanobacterial population in direct contact with these organisms in the microbial mats. The end products and the exudates produced by the cyanobacteria probably comprise the major source of organic nutrients for the layer underneath. Thus, the nature and types of these products undoubtedly would play a role in determining the nutritional modes and abilities of those organisms that they support nutritionally.

As is the case with some thermophilic microorganisms, our isolates turned out to be quite generous in terms of production of enzymes. Their high potential for the production of amylolytic, proteolytic and lipolytic enzymes is quite in-

teresting since nothing has been reported before on the ability of any of the Chloroflexaceae to produce enzymes and in such large amounts. However such an ability is expected from these organisms especially if we regard the niche that these organisms occupy within the microbial mats. The high potential for enzyme production possibly aids these organisms in regulating their own microenvironment in order to maintain a beneficial status. Through enzymatic activities, they may regulate the degree of shading exerted by the upper cyanobacterial layer by degrading excess organic matter that accumulates as dead cells and debris, thus maintaining a certain range of light intensity to be used for photosynthesis as well as maintaining a constant supply of simple organic compounds for their nutrition.

The pigment analyses carried out by both spectrophotometry and chromatography indicated that all our isolates possessed both bacteriochlorophylls a and c in addition to carotenoids as their major photosynthetic pigments. The pigments were found in similar ratios in all six isolates with bacteriochlorophyll c being always present in an amount exceeding that of bacteriochlorophyll a. The minor shifts in the range of some peaks however, may imply slight differences among the cultures. These differences may be due to the conjunction of the pigments with various proteins whether in their intact or in their extracted forms. Another possibility for these differences may be due to some

variation in the light wavelengths that are most efficiently absorbed by the various isolates. These wavelengths are most probably determined by the cyanobacterial population that overlies these organisms *in situ* as different types of cyanobacteria may allow different wavelengths to pass through to reach the underlying layer. Our cultures have been isolated from several types of mats each with a different cyanobacterial population, thus they may have been receiving different wavelengths in their original environment and so, these differences probably correspond to those obtained in the spectrophotometric scans.

Comparing scans of our isolates with those of *C. aurantiacus* (strain J-10-fl) - the only strain for which absorption spectra of methanolic extracts and of sonically disrupted cells have been published (Pierson & Castenholz, 1974 a) - indicated similar patterns with similar absorption maxima except for isolate HRD-5, which amongst all isolates was the only one to show an extra shoulder at 453 nm in its methanolic extract scan. The several small peaks appearing in the range of 572 - 630 nm in acetone extracts of all isolates (resolved into one peak, in the range of 610 - 633 nm in methanolic extracts), have never been discussed previously in literature. These peaks may correspond to some components that have not been observed previously due to their presence in minute quantities as demonstrated by the height of the peaks. A possibility for such components are the additional slow migrating light green bands that ap-

peared in the TLC chromatograms of our isolates. These bands in addition to the fast migrating dark green band have been identified as bacteriopheophytin c since their spectral characteristics appeared to be identical to bacteriopheophytin c isolated from C. aurantiacus (strain J-10-f1). The exact identity of the brown green pigment remains unsure although its absorption scan showed the two major bands at 667 nm and 411 nm corresponding to those of bacteriopheophytin c, however, its identity still needs to be verified. The fast migrating dark green band was the most concentrated and possibly the purest, while the slow migrating ones probably consisted of moieties of bacteriopheophytin c associated with some form of large molecules (probably proteins) that slowed down their migration. This explanation may be manifested taking into consideration the complex structure of the chlorosome in which this pigment is located (Fig. 1). The maroon band which was identified as bacteriopheophytin a furthermore verifies this notion since this pigment is basically located in the cytoplasmic membrane and in a simpler construction thus its extraction and separation from its adjoining molecules would be much easier (as demonstrated by faster migration and the formation of one single band). The only difference that could be distinguished between our isolates and the reference strain in this respect, is the presence of one extra shoulder at 453 in the scans of bacteriopheophytin a obtained from all our isolates.

It is worth mentioning at this point that the method used in this work (modified from the method of Gloe et al., 1975) is the first time to be used for the separation of pigments of Chloroflexus. From the results it is apparent that this method is more suitable than the method previously used by Pierson and Castenholz (1974b) (modified from Stanier and Smith (1960), and Sistrom (1966)) which involved the use of powdered sugar columns and which apparently separated the pigments into only two bands: one fast migrating blue band, identified as bacteriochlorophyll a and another slow migrating green band indetified as bacteriochlorophyll c.

The analysis of membrane fatty acids using the MIS-gas chromatographic system revealed a similar pattern for all six isolates. They all exhibited an abundance of highly saturated long chain fatty acids (C_{16} and C_{18}) with 18:1 monenoic acid and 16:0 and 18:0 saturated acids being the dominant types. Similar results have been reported by Kenyon and Gray (1974) who used three strains of phototrophically grown Chloroflexus aurantiacus. The abundance of these types of fatty acids most probably correlates with the thermophilic nature of these organisms (Hensel et al., 1986). Comparing the results of the fatty acid analysis of our isolates with those of the reference strain obtained from Dr. B. Tindall (Germany), it appears that there are some differences in the ratios of the major fatty acids. In our isolates, the major fatty acid is oleic acid

(18:1 cis 9) while in the reference strain, stearic acid (18:0) predominates. As for the unknown fatty acid (18:3 cis 6,12,14) it is found in the reference strain in much lesser amounts than in our isolates. The reason for these differences may be attributed to several aspects ranging from differences in the original environment to possible differences in the origin of the isolates themselves.

Taking all the above mentioned into consideration, it becomes clear that our isolates (isolated from the Zara hot springs in Jordan), possess a unique assemblage of characteristics in comparison with known kinds of phototrophic bacteria. Based on morphology, physiology and pigment absorption characteristics, it is evident that the six isolates are closely related to each other but are not identical. Comparison with other photosynthetic organisms belonging to the Chloroflexaceae reveals that our isolates bear a close resemblance to Chloroflexus. Although the fatty acid analysis supports this relationship, it indicates however, that the differences between our isolates are enough to separate them into two separate species.

VII. SUMMARY AND CONCLUSION

In this study we report on the isolation and characterization of gliding filamentous phototrophic bacteria from the Zara hot springs in Jordan.

Six pure cultures, HRD-1, HRD-2, HRD-3, HRD-4, HRD-5 and HRD-6, were successfully isolated from the microbial mats found growing in the Zara hot springs at a temperature range of 54 - 63°C, and at a pH range of 6.3 - 7.1.

All the isolates were characterized by having a filamentous shape with a length of more than 300 μm and a width that ranged from 0.6 - 1.2 μm. The filaments showed neither differentiation nor branching; and they all appeared to have a thin sheath surrounding the trichome.

Detailed microscopic studies revealed that all the filaments consisted of cells uniseriately arranged in multicellular filaments. Numerous electron dense inclusions and bodies were observed scattered all over the cytoplasm in addition to chlorosomes which were also seen in all the isolates.

Optimization of growth conditions revealed that all the isolates grew best as photoheterotrophs under semiaerobic

conditions and with a medium light intensity equivalent to 6.72 W/cm^3. The optimum temperature for growth was 50-55 °C while the range was from 35 °C up to 70 °C. The optimum pH for growth was 6.5 - 8.5. No measurable growth was obtained below pH 5 or above pH 10.

Growth rate studies revealed a distinguished pattern of growth whereby the filaments go through a series of morphological changes taking about 200 hours to complete the growth curve.

Growth experiments carried out to determine the nutritional requirements of the isolates, indicated the clear need for the presence of at least one organic substrate to support the growth of the isolates since none of them was able to grow with HCO_3^- serving as the sole source of carbone.

Screening for enzyme production revealed that the majority of the isolates had a high potential for the production of amylolytic, proteolytic and lipolytic enzymes. The isolates showed no signs of antimicrobial activities, neither against Gram-positive or Gram-negative bacteria nor against yeasts.

Analysis of the pigment content of all six isolates was carried out by both spectrophotometry and thin-layer

chromatography. It indicated the presence of both bacteriochlorophylls a and c as well as carotenoids as the major photosynthetic pigments of these organisms. The pigments were found in similar ratios in all six isolates with bacteriochlorophyll c being always present in an amount exceeding that of bacteriochlorophyll a.

The analysis of cell membrane fatty acids by gas chromatorgraphy revealed that all the isolated exhibited an abundance of highly saturated long chain fatty acids. Cluster analysis, which depended on the fatty acid constituents, indicated that the six isolates could be separated into two distinct groups, each one of them possibly representing a different species.

In conclusion, we have succeeded in isolating six pure cultures of thermophilic gliding filamentous phototrophic bacteria that bear a close resemblance to Chloroflexus aurantiacus. Based on morphology, physiology and pigment absorption characteristics, it appears that the six isolates are clearly related to each other. Fatty acid analysis, on the other hand, revealed that in spite of the similarities, the isolates exhibited some differences amongst them which are enough for them to be separated into two species. Thus, this finding, along with the other unique characteristics of these organisms make them suitable candidates for more studies and research.

VIII. LITERATURE CITED

Achenbach-Richter, L., R. Gupta, K.O. Stetter, and C.R. Woese. 1987. Were the original eubacteria thermophiles?. System. Appl. Microbiol. 9: 34-39.

Anderson, K.L., T. A. Tayne, and D.M. Ward. 1987. Formation and fate of fermentation products in hot spring cyanobacterial mats. Appl. Environ. Microbiol. 53: 2343-2352.

Awramik, S.M. 1981. The pre-Phanerozoic biosphere-three billion years of crises and opportunities. In M. H. Nitecki (ed.), "Biotic Crises in Ecological and Evolutionary Time." Academic Press, 83-102.

Awramik, S.M. 1984. Ancient stromatolites and microbial mats. In Y. Cohen, R.W. Castenholz, and H.O Halvorson (eds.), Microbial Mats : Stromatolites, Alan R. Liss, 1-22.

Bauld, J., and T.D. Brock. 1973. Ecological studies of Chloroflexis, a gliding photosynthetic bacterium. Arch. Mikrobiol. 92: 267-284.

Bauld, J. and T.D. Brock. 1974. Algal excretion and bacterial assimilation in mats of alkaline hot spring effluents. J. Phycol. 10: 101-106.

Betti, J.A., R.E. Blankenship, L.V. Natarajan, L.C. Dickinson, and R.C. Fuller. 1982. Antenna organization and evidence for the function of a new antenna pigment species in the green photosynthetic bacterium Chloroflexux aurantiacus. Biochim. Biophys. Acta. 680: 194-201.

Blankenship, R.E., R. Feick, B.D. Bruce, C. Kirmaier, D. Holten, and R.C. Fuller. 1983. Primary photochemistry in the facultative green photosynthetic bacterium Chloroflexux aurantiacus. J. Cell. Biochem. 22: 251-261.

Blankenship, R.E., and R. C. Fuller. 1986. Membrane topology and photochemistry of the green photosynthetic bacterium Chloroflexux aurantiacus. In L.A. Staehelin , and C. J. Arntzen (eds.) Encyclopedia of Plant Physiology New Series, vol. 19, Photosynthesis III, Springer-Verlag, 390-399.

Boon, J.J., H. Hines, and A. L. Burlingame, J. Klok, W. I. Rijpstra, J. W. DE Leeuw, K.E Edmunds, and G. Eglinton. 1981. Organic geochemical studies of Solar Lake laminated cyanobacterial mats. In Advances in Organic Geochemistry, John-Wiley and Sons, 207-227.

Brock, T.D. 1969. Vertical zonation in hot spring algal mats. Phycologia. 8: 201-205.

Brock, T.D. 1970. High temperature systems. Ann. Rev. Ecol. Syst. 1: 191-220.

Brock, T.D. 1978. "Thermophilic Microorganisms and Life at High Temperatures", Springer-Verlag.

Brock, T.D. 1986. Introduction: An overview of the thermophiles. In T.D. Brock (ed.), Thermophiles: General, Molecular, and Applied Microbiology, Wiley-Interscience, 1-16.

Brock, T.D., and M.L. Brock. 1966. Temperature optima for algal development in Yellowstone and Iceland hot springs. Nature (Lond.). 209: 733-734.

Brockmann H., and A. Lipinski. 1983. Bacteriochlorophyll g. A new bacteriochlorophyll from Heliobacterium chlorum. Arch. Microbiol. 136: 17-19.

Castenholz, R.W. 1969. Thermophilic blue-green algae and the thermal environment. Bacteriol. Rev. 33: 476-504.

Castenholz, R.W. 1973. The possible photosynthetic use of sulfide by the filamentous phototrophic bacteria of hot springs. Limnol. Oceanogr. 18: 863-876.

Castenholz, R.W. 1984. Habitats of Chloroflexux and related organisms. In M.J. Klug, and C.A.Reddy (eds.), Current Perspectives in Microbial Ecology, American Society of Microbiology, 196-200.

Castenholz, R.W. and B.K. Pierson. 1981. Isolation of members of the family Chloroflexaceae. In M.P. Starr, H. Stolp, H.G. Trüper, A. Balows, and H.G. Schlegel (eds.), The Prokaryotes, vol. I. Springer-Verlag, 290-298.

Doemel, W.N., and T.D. Brock. 1974. Bacterial stromatolites: origin of laminations. Science. 184: 1083-1085.

Doemel, W.N., and T.D. Brock. 1976. Vertical distribution of sulfur species in benthic algal mats. Limnol. Oceanogr. 21: 237-244.

Doemel, W.N., and T.D. Brock. 1977. Structure, growth, and decomposition of laminated algal-bacterial mats in alkaline hot springs. Appl. Environ. Microbiol. 34: 433-452.

Dubinina, G.A. and V.M. Gorlenko. 1975. New filamentous photosynthetic green bacteria containing gas vacuoles. Microbiology. 44: 511-517.

Fager, E.W. 1969. Recurrent group analysis in the classification of Flexibacteria. J. Gen. Microbiol. 58: 179-187.

Feick, R.G., and R.C. Fuller. 1984. Topography of the photosynthetic apparatus of Chloroflexux aurantiacus. Biochemistry. 23: 3693-3700.

Fox, G.E., E. Stackebrandt, R.B.Hespell, J.Gibson, J. Maniloff, T.A.Dyer, R.S. Wolfe, W.E. Balch, R.S. Tanner, L.J. Magrum, L.B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B.J. Lewis, D.A. Stahl, K.R. Luehrsen, K.N. Chen, and C. R. Woese. 1980. The phylogeny of prokaryotes. Science. 209: 457-463.

Gest, H., and J.L. Favinger. 1983. Heliobacterium chlorum, an anoxygenic brownish-green photosynthetic bacterium containing a "new" form of bacteriochlorophyll. Arch. Microbiol. 136: 11-16.

Gibson,J., W. Ludwig, E. Stackebrandt, and C.R. Woese. 1985. The phylogeny of the green photosynthetic bacteria: absence of a close relationship between Chlorobium and Chloroflexus. System. Appl. Microbiol. 6: 152-156.

Giovannoni, S.J., N.P. Revsbech, D.M. Ward, and R.W. Castenholz. 1987. Obligately phototrophic Chloroflexus: Primary production in anaerobic hot spring microbial mats. Arch. Microbiol. 147: 80-87.

Gloe, A., N. Pfennig, H. Brockmann, and W. Trowitzsch. 1975. A new bacteriochlorophyll from brown-colored Chlorobiaceae. Arch. Microbiol. 102: 103-109.

Gloe, A., and N. Risch. 1978. Bacteriochlorophyll cs, a new

bacteriochlorophyll from Chloroflexus aurantiacus. Arch. Microbiol. 118: 153-156.

Gorlenko,V.M. 1975. Characteristics of filamentous phototrophic bacteria from freshwater lakes. Microbiology. 44: 756-768.

Gorlenko, V.M., and T.A. Pivovarova. 1977. On the belonging of blue-green alga Oscillatoria coeurulescens Gicklhorn, 1921 to a new genus of Chlorobacteria Oscillochloris nov. gen. Izv. Akad. Nauk SSSR, Ser. Biol. 3: 396-409 (In Russian with an English Summary).

Halfen, L.N., B.K.Pierson, and C.W. Francis. 1972. Carotenoids of a gliding organism containing bacteriochlorophylls. Arch. Mikrobiol. 82: 240-246.

Hensel, R., W.Demharter, O.Kandler, R.Kroppenstedt, and E. Stackebrandt. 1986. Chemotaxonomic and molecular-genetic studies of the genus Thermus: evidence for a phylogenetic relationship of Thermus aquaticus and Thermus ruber to the genus Deinococcus. Appl. Environ. Microbiol. 36: 444-453.

Holo, H., and R. Sirevåg. 1986. Autotrophic growth and CO_2 fixation of Chloroflexux aurantiacus. Arch. Microbiol. 145: 173-180.

Holt, J. G., and R.A. Lewin. 1968. Herpetosiphon aurantiacus gen. et sp. n., a new filamentous gliding organism. J. Bacteriol. 95: 2407-2408.

Imhoff, J.F. 1988. Anoxygenic phototrophic bacteria. In B. Austin (ed.), Methods in Aquatic Bacteriology, John-Wiley & Sons Ltd., 207-240.

Jørgensen, B.B., N.P. Revsbech, and Y. Cohen. 1983. Photosynthesis and structure of benthic microbial mats: microelectrodes and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. 28: 1075-1093.

Kenyon, C.N., and A.M. Gray. 1974. Preliminary analysis of lipids and fatty acids of green bacteria and Chloroflexus aurantiacus. J. Bacteriol. 120: 131-136.

Kleinig, H., and H. Reichenbach. 1977. Carotenoid glucosides and menaquinones from the gliding bacterium Herpetosiphon giganteus Hp a2. Arch. Microbiol. 112: 307-310.

Knudsen, E., E. Jantez, K. Bryn, J. Ormerod, and R. Sirevåg. 1982. Quantititive and structural characteristics of lipids in Chlorobium and Chloroflexus.Arch. Microbiol.132: 149-154.

Korthals, H.J., and C.L. Steenbergen. 1985. Separation and quantification of pigments from natural phototrophic

microbial populations. FEMS Microbiology Ecology. 31: 177-185.

Kroppenstedt, R.M. 1988. Chemotaxonomia: III. Reunion del Grupo de Taxonomia Bacteriana de la SEM, Madrid.

Laakmann-Ditges, G., and J.H. Klemme. 1988. Amino acid metabolism in the thermophilic phototroph, Chloroflexus aurantiacus: properties and metabolic role of two L-threonine (L-serine) dehydratases. Arch. Microbiol. 149: 249-254.

Lewin, R.A. 1969. A classification of Flexibacteria. J. Gen. Microbiol. 58: 189-206.

Lewin, R.A. 1970. New Herpetosiphon species (Flexibacterales). Can. J. Microbiol. 16: 517-520.

Lewin, R.A., and D.M. Lounsbery. 1969. Isolation, cultivation and characterization of Flexibacteria. J. Gen. Microbiol. 58: 145-170.

Madigan, M.T., and T.D. Brock. 1976. Quantitative estimation of bacteriochlorophyll c in the presence of chlorophyll a in aquatic environments. Limnol. Oceanogr. 21: 462-476.

Madigan, M.T., and T.D. Brock. 1977. Adaptation by hot spring phototrophs to reduced light intensities. Arch. Microbiol. 113: 111-120.

Madigan, M.T., and T.D. Brock. 1977. "Chlorobium-type" vesicles of photosynthetically grown Chloroflexus aurantiacus observed using negative staining techniques. J. Gen. Microbiol. 102: 279-285.

Madigan, M.T., S.R. Petersen, and T.D. Brock. 1974. Nutritional studies on Chloroflexus, a filamentous photosynthetic, gliding bacterium. Arch. Microbiol. 100: 97-103.

Marshall, J.H., and G.J. Wilmoth. 1981. Pigments of Staphylococcus aureus, a series of triterpenoid carotenoids. J. Bacteriol. Sept. 900-913.

Mayer, H., and H. Reichenbach. 1978. Restriction endonucleases: general survey procedure and survey of gliding bacteria. J. Bacteriol. 136: 708-713.

Meissner, J., J.H. Krauss, U.J. Jurgens, and J. Weckesser. 1988. Absence of a characteristic cell wall lipopolysaccharide in the phototrophic bacterium Chloroflexus aurantiacus. J. Bacteriol. 170: 3213-3216.

Mitchell, R. 1974. The evolution of thermophily in hot springs. Q. Rev. Biol. 49: 229-242.

Montesinos, E., R. Guerrero, C. Abella, and I. Esteve. 1983. Ecology and physiology of the competition for light between

Madigan, M.T., and T.D. Brock. 1977. "Chlorobium-type" vesicles of photosynthetically grown Chloroflexus aurantiacus observed using negative staining techniques. J. Gen. Microbiol. 102: 279-285.

Madigan, M.T., S.R. Petersen, and T.D. Brock. 1974. Nutritional studies on Chloroflexus, a filamentous photosynthetic, gliding bacterium. Arch. Microbiol. 100: 97-103.

Marshall, J.H., and G.J. Wilmoth. 1981. Pigments of Staphylococcus aureus, a series of triterpenoid carotenoids. J. Bacteriol. Sept. 900-913.

Mayer, H., and H. Reichenbach. 1978. Restriction endonucleases: general survey procedure and survey of gliding bacteria. J. Bacteriol. 136: 708-713.

Meissner, J., J.H. Krauss, U.J. Jurgens, and J. Weckesser. 1988. Absence of a characteristic cell wall lipopolysaccharide in the phototrophic bacterium Chloroflexus aurantiacus. J. Bacteriol. 170: 3213-3216.

Mitchell, R. 1974. The evolution of thermophily in hot springs. Q. Rev. Biol. 49: 229-242.

Montesinos, E., R. Guerrero, C. Abella, and I. Esteve. 1983. Ecology and physiology of the competition for light between

pigments in holomictic lake Ciso, Spain. Appl. Environ. Microbiol. 46: 999-1006.

Pfennig, N. 1967. Photosynthetic bacteria. Ann. Rev. Microbiol. 21: 285-325.

Pfennig, N. 1988. Genus Chloroflexus. In J.G. Holt (ed.), Bergey's Manual of Systematic Bacteriology, vol. III, Williams and Wilkins, 1698-1702.

Pierson, B.K. 1985. Cytochromes in Chloroflexus aurantiacus grown with and without oxygen. Arch. Microbiol. 143: 260-265.

Pierson, B.K. and R. Castenholz. 1971. Bacteriochlorophylls in gliding filamentous prokaryotes from hot springs. Nature (New Biology) 233: 25-27.

Pierson, B.K. and R.W. Castenholz. 1974a. A phototrophic gliding filamentous bacterium of hot springs, Chloroflexus aurantiacus gen. and sp. nov. Arch. Microbiol. 100: 5-24.

Pierson, B.K. and R.W. Castenholz. 1974b. Studies of pigments and growth in Chloroflexus aurantiacus, a phototrophic filamentous bacterium. Arch. Microbiol. 100: 283-305.

Pierson, B.K. and H.M. Howard. 1972. Detection of bacterio-

pigments in holomictic lake Ciso, Spain. Appl. Environ. Microbiol. 46: 999-1006.

Pfennig, N. 1967. Photosynthetic bacteria. Ann. Rev. Microbiol. 21: 285-325.

Pfennig, N. 1988. Genus Chloroflexus. In J.G. Holt (ed.), Bergey's Manual of Systematic Bacteriology, vol. III, Williams and Wilkins, 1698-1702.

Pierson, B.K. 1985. Cytochromes in Chloroflexus aurantiacus grown with and without oxygen. Arch. Microbiol. 143: 260-265.

Pierson, B.K. and R. Castenholz. 1971. Bacteriochlorophylls in gliding filamentous prokaryotes from hot springs. Nature (New Biology) 233: 25-27.

Pierson, B.K. and R.W. Castenholz. 1974a. A phototrophic gliding filamentous bacterium of hot springs, Chloroflexus aurantiacus gen. and sp. nov. Arch. Microbiol. 100: 5-24.

Pierson, B.K. and R.W. Castenholz. 1974b. Studies of pigments and growth in Chloroflexus aurantiacus, a phototrophic filamentous bacterium. Arch. Microbiol. 100: 283-305.

Pierson, B.K. and H.M. Howard. 1972. Detection of bacterio-

chlorophyll-containing microorganisms by infrared fluorescence photomicrography. J. Gen. Microbiol. 73: 359-363.

Pierson, B.K. and J.P. Thronber. 1983. Isolation and spectral characterization of photochemical reaction centers from thermophilic green bacterium Chloroflexus aurantiacus strain J-10-fl. Proc. Natl. Acad. Sci. USA. 80: 80-84.

Pierson, B.K., S.J. Giovannoni, and R.W. Castenholz. 1984. Physiological ecology of a gliding bacterium containing bacteriochlorophyll a. Appl. Environ. Microbiol. 47: 576-584.

Pierson, B.K., S.J. Giovannoni, D.A. Stahl, and R.W. Castenholz. 1985. Heliothrix oregonensis, gen. nov., sp. nov., a phototrophic filamentous gliding bacterium containing bacteriochlorophyll a. Arch. Microbiol. 142: 164-167.

Pierson, B.K., A. Oesterle, and G.L. Murphy. 1987. Pigments, light penetration, and photosynthetic activity in the multilayered microbial mats of Great Sippewissett salt march, Massachusetts. FEMS Microbiology Ecology. 45: 365-376.

Pivovarova, T.A., and V.M. Gorlenko. 1977. Fine structure of Chloroflexus aurantiacus var. mesophilus (nom. prof.) growth in light under aerobic and anaerobic conditions. Microbiology. 46: 329-334.

Reichenbach, H., and J.R. Golecki. 1975. The fine structure of Herpetosiphon, and a note on the taxonomy of the Genus. Arch. Microbiol. 102: 281-291.

Reichenbach, H., and M. Dworkin. 1981. Introduction to the gliding bacteria. In M.P. Starr, H.Stolp, H.G. Trüper, A. Balows, and H.G. Schlegel (eds.), The Prokaryotes, Springer-Verlag, 315-327.

Reichenbach, H., W. Ludwig, and E. Stackebrandt. 1986. Lack of relationship between gliding cyanobacteria and filamentous gliding heterotrophic eubacteria: comparison of 16S rRNA catalogues of Spirulina, Saprospira, Vitreoscilla, Leucothrix, and Herpetosiphon. Arch. Microbiol. 145: 391-395.

Richardson, L.L., and R.W. Castenholz. 1987. Diel vertical movements of the cyanobacterium Oscillatoria terebriformis in a sulfide-rich hot spring microbial mat. Appl. Environ. Microbiol. 53: 2142-2150.

Rimawi, O., and E. Salameh. 1988. Hydrochemistry and groundwater system of the Zerka Ma'in-Zara thermal field, Jordan. J. Hydrol. 98: 147-163.

Salameh, E.S. 1986. Curative water in Jordan. Bulletin # 7. The Water Research and Study Center, University of Jordan, Amman.

Salameh, E. S, and K.A. Khudeir. 1983. Thermal water system in Jordan. N. Jb. Geol. Palaont. Mh. 4: 249-256.

Schmidt, K. 1980. A comparative study on the composition of chlorosomes (chlorobium vesicles) and cytoplasmic membranes from Chloroflexus aurantiacus strain OK-70-fl and Chlorobium limicola f. thiosulfatophilum strain 6230. Arch. Microbiol. 124: 21-31.

Sharp, R.J., and M.J. Munster. 1983. Biotechnological implications for microorganisms from extreme environments. In R.A. Herbert, and G.A. Codd (eds.), Microbes in Extreme Environments, Academic Press, 215-295.

Sheridan, R.P. 1966. Photochemical and dark reduction of sulfate and thiosulfate to hydrogen sulfide in Synechococcus lividus. Ph.D. Thesis, University of Oregon, Eugene, U.S.A.

Shraideh, Z. 1979. Photosynthetic bacteria in Jordan. M.Sc. Thesis. University of Jordan, Amman.

Sirevåg, R., and R. Castenholz. 1979. Aspects of carbon metabolism in Chloroflexus. Arch. Microbiol. 12: 151-153.

Sistrom, W.R. 1966. The spectrum of bacteriochlorophyll in vivo: observations on mutants of Rhodopseudomonas spheroides

unable to grow phototrophically. Photochem and Photobiol. 5: 845-856.

Sprague, S.G., L.A. Staehelin, and R.C. Fuller. 1981. Semi-anaerobic induction of bacteriochlorophyll synthesis in the green bacterium Chloroflexus aurantiacus. J. of Bacteriol. Sept. 1032-1039.

Sprague, S.G., L.A. Staehelin, M.J. Dibartolomeis, and R.C. Fuller. 1981. Isolation and development of chlorosomes in the green bacterium Chloroflexus aurantiacus. J. of Bacteriol. 147: 1021-1031.

Staehelin, L.A., J.R. Golecki, R.C. Fuller, and C. Drews. 1978. Visualization of the supramolecular architecture of chlorosomes (chlorobium type vesicles) in freeze-fractured cells of Chloroflexus aurantiacus. Arch. Microbiol. 119: 269-277.

Stanier, R.Y., and J.H. Smith. 1960. The chlorophylls of green bacteria. Biochim. Biophys. Acta (Amst.) 41: 478-484.

Stanier, R.Y., N. Pfennig, and H.G. Trüper. 1981. Introduction to the phototrophic prokaryotes. In M. P. Starr, H. Stolp, H.G. Trüper, A. Balows, and H.G. Schlegel (eds.). The Prokaryotes. Springer-Verlag. 197-211.

Stanley, S.M. 1973. An ecological theory for the sudden origin of multicellular life in the late precambrian. Proc. Natl. Acad. Sci. USA. 70: 1486-1489.

Stolz, J.F. 1984. Fine structure of the stratified microbial community at Laguna Figueroa, Baja California, Mexico: II. Transmission electron microscopy as a diagnostic tool in studying microbial communities in situ. In Y. Cohen, R.W. Castenholz, and H.O. Halvorson (eds.) Microbial Mats: Stromatolites, Alan R. Liss, 23-38.

Tansey, M.R., and T.D. Brock. 1972. The upper temperature limit for eukaryotic organisms. Proc. Natl. Acad. Sci. USA. 69: 2426-2428.

Thornber, J.P., T.L. Trosper, and C.E. Strouse. 1978. Bacteriochlorophyll in vivo: relationship of spectral forms to specific membrane components. In R.K. Clayton, and W.R. Sistrom (eds). The Photosynthetic Bacteria, Plenum Press, 133-160.

Trüper, H.G. 1976. Higher taxa of the phototrophic bacteria: Chloroflexaceae fam. nov., a family for the gliding, filamentous, phototrophic "green" bacteria. Int. J. Syst. Bacteriol. 26: 74-75.

Ward, D.M., E. Beck, N.P. Revsbech, K.A. Sandbeck, and M.R.

Winfrey. 1984. Decomposition of hot spring microbial mats. In Y. Cohen, R.W. Castenholz, and H.O. Halvorson (eds.), Microbial Mats: Stromatolites, Alan R. Liss, 191-214.

Waring, G.A. 1965. Thermal springs of the United States and other countries of the world-a summary. U.S. Geological Survey Professional Paper 492.

Woese, C.R., B.A. Debrunner-Vossbrink, H. Oyaizu, E. Stackebrandt, and W. Ludwig. 1985. Gram-positive bacteria: Possible photosynthetic ancestry. Science. 229: 762-765.

Appendix I

Utilization of organic compounds under aerobic conditions (Madigan *et al*., *1974)*

Carbon source	Strain							
	OK-70-fl		J-10-fl		254-2		396-1	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1. 4% Formalin - killed control	-	-	-	-	-	-	-	-
2. Glutamate	+	+	++	+	++	+	+	+
3. Aspartate	++	+	+	+	+	+	+	+
4. Glycyl-glycine	-	-	-	-	-	-	-	-
5. Acetate	+	-	+	+	++	+	+	+
6. Pyruvate	++	+	+	+	+	+	++	+
7. Lactate	-	-	++	++	++	+	++	++
8. Succinate	+	+	+	-	+	+	++	+
9. Malate	+	+	++	++	++	+	+	+
10. Butyrate	++	-	++	+	++	+	+	+

Appendix I (contd.)

Carbon source	Strain							
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
11. Citrate	+	+	–	–	–	–	–	–
12. Ribose	++	+	+	+	+	+	+	+
13. Glucose	++	+	++	++	++	++	++	++
14. Galactose	++	+	++	++	++	+	++	++
15. Ethanol	+	+	+	–	+	–	+	–
16. Glycerol	+	–	++	++	+	+	++	++
17. Mannitol	++	++	+	+	+	+	+	+
18. Yeast extract	++	++	++	++	++	++	++	++
19. Casamino acids	++	++	++	+	++	++	++	+
20. HCO_3	–	–	–	–	–	–	–	–

Appendix II

Utilization of organic compounds under anaerobic conditions (Madigan *et al., 1974)*

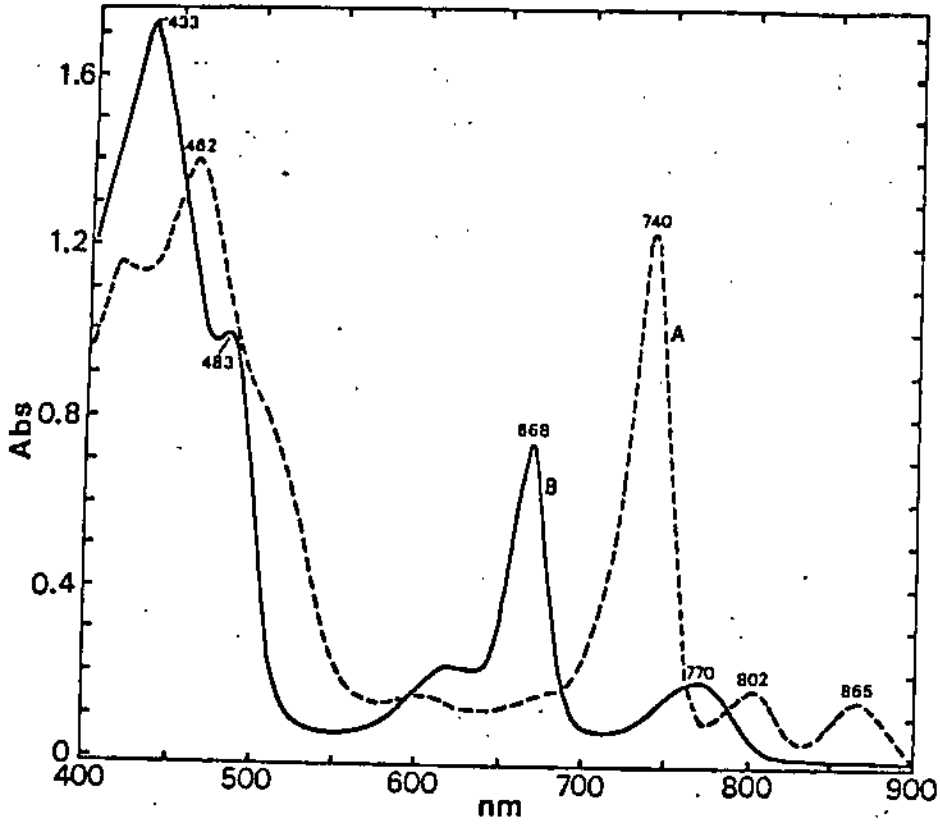
Carbon source	Strain							
	OK-70-fl		J-10-fl		254-2		396-1	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1. 4% Formalin - killed control	-	-	-	-	-	-	-	-
2. Glutamate	+	-	++	-	+	-	+	-
3. Aspartate	++	-	+	-	+	-	+	-
4. Glycyl-glycine	+	-	+	-	+	-	+	-
5. Acetate	+	-	+	-	+	-	+	-
6. Pyruvate	+	-	++	-	+	-	++	-
7. Lactate	-	-	+	-	+	-	-	-
8. Succinate	+	-	+	-	+	-	+	-
9. Malate	+	-	+	-	+	-	+	-
10. Butyrate	+	-	+	-	+	-	+	-

Appendix II (contd.)

Carbon source	Strain							
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
11. Citrate	+	–	+	–	+	–	–	–
12. Ribose	+	–	+	–	+	–	+	–
13. Glucose	++	+	++	–	++	–	++	–
14. Galactose	+	–	+	–	+	–	+	–
15. Ethanol	+	–	+	–	+	–	–	–
16. Glycerol	++	–	+	–	+	–	+	–
17. Mannitol	+	–	+	–	+	–	+	–
18. Yeast extract	++	–	++	–	++	–	++	–
19. Yeast extract no sulfide	++	–	++	–	++	–	++	–
20. Casamino acids	++	–	++	–	++	–	++	–
21. HCO_3	+	–	–	–	–	–	–	–

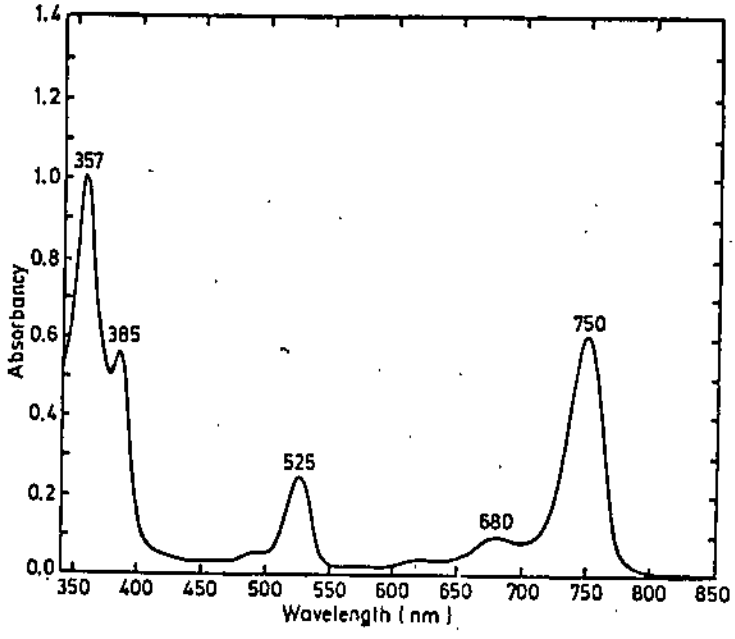
Appendix III

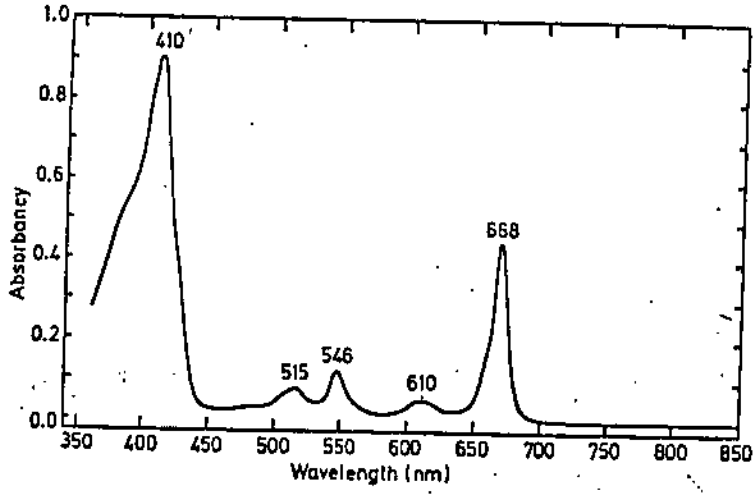
Absorption spectra of a) sonically disrupted cells and b) methanol extracts of Chloroflexus aurantiacus strain J-10-fl (Pierson & Castenholz, 1974 a).



Appendix IV

Absorption spectra of bacteriopheophytins a & c obtained from Chloroflexus aurantiacus strain J-10-fl (Pierson & Castenholz, 1974 b).





Appendix V

MIS computer print-out sheets of fatty acid pattern of all six isolates.

Microbial Identification System (Rev: 3.0) (Sw s/n: 2307A10033) (Hw s/n: 2529R29741) 19-FEB-89 15:56:01

ID: 33 01-PANIA HRD-1 Date of run: 19-FEB-89 19:30:52
Bottle: 7 SAMPLE [AEROBIC]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.516	39163000	0.078	. . .	6.999	SOLVENT PEAK	. . .	< min rt	
1.879	2306	0.026	. . .	7.757	. . . . .	. . .	< min rt	
2.084	655	0.027	. . .	8.185	. . . . .	. . .	< min rt	
4.613	732	0.036	1.037	12.097	11:0 ISO 3OH	0.36	ECL deviates 0.007	
6.934	941	0.063	. . .	13.981	. . . . .	. . .		
8.476	1184	0.064	0.951	15.001	15:0	0.53	ECL deviates 0.001	Reference 0.000
9.121	1083	0.060	0.945	15.392	16:1 ISO E	0.48	ECL deviates 0.006	
9.429	1657	0.057	. . .	15.579	. . . . .	. . .		
9.749	5665	0.051	0.940	15.773	16:1 B	2.50	ECL deviates -0.001	
10.121	38244	0.047	0.937	15.999	16:0	16.81	ECL deviates -0.001	Reference -0.001
10.881	1339	0.082	0.934	16.442	17:1 ISO G	0.59	ECL deviates 0.009	
11.483	2356	0.066	0.932	16.793	17:1 B	1.03	ECL deviates 0.001	
11.838	4843	0.050	0.931	17.000	17:0	2.11	ECL deviates 0.000	Reference -0.001
12.143	10200	0.060	. . .	17.174	. . . . .	. . .		
12.481	1348	0.068	. . .	17.367	. . . . .	. . .		
12.568	2534	0.052	0.930	17.417	18:1 ISO F	1.11	ECL deviates 0.007	
12.679	1786	0.066	. . .	17.480	. . . . .	. . .		
12.849	16495	0.056	0.931	17.577	18:3 CIS 6,12,14 . .	7.20	ECL deviates 0.000	
13.087	1908	0.050	0.931	17.713	Sum In Feature 6 . .	0.83	ECL deviates -0.007	18:2 CIS 9,12/18:0a
13.186	75858	0.055	0.931	17.769	18:1 CIS 9	33.12	ECL deviates 0.000	
13.589	56394	0.050	0.932	18.000	18:0	24.64	ECL deviates -0.000	Reference -0.001
14.827	4793	0.054	. . .	18.709	. . . . .	. . .		
14.907	1367	0.049	0.937	18.755	Sum In Feature 8 . .	0.60	ECL deviates -0.001	unknown 18.756/19:1
14.979	1967	0.053	. . .	18.796	. . . . .	. . .		
15.101	1575	0.063	0.939	18.866	Sum In Feature 9 . .	0.69	ECL deviates -0.001	19:0 CYCLO C9-10/un
15.337	3499	0.050	0.940	19.002	19:0	1.54	ECL deviates 0.002	Reference 0.001
16.548	8405	0.054	. . .	19.703	. . . . .	. . .		
16.663	11154	0.062	0.951	19.770	20:1 CIS 11	4.97	ECL deviates -0.000	
17.059	1995	0.051	0.955	19.999	20:0	0.89	ECL deviates -0.001	Reference -0.002
18.234	9710	0.051	. . .	20.680	. . . . .	. . .	> max rt	
18.609	3284	0.148	. . .	20.897	. . . . .	. . .	> max rt	
19.876	10289	0.054	. . .	21.630	. . . . .	. . .	> max rt	
*******	1908	. . . . .		. . .	SUMMED FEATURE 6 . .	0.83	18:2 CIS 9,12/18:0a	18:0 ANTEISO/18:2 c
*******	1367	. . . . .		. . .	SUMMED FEATURE 8 . .	0.60	unknown 18.756/19:1	19:1 CIS 10/18.576
*******	1575	. . . . .		. . .	SUMMED FEATURE 9 . .	0.69	un 18.846/18.858	un 18.858/.846/19cy
*******	. . . . .	. . . . .		. . .	. . . . .	. . .	19:0 CYCLO C9-10/un	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
39163000	258822	228225	88.18	213266	6	0.004	0.001

TSBA [Rev 3.0] * NO MATCH *

Microbial Identification System (Rev: 3.0) (Sw s/n: 2907A40033) (Hw s/n: 2529A24741) 19-FEB-89 15:56:01

ID: 34 OA-RANIA HRD-2 Date of run: 19-FEB-89 20:00:42
Bottle: 8 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.516	39125000	0.078	. . .	6.998	SOLVENT PEAK	. . .	< min rt	
1.879	1706	0.025	. . .	7.756	. . . . .	. . .	< min rt	
2.084	465	0.026	. . .	8.184	. . . . .	. . .	< min rt	
4.613	653	0.035	1.037	12.097	11:0 ISO 30H	0.29	ECL deviates 0.007	
8.476	1023	0.049	0.951	15.000	15:0	0.42	ECL deviates 0.000	Reference 0.000
9.430	2479	0.074	. . .	15.579	. . . . .	. . .		
9.749	6069	0.049	0.940	15.773	16:1 8	2.44	ECL deviates -0.001	
10.123	46237	0.046	0.937	16.000	16:0	18.54	ECL deviates 0.000	Reference 0.000
10.879	915	0.058	0.934	16.441	17:1 ISO 6	0.37	ECL deviates 0.000	
11.483	2270	0.060	0.932	16.793	17:1 8	0.91	ECL deviates 0.001	
11.838	5044	0.051	0.931	17.000	17:0	2.01	ECL deviates -0.000	Reference -0.001
12.143	10956	0.059	. . .	17.174	. . . . .	. . .		
12.243	2136	0.064	0.931	17.231	16:0 20H	0.85	ECL deviates -0.004	
12.481	2215	0.059	. . .	17.367	. . . . .	. . .		
12.567	3091	0.052	0.930	17.416	18:1 ISO f	1.23	ECL deviates 0.006	
12.849	22759	0.055	0.931	17.577	18:3 CIS 6,12,14 . .	9.06	ECL deviates 0.000	
13.072	4512	0.053	. . .	17.704	. . . . .	. . .		
13.187	81912	0.054	0.931	17.770	18:1 CIS 9	32.63	ECL deviates 0.001	
13.280	2469	0.047	0.931	17.823	Sum In Feature 7 . .	0.98	ECL deviates 0.001	18:1 CIS 11/t 9/t 6
13.589	59595	0.052	0.932	17.999	18:0	23.76	ECL deviates -0.001	Reference -0.001
14.829	2263	0.051	. . .	18.710	. . . . .	. . .		
14.908	1380	0.049	0.937	18.756	Sum In Feature 8 . .	0.55	ECL deviates -0.000	unknown 18.756/19:1
15.103	1358	0.055	0.939	18.867	Sum In Feature 9 . .	0.55	ECL deviates 0.000	19:0 CYCLO C9-10/un
15.336	2866	0.050	0.940	19.001	19:0	1.15	ECL deviates 0.001	Reference 0.000
16.547	4414	0.052	. . .	19.702	. . . . .	. . .		
16.663	9126	0.066	0.951	19.770	20:1 CIS 11	3.71	ECL deviates -0.000	
17.060	1357	0.053	0.955	20.000	20:0	0.55	ECL deviates -0.000	Reference -0.002
18.233	4879	0.050	. . .	20.679	. . . . .	. . .	> max rt	
18.603	4405	0.174	. . .	20.893	. . . . .	. . .	> max rt	
19.876	6002	0.063	. . .	21.631	. . . . .	. . .	> max rt	
*******	2469	. . . . .	. . .	SUMMED FEATURE 7 . .	0.98	18:1 CIS 11/t 9/t 6	18:1 TRANS 9/t6/c11	
*******	. . . . .	. . . . .	. . .	. . . . .	. . .	18:1 TRANS 6/t9/c11		
*******	1380	. . . . .	. . .	SUMMED FEATURE 8 . .	0.55	unknown 18.756/19:1	19:1 CIS 10/18.576	
*******	1358	. . . . .	. . .	SUMMED FEATURE 9 . .	0.55	un 18.846/18.858	un 18.858/.846/19cy	
*******	. . . . .	. . . . .	. . .	. . . . .	. . .	19:0 CYCLO C9-10/un		

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
39125000	277107	250268	90.31	233752	6	0.003	0.001

TSBA ERev 3.03 * NO MATCH *

Microbial Identification System (Rev: 3.0) (Sw s/n: 2907A40033) (Hw s/n: 2529M24741) 19-FEB-89 15:56:01

ID: 35 OB-RANIA HRD-3 Date of run: 19-FEB-89 20:30:34
Bottle: 9 SAMPLE [AEROBE]

RI	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.519	38222000	0.076	. . .	7.006	SOLVENT PEAK	. . .	< min rt	
1.706	1351	0.023	. . .	7.397	. . . . .	. . .	< min rt	
1.827	450	0.025	. . .	7.649	. . . . .	. . .	< min rt	
1.878	1655	0.025	. . .	7.756	. . . . .	. . .	< min rt	
2.083	423	0.025	. . .	8.184	. . . . .	. . .	< min rt	
4.613	548	0.031	1.037	12.098	11:0 ISO 3OH	0.44	ECL deviates 0.008	
9.421	1971	0.079	. . .	15.575	. . . . .	. . .		
9.749	3944	0.049	0.940	15.774	16:1 B	2.89	ECL deviates -0.000	
10.121	19934	0.046	0.937	16.000	16:0	14.58	ECL deviates -0.000	Reference -0.001
11.483	1374	0.060	0.932	16.793	17:1 B	1.00	ECL deviates 0.001	
11.840	4158	0.049	0.931	17.001	17:0	3.02	ECL deviates 0.001	Reference 0.001
12.142	3915	0.060	. . .	17.173	. . . . .	. . .		
12.481	1929	0.055	. . .	17.367	. . . . .	. . .		
12.568	1678	0.054	0.930	17.416	18:1 ISO F	1.22	ECL deviates 0.006	
12.849	14740	0.056	0.931	17.577	18:3 CIS 6,12,14 . .	10.70	ECL deviates -0.000	
13.071	2044	0.054	. . .	17.703	. . . . .	. . .		
13.184	40245	0.056	0.931	17.768	18:1 CIS 9	29.23	ECL deviates -0.001	
13.590	40862	0.051	0.932	17.999	18:0	29.71	ECL deviates -0.001	Reference -0.001
14.625	2083	0.080	. . .	18.592	. . . . .	. . .		
14.829	1965	0.063	. . .	18.709	. . . . .	. . .		
15.104	1621	0.057	0.939	18.867	Sum In Feature 9 . .	1.19	ECL deviates -0.000	19:0 CYCLO C9-10/un
15.337	2508	0.050	0.940	19.000	19:0	1.84	ECL deviates 0.000	Reference 0.001
16.546	2460	0.051	. . .	19.700	. . . . .	. . .		
16.664	5645	0.065	0.951	19.769	20:1 CIS 11	4.19	ECL deviates -0.001	
18.231	2685	0.051	. . .	20.676	. . . . .	. . .	> max rt	
19.876	6936	0.115	. . .	21.629	. . . . .	. . .	> max rt	
*******	1621	. . . . .		. . .	SUMMED FEATURE 9 . .	1.19	un 18.846/18.858	un 18.858/.846/19cy
*******	. . . . .			. . .	. . . . .	. . .	19:0 CYCLO C9-10/un	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
38222000	153624	137257	89.35	128189	4	0.003	0.001

TSBA [Rev 3.0] * NO MATCH *

ID: 36 QC-RANIA HRD-4 Date of run: 19-FEB-89 21:00:22
Bottle: 10 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.519	38795000	0.077	. . .	7.006	SOLVENT PEAK	. . .	< min rt	
1.879	2126	0.026	. . .	7.758	. . . . .	. . .	< min rt	
2.085	572	0.026	. . .	8.188	. . . . .	. . .	< min rt	
4.613	686	0.036	1.037	12.097	11:0 ISO 3OH	0.44	ECL deviates 0.007	
9.429	1606	0.061	. . .	15.579	. . . . .	. . .		
9.749	3794	0.054	0.940	15.773	16:1 B	2.20	ECL deviates -0.001	
10.123	28496	0.046	0.937	16.000	16:0	16.48	ECL deviates 0.000	Reference 0.000
11.481	1235	0.063	0.932	16.791	17:1 B	0.71	ECL deviates -0.001	
11.839	2915	0.049	0.931	17.000	17:0	1.67	ECL deviates 0.000	Reference 0.000
12.144	9993	0.059	. . .	17.174	. . . . .	. . .		
12.244	1685	0.062	0.931	17.231	16:0 2OH	0.97	ECL deviates -0.004	
12.569	1892	0.052	0.930	17.417	18:1 ISO F	1.09	ECL deviates 0.007	
12.851	12205	0.060	0.931	17.577	18:3 CIS 6,12,14 . .	7.01	ECL deviates 0.000	
13.073	3917	0.057	. . .	17.704	. . . . .	. . .		
13.187	55274	0.055	0.931	17.769	18:1 CIS 9	31.74	ECL deviates 0.000	
13.590	47456	0.050	0.932	17.999	18:0	27.28	ECL deviates -0.001	Reference -0.001
14.827	1997	0.048	. . .	18.708	. . . . .	. . .		
15.104	1060	0.056	0.939	18.867	Sum In Feature 9 . .	0.61	ECL deviates 0.000	19:0 CYCLO C9-10/un
15.338	3119	0.051	0.940	19.001	19:0	1.81	ECL deviates 0.001	Reference 0.001
16.548	3745	0.050	. . .	19.702	. . . . .	. . .		
16.664	11136	0.061	0.951	19.769	20:1 CIS 11	6.53	ECL deviates -0.001	
17.062	2499	0.055	0.955	20.000	20:0	1.47	ECL deviates -0.000	Reference -0.001
18.232	4205	0.051	. . .	20.677	. . . . .	. . .	> max rt	
19.878	5810	0.067	. . .	21.630	. . . . .	. . .	> max rt	
*******	1060	. . .	. . .	. . .	SUMMED FEATURE 9 . .	0.61	un 18.846/18.858	un 18.858/.846/19cy
*******	. . . . .	. . .	. . .	. . .	. . . . .	. . .	19:0 CYCLO C9-10/un	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Mbr Ref	ECL Deviation	Ref ECL Shift
38795000	194710	173452	89.08	162132	5	0.003	0.001

TSBA [Rev 3.0] * NO MATCH *

Microbial Identification System (Rev: 3.0) (Sw s/n: 2907R40033) (Hw s/n: 2529A24741) 19-FEB-89 15:56:01

ID: 37 CX-RANIA HRD-5 Date of run: 19-FEB-89 21:30:15
Bottle: 11 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.519	38120000	0.076	. . .	7.007	SOLVENT PEAK	. . .	< min rt	
1.878	1852	0.025	. . .	7.756	. . . . .	. . .	< min rt	
2.084	473	0.025	. . .	8.186	. . . . .	. . .	< min rt	
4.612	665	0.037	1.037	12.097	11:0 ISO 3OH	0.40	ECL deviates 0.007	
9.428	2704	0.104	. . .	15.580	. . . . .	. . .	> max ar/ht	
9.749	4914	0.053	0.940	15.774	16:1 B	2.66	ECL deviates 0.000	
10.122	33041	0.047	0.937	16.000	16:0	17.81	ECL deviates 0.000	Reference -0.001
11.839	1913	0.051	0.931	16.999	17:0	1.02	ECL deviates -0.001	Reference 0.000
12.144	7560	0.060	. . .	17.173	. . . . .	. . .		
12.483	1689	0.052	. . .	17.367	. . . . .	. . .		
12.569	1691	0.051	0.930	17.416	18:1 ISO F	0.90	ECL deviates 0.006	
12.850	16683	0.052	0.931	17.576	18:3 CIS 6,12,14 . .	8.93	ECL deviates -0.001	
13.071	1182	0.050	. . .	17.702	. . . . .	. . .		
13.188	62772	0.054	0.931	17.769	18:1 CIS 9	33.61	ECL deviates 0.000	
13.592	51901	0.050	0.932	18.000	18:0	27.81	ECL deviates -0.000	Reference 0.001
14.830	903	0.050	. . .	18.709	. . . . .	. . .		
15.339	1573	0.051	0.940	19.001	19:0	0.85	ECL deviates 0.001	Reference 0.002
16.548	1743	0.052	. . .	19.702	. . . . .	. . .		
16.664	9375	0.065	0.951	19.769	20:1 CIS 11	5.13	ECL deviates -0.001	
17.061	1620	0.052	0.955	20.000	20:0	0.89	ECL deviates -0.000	Reference -0.001
18.233	1952	0.051	. . .	20.680	. . . . .	. . .	> max rt	
19.876	9944	0.195	. . .	21.628	. . . . .	. . .	> max rt	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
38120000	201929	186148	92.18	173914	5	0.003	0.001

TSBA [Rev 3.0] * NO MATCH *

Microbial Identification System (Rev: 3.0) (Sw s/n: 2907A40033) (Hw s/n: 2529A24741) 19-FEB-89 15:56:01

ID: 38 I-RANIA HRD-6 Date of run: 19-FEB-89 22:30:01
Bottle: 12 SAMPLE [AEROBE]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.519	38057000	0.076	. . .	7.002	SOLVENT PEAK		< min rt	
1.878	2022	0.026	. . .	7.751	. . . . .		< min rt	
1.938	439	0.027	. . .	7.877	. . . . .		< min rt	
2.084	561	0.026	. . .	8.182	. . . . .		< min rt	
4.613	687	0.036	1.041	12.097	11:0 ISO 3OH	0.33	ECL deviates 0.007	
9.426	3603	0.096	. . .	15.578	. . . . .			
9.749	4996	0.051	0.939	15.774	16:1 B	2.15	ECL deviates -0.000	
10.122	45438	0.046	0.936	16.000	16:0	19.52	ECL deviates 0.000	Reference -0.002
11.839	2186	0.053	0.929	17.000	17:0	0.93	ECL deviates 0.000	Reference -0.002
12.143	15363	0.058	. . .	17.174	. . . . .			
12.481	1920	0.054	. . .	17.367	. . . . .			
12.570	2723	0.051	0.928	17.418	18:1 ISO F	1.16	ECL deviates 0.008	
12.850	17251	0.056	0.928	17.577	18:3 CIS 6,12,14 . .	7.34	ECL deviates 0.000	
13.189	75305	0.057	0.928	17.771	18:1 CIS 9	32.06	ECL deviates 0.002	
13.279	2217	0.045	0.928	17.823	Sum In Feature 7 . .	0.94	ECL deviates 0.001	18:1 CIS 11/t 9/t 6
13.589	67316	0.051	0.928	18.000	18:0	28.68	ECL deviates -0.000	Reference -0.002
15.105	831	0.052	0.934	18.868	Sum In Feature 9 . .	0.36	ECL deviates 0.001	19:0 CYCLO C9-10/un
15.336	1547	0.051	0.935	19.000	19:0	0.66	ECL deviates 0.000	Reference -0.002
16.548	765	0.045	. . .	19.703	. . . . .			
16.664	11681	0.064	0.945	19.771	20:1 CIS 11	5.07	ECL deviates 0.001	
17.059	1801	0.050	0.949	20.000	20:0	0.78	ECL deviates -0.000	Reference -0.003
18.233	1286	0.065	. . .	20.681	. . . . .		> max rt	
19.876	969	0.056	. . .	21.633	. . . . .		> max rt	
*******	2217	. . . . .		. . .	SUMMED FEATURE 7 . .	0.94	18:1 CIS 11/t 9/t 6	18:1 TRANS 9/t6/c11
*******	. . . . .			. . .	. . . . .		18:1 TRANS 6/t9/c11	
*******	831	. . . . .		. . .	SUMMED FEATURE 9 . .	0.36	un 18.846/18.858	un 18.858/.846/19cy
*******	. . . . .			. . .	. . . . .		19:0 CYCLO C9-10/un	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
38057000	255630	233979	91.53	217910	5	0.003	0.002

TSBA [Rev 3.0] * NO MATCH *

عزل وتنقية البكتيريا الخيطية المنزلقة ذات التمثيل الضوئي من ينابيع زارة الحارة

رسالة ماجستير مقدمة من

رانيه معاذ ديرانية

حزيران ١٩٩٠

باشراف

الدكتور فؤاد انستي حشوة

قدمت هذه الدراسة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في الجامعة الاردنية

عمان / الاردن

ملخص

تم في هذه الدراسة عزل وتنقية ومعرفة خواص بعض البكتيريا الخيطية المنزلقة ذات التمثيل الضوئي من الينابيع الحارة في منطقة زارة - الاردن .

ست عزلات نقية، HRD-1, HRD-2, HRD-3, HRD-4, HRD-5 و HRD-6 تم عزلها بنجاح من الاغطية الميكروبية التي تتواجد في تلك المنطقة والتي تنمو في مياه الينابيع في درجات حرارة تتراوح مابين ٥٤ - ٦٢ درجة مئوية وفي درجة حموضة تتراوح مابين ٦,٢ - ٧,١ .

تمتاز جميع العزلات بكونها خيطية الشكل ، طولها يزيد عن ٣٠٠ ميكروميتر وعرضها يتراوح مابين ٠,٦ - ١,٢ ميكروميتر ، وهي لاتظهر اي نوع من التخصص او التشعب . وعلى مايبدو ان جميع هذه الكائنات الخيطية لديها غشاء رقيق يحيط بها من الخارج .

الدراسة المجهرية الدقيقة لهذه الكائنات اظهرت ان الاشكال الخيطية تتكون من مجموعة من الخلايا المتلاصقة والمتصلة ببعضها واحدة تلو الاخرى لتكون مايشبه الخيوط . وقد لوحظ وجود عدة اجسام واشكال ذات كثافة الكترونية عالية موزعة في سيتوبلازم الخلايا ، اضافة الى وجود الكلوروسومات في جميع العزلات .

وفي محاولة لايجاد الظروف الملائمة لنمو هذه الكائنات ، وجد ان افضل نمو لها يتم تحت ظروف شبه هوائية وبوجود ضوء معتدل الكثافة يعادل مامقداره ٦,٧٢ واط / سم٢ . افضل درجة حرارة لنمو هذه الكائنات تتراوح مابين ٥٠ - ٥٥ درجة مئوية ، كما وتستطيع

النمو ايضا على مدى يتراوح ما بين ٢٥ - ٧٠ درجة مئوية . افضل درجة حموضة (pH) لنمو هذه الكائنات تتراوح مابين ٦,٥ - ٨,٥ . لم نستطع ملاحظة اي نمو لهذه الكائنات تحت درجة حموضة ٥ او فوق درجة حموضة ١٠ .

تبين من دراسة مراحل النمو المختلفة ان هذه الكائنات تمر عبر مراحل متسلسلة بحيث يطرا عليها اثناء هذه المراحل عدة تغييرات مورفولوجية تؤدي الى اختلاف الاشكال الخيطية من حيث الطول والعرض كما وتبين انها تحتاج الى حوالي مائتي ساعة لانهاء مختلف مراحل منحنى النمو .

التجارب التي اجريت لمعرفة الحاجات الغذائية لهذه الكائنات ، اثبتت الحاجة الضرورية لوجود مركب عضوي واحد على الاقل لضمان استمرارية نمو الكائنات حيث انه لم تستطع اي من العزلات ان تنمو بوجود البايكربونات كالمصدر الوحيد للكربون .

تبين ايضا من خلال الدراسة ان معظم العزلات تمتلك مقدرة عالية على انتاج انزيمات هاضمة للنشويات ، للبروتينات ، وللدهنيات . لم تستطع اي من العزلات انتاج مواد مضادة للبكتيريا الموجبة او السالبة لصبغ جرام او حتى مضادة للخمائر .

تم التعرف على الصبغات المختلفة لتلك العزلات والمتعلقة بعملية التمثيل الضوئي بواسطة تحليل الطيف الضوئي المرئي ، وبواسطة كروماتوجرافيا الطبقة الرقيقة . وقد تبين ان جميع العزلات تحتوي بشكل رئيسي على نوعي اليخضور البكتيري a و c بالاضافة الى الكاروتينات . وقد لوحظ ان الصبغات تتواجد في العزلات بنسب متشابهة وبحيث يكون اليخضور البكتيري c نسبة اكبر من

اليخضور البكتيري a .

تبين من تحليل الاحماض الدهنية المكونة لغلاف الخلايا ان جميع العزلات تحتوي وبشكل كبير على احماض دهنية مشبعة وطويلة السلسلة .

اما بالنسبة لتحليل المجموعات والذي يعتمد على محتوى الاحماض الدهنية فقد اظهر ان العزلات الست يمكن تقسيمها الى مجموعتين منفردتين بحيث يمكن ان تمثل كل مجموعة منها صنف مختلف من البكتيريا .

والخلاصة ، اننا قد نجحنا في عزل وتنقية ست عزلات من البكتيريا الخيطية المنزلقة ذات التمثيل الضوئي والمحبة للحرارة والتي تشبه الى حد كبير البكتيريا من نوع Chloroflexus aurantiacus . بالاعتماد على المورفولوجية ، الفسيولوجية وخواص الصبغات المختلفة ، يتبين ان العزلات الست متقاربة من بعضها البعض من حيث الصفات ، وبالرغم من ان تحليل الاحماض الدهنية يظهر بعض التشابه فيما بين العزلات الا انه اظهر وجود عدة اختلافات فيما بينها ، وان هذه الاختلافات كافية لان تقسم العزلات الى مجموعتين بحيث تمثل كل مجموعة صنفا مختلفا من البكتيريا . وهكذا فان هذه النتيجة بالاضافة الى ماتمتاز به هذه العزلات من الصفات والخواص الفريدة تجعلها مؤهلة لمزيد من الدراسات والبحث .