UPRICO/WRRI/FR/72/16/1
A-016-PR

Comparative Study of the Benthic Microbial Biota in Some Coastal Areas of Puerto Rico

by

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December 1972

"The work upon which this publication is based was supported in part by funds provided by the United States Department of the Interior as authorized under the Water Resources Research Act of 1964, Public Law 88-379."

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Part of this work has been presented at the IV Latinamerican Congress of Microbiology, Punta del Este, Uruguay, December 1971; the remaining portion shall be presented at the I International Congress of Bacteriology to take place in Jerusalem, Israel, September 1973.

ACKNOWLEDGMENTS

Through this means I would like to gratefuly acknowledge the valuable help and services rendered by: Dr. Ernesto Colón Yordán, Secretary of Health and Dr. Angel A. Colón, former Assistant Secretary for Environmental Health and Consumer Protection, for allowing me to complete this work; Dr. Antonio Santiago Vázquez, Secretary of Public Works and former Director of the W. R. R. I., for his encouragement at the outstart of this undertaking;Dr. José A. Ramos, Director of the Graduate School, University of Puerto Rico at Mayaguez, for suggesting the problem which led to this work; Dr. Virgilio Biaggi, Chairman of the Biology Department, University of Puerto Rico, at Mayaguez, for the responsible execution of his administrative duties which greatly facilitated the experimental labor; Dr. Frank Lowman, Director of the Biology Division of P. R. N. C. at Mayaguez, for facilitating his navigational equipment, laboratory equipment and personnel who voluntarily offered their services; Ernesto F. Colón, Professor of Engineering and former Director of W. R. R. I., for his unending stimulus and understanding; Lolita Lugo de Danielsen, Head of the Bacteriology Section of the Institute of Health Laboratories, Department of Health, San Juan, Puerto Rico, for her extraordinary and highly specialized technical assistance, without whose help this work would have been impossible to complete; Dr. James Sanabia, Head, Chemistry Division of the Institute of Health Laboratories, Departament of Health, San Juan, for the edition of this work.

And to all those who in one way or another contributed in a large or samll fashion on a professional or personal level.

I - ABSTRACT

In this work we have chosen two large groups of bacteria: one the gram-positive, non spore-forming, unicellular eubacteria known as the lactic acid bacteria since they are able to synthesize only a few of the building blocks necessary for growth, and accordingly must be supplied with large numbers of organic compounds by the environment. The lactic acid bacteria include both rod-shaped organisms and cocci; the latter with its marked tendency to appear in bunches and short chains, has been the group studied in this work, following the papers of Baird-Parker (1) and with the modifications introduced by Desnues et al. (2) in addition to the recommendations from the subcommittee on taxonomy of Staphylococcus and Micrococcus (3).

The other main group is the aerobic pseudomonadaceae. They are gram-negative, polar flagellated rods (4) whose only energy metabolism is the respiration of organic compounds. Several aerobic pseudomonads can carry on anaerobic respiration using nitrate as the terminal hydrogen acceptor. Most of these organisms can synthesize all the compounds necessary for growth from a single organic compound, almost the opposite of the general characteristics of the previously described lactic forming group.

The pseudomonads as a whole can utilize an extremely wide range of carbon compounds. Such chemically diverse compounds as alcohol, amines, aliphatic, and aromatic carboxylic acids, sugar and hydrocarbons can serve as substrates for one or more pseudomonads. On the other hand, certain pseudomonads can grow on a very restricted number of compounds. Because of the very large number of carbon compounds they can oxidize, the pseudomonads are one of the most important links in the cycle of carbon in nature.

All determinations were made following in part the scheme devised by Simidu and Aiso (5) and the schemes of Bonde (6) Shewan et al (7) and the general description of grouping published by Quigley and Colwell (8).

The distinction between pseudomonads and achromobacter were achieved by using the Hugh and Leifson test (9), the motility test (10), the flagellar Stain (11) (12), or the electron microscope (Hitachi-Perkin Elmer Hu II). The regular occurrence of Achromobacter cells in pairs has often been utilized as an important determinative characteristic of the group (13), and although single cells are observed by light and electron microscope the predominant cell arrangement are pairs.

If peritrichous pseudomonads were found, the differentiation with Achromobacter was made following the scheme of King (14); if differentiation from Enterobacteriaceae was necessary, the scheme of Ewing (15) was followed and if Vibrio was suspected, either gram-stain (16), flagellar stain (12, 14), electron microscopy or the use of penicillin, terramycin and 0/129, as proposed by Shewan and Hodgkiss, were performed (17); to differenciate Pseudomonadaceae, Achromobacter and Bacteriaceae, the Shewan and Kodgkiss scheme was followed, op. cit.

The enhancement of pigment production was obtained by the Paton's media (18) and the oxidase reaction was made following the recommendations of Kovacs (19).

The use of "aged" sea water (20) media in these experiments represented respect for the environmental source from which these organisms were isolated. Not all strains could be grown on media prepared with ordinary distilled water. This would indicate that these group of organisms were highly adapted to their marine environment and considered to be

true marine forms (21, 22).

Rincón coast presents an area where Pseudomonas group III predominates.

In Mayaguez bay no Pseudomonas group I could be isolated. No Vibrios were identified at any station sampled.

Tallaboa bay presents two special characteristics, one is that there are several stations where only one species could be isolated. The other and perhaps the most important is that an ample flora of micrococcus grew in several stations in sharp contrast with Mayaguez bay and Rincón coast where no gram positive cocci could be isolated.

Several physiological characteristics of both the gram negative and gram positive micrococci isolated are quite different from the biochemical and physiological characteristics reported by researchers that sample from temperate seas.

Due to the fast development of industry in Puerto Rico with its consequently coastal localization and increased energy demand, it is urgent to continue the study of the characteristics of the coastal areas, mainly in relation with the bacterial ecology about which there is a general lack of knowledge of its function and importance as the first link in the fundamental biological energy conversion chain.

II - INTRODUCTION

During recent years, the use of marine outfalls for the disposal of sanitary and industrial waste have greately increased.

It has been found that those benthic organisms which are sessile or which move only relative short distances, give the most accurate indication of the condition of the water over a period of time. Therefore information regarding the bacterial flora on the sea bottom has assumed a great significance, since plankton and chemical analysis give only the conditions of the water at the time of sampling.

Bacteria which enter the oceans by way of land drainage and sewage out falls, die quickly (23). Apparently the benthic bacterial flora is much more stable as source of information about the environmental pressures exerted, if any, by the discharges that come from domestic, industrial or natural stream flows.

In the current study we have investigated the contribution of the indigenous marine bacterial microflora in polluted areas such as Mayaguez bay, in which raw sewage is discharged (24); Tallaboa bay, where the outlets of cooling water, mixed with other substances, from a big petrochemical complex are discharged (25), and a third area such as Rincón coast, where there is no known major source of animal or chemical discharge of pollutants.

Some chemical determinations were made in the water samples in order to relate which were the characteristics of the aquatic environment near which the bacterial isolates lived. Bacterial ecology has one very large handicap compared to the ecology of macroorganisms: the minute size of the environment of a microbe. The environment of any organisms is of the

same scale as the organism. Thus the environment of a bacterium is really only a few cubic microns in extent. This means that it is very difficult to describe accurately the environment of a bacterium in nature (26). This handicap to the study of bacterial ecology is counter balanced by the relative ease with which we can determine the bacteria that are best adapted to particular environment. A given environment is chosen and inoculated with many kinds of bacteria (a bit of sea bottom core, for example) and the form best adapted to the environment will come to the fore and without much difficulty can be isolated and studied.

We chose to determine part of the benthic bacterial flora by using in this case the pseudomonadaceae and the micrococacceae, because the effects of the settled sludge upon that flora are and can be more readily noted.

If changing conditions, such as pollution, are unfavorable, organisms must resist these changes, migrate or be destroyed. But if conditions are favorable for certain organisms, these will thrive and build up their populations. For this reason, the society of organisms found is zones of pollution is highly significant. It offers clues to the intensity of pollution and the degree of recovery.

As such, a study of the pseudomonadaceae and the micrococaceae was undertaken in an attempt to evaluate the degree of pollution of the sediment and thereby relate this with other ecological phenomena potentially affecting the bottom community life rather than being able to measure the net physical-chemical effects of the sludge upon the surroundings waters.

Biological indicators, as part of the species compositions of the aquatic environment, are determined by the condition in a given area,

and can indicate past environmental conditions. Single species however do not possess a high index value. Tarzwell and Col have concluded that it is the qualitative and the quantitative compositions of the population which is of importance in denoting pollutional conditions (27). Thus the presence and possible variation of certain species of bacteria, used as indicators, should be determined.

Studies on the whole microbial ecology of the benthos involve difficulties of taxonomic and technical nature. A study of the whole microbial ecology is interesting and necessary, but due to the lack of time and resources we were obliged to limit this work to a few species that can be grown, compared and identified at the same time. This can be achieved by studying the Pseudomonadaceae and Micrococcaceae group, where the combined taxonomic and special growth requirements, if any, can be partially overcome.

There are problems like the partial identification of members not of one taxonomic group but of several different, not always closely related groups, that will cause trouble and confusion in the estimation of the indigenous benthic bacterial flora present at a certain moment in selected areas of tropical seas supossedly affected by chemical or sanitary discharges.

For those reasons, and also because there is not enough data available yet to give us a scientific account of the benthic bacteria in tropical seas, the authors have emphasized its search for two groups not only well known to be common inhabitants of the benthos in northern seas, but about which its biological, physiological and mayor taxonomic characteristics are known.

The equally interesting question of the presence of virus or yeast in the benthos will not be entered into in this report. The reader is referred to papers that will be published elsewhere. The difference between laboratory and field conditions was stressed already by Nusbaum and Garver (28) and the authors do not pretend to simulate natural conditions in any of their results, except that the cores obtained by sampling were kept undisturbed as much as possible through the time these studies were performed.

In most cases we have used standard bacteriological methods. However a few procedures have been modified to facilitate the studies on bacterial physiology or biochemistry.

We are aware that innumerable interrelated factors such as salinity, nutrients, hydrogen sulfide, climate (temperature, storms, ilumination) depth of waters, distance from the shore, currents, and the production and the composition of plants and animal material cause an uneven micro and macro zonal distribution of benthic bacteria.

Nevertheless we have tried to isolate and identify bacterial strains already well known and isolated previously from other seas (7) mainly from northern seas. We did not attempt to determine the viable bacterial population and it should be noted that except for the publications of Gastelvi (29) there are no published works known to us with the characterization of the marine benthic bacterial biota in tropical seas.

Bacteria play essential roles in the cycles of all the biologically important elements, namely: carbon, oxygen, nitrogen and sulfur. In the oxygen and carbon cycle its esential role is to return the carbon dioxide to the atmosphere. Of course much of the carbon goes to form bacteria

themselves, but this carbon too is eventually released.

In the nitrogen cycle, nitrogen is lost by the action of denitrifying bacteria and also when plants are harvested and removed from the ground on which they grew. Both these losses are made up by nitrogen fixation. A number of microorganisms are responsible, mainly the symbiotic nitrogen fixing bacteria. In tropical areas, the blue green algae are probably responsible for a lot of the nitrogen fixation.

Finally, the sulfur cycle is probably not so critical as the nitrogen cycle, but the interconversions made by a large number of microorganisms are also of considerable chemical importance.

III - DISCUSSION

We have been concerned in our laboratory with the distribution of the benthic Gram negative bacilli and Gram positive micrococci flora from three different areas in tropical seas. We believe they are representative of part of the marine microbiota at the moment of sampling and at the sites sampled since it has been clearly demostrated (30) that the elimination of non-marine organisms in the sea is directly related to the size of the marine microbial population. In the deep sea, where the microorganisms, are sparse there is a minimal effect on non-marine microorganisms, however, water sampled from coastal zones had a strong killing effect.

Our samples were taken from coastal zones except that we dealt only with benthic communities.

Also the microorganisms which are foreign to the marine environment, carried into the sea in sewage and in surface drainage waters, rapidly disappear in the sea. Hypotheses proposed to account for this decline include dilution by ocean currents (31), physicochemical factors (32), and toxic products of marine microorganisms (33).

We do not pretend to present our bacteriological findings as indicator organisms. The "state of the art" today is such that sorting and enumeration of indicator organisms is, even for the expert, extremely time consuming. For many areas, check lists are not available and we were confronted with the difficult task of developing our own reference sources. This problem must be alleviated by developing adequate bacterial identification lists. Until this has been accomplished, the use of bacteria as indicator organisms can not be exploited to its full potential.

The chemical analysis are relatively limited first, because that was not the main purpose of this study and second, because in general, we believe that organisms by themselves, reveal more about water quality than the chemical analysis. This is specially true when the area being investigated is under direct influence of an identifiable waste stream. Even the most specific chemical analysis can do no more than indicate conditions existent at the time of the sampling. On the other hand organisms that spend the majority of their life cycle in the area under investigation represent conditions that have prevailed at least over the past months.

So far we only dealt with two main types of marine bacteria, which aside from the argument over definition, presented to us, as to most workers in marine microbiology, great difficulties in identifying the isolates, regardless of which determinative keys were used. Whether the differences and difficulties are real or not have resulted in the publication of quite different determinative keys. Moreover, except for the short mention of Castelvi (29) there is no published work known to us in relation to the taxonomic study of benthic marine tropical microbial flora. Therefore we followed the identification to the generic level, following the determinative keys prepared for Gram negative bacteria isolated from water samples in temperate seas.

As for micrococci, with a very few exceptions (1, 2, 3, 8) there is a general lack of detailed work on taxonomy of benthic micrococci. Further identification to the species level is an intractable problem, in which the statistical approach is likely to lead to some worthwhile results (34).

Bottom cores were taken with a Gemware Phleger Gravity Corer with a

weight of 55 lbs., plastic liners and bronze core catchers. The cores were put immediately in dry ice (35) and kept frozen until used for bacteriological plating.

Water samples were taken from the bottom at the same stations where benthic cores were obtained. Several determinations were made "in situ" and several were performed at our laboratory in an intent to gather some basic general knowledge of the prevailing physical and chemical environmental conditions at the different sampling sites. Since no data, known to us, has yet been published in relation to the characteristics of the water at the bottom in coastal areas of tropical seas such as Puerto Rico's west and south coasts, no comparison could be done to check the validity of our results.

The superficial current direction was studied by using green fluorescein powdered stain (U. S. Navy surplus) and the sampling station distribution followed the pattern of the general sweeping of the currents.

We fully realized that when testing a solid or semisolid such as muds, broken dead corals or sediments, the problem of sampling error is large. Lyn and Yound (36) showed that there exists a considerable amount of error in using a sampling technique very similar to ours, pointing out that the major errors occur with samples containing larger solids than anticipated. The total problem of sampling is complex when consideration is given to the potencial sampling errors and the conclusions to be drawn from the results of the samples examined. It would be unwise to seek precision with less than 5% variation, when core samples might reflect 100% to 200% variation when taken distances 5 feet apart. Nevertheless, since there were no better methods for sampling but experimental ones, we decided

to use the one that had been statistically analysed and its drawbacks well known.

Also, quantitative sampling for microorganisms present very difficult problems. The situation becomes acute because if the samples are not properly quantitized the value of the data proportionally decreases. Unfortunately extensive sampling is time and money consuming and as sometime happens, the pressure of the problem can not await its completions. One way to solve this dilemma (37) is to select a control area which is completely or nearly free of the substances or substances which affect the area or areas under study. That was the reason why we have included Rincón Coast in our studies.

Three areas were chosen in order to compare which, if any, would be the main differential characteristics of the benthic bacterial flora when generally compared with each other.

Area one (see map Rincón Coast) was Rincón Coast. This is a low sandy coast with an approximate length of 5 miles, which extends from north to south at the western part of the island and is located approximately seven miles north of Mayaguez bay.

The superficial sea currents run continuously northward at a speed of 0.4 - 0.6 knots /hour, corresponding generally with the deep currents (38). There are no major sources of pollution and the experimental nuclear thermoelectric plant located at the northern edge of the area at Punta Higuera closed its operation eight months before our studies began. The sea bottom is sandy and rocky, no sludge deposited or recovered in any sample, its continental shelf being very narrow and its continental slope becoming very steep not far from the coastal line.

The second area chosen was Mayaguez bay (see map Mayaguez bay). It is a bay located about halfway along the 34 mile stretch of the west coast between Cabo Rojo and Punta Borinquen. It is an ample bay that extends from Punta Cadena to Punta Guanajibo with an approximate length of five miles. Pollutants enter Mayaguez bay from four main sources (24, 25): Yaguez river, untreated sewage from Mayaguez Municipal Sewage system outfall, located 926 meters from the shore southwest of the Yaguez river mouth, wastes from tuna canneries and Guanajibo river outflow; also (25) direct discharges of sewage and garbage into the sea from homes, restaurants and gasoline stations located along its shore. The currents prevailing in the bay have a velocity of about 1 knot /hr. and sets northward and southward across the entrance to the bay. Farther out from shore a northerly current has been observed to prevail (25). A large superficial swirling was observed at the sewage discharge outlet.

The third area chosen was Tallaboa bay (see map Tallaboa bay). It is an open bay located on the southern coast next to Guayanilla bay. The area under study extended from the eastern margin of the CORCO petrochemical complex to Punta Guayanilla, approximately five miles west. Tallaboa bay is a generally shallow, open bay somewhat protected by small mangrove islands and coral reefs, its waters are turbid and it has muddy bottom sediments. Its superficial current pattern, as measured by us, is mainly westward during the day at a speed of 0.5 knots /hour. Being the tidal range of this bays only 35 cm, the wind force will be more stronger than the tidal force (25). Since the waste discharge to the bay waters is generally of a lower density than sea water, the contaminants will follow the general pattern of the superficial currents.

Our findings in relation with speed and current direction are in agreement with those found by other investigators (38).

The great share of pollution entering the bay comes from the industrial discharges of the Commonwealth Oil Refining Co. and Union Carbide Caribe which, besides discharging 80,000 galloas/minute of hot water from cooling and condensing operations, have a combined BOD output equivalent, in terms of population, to the domestic wastes discharged by a city of 540,000 inhabitants (39).

Sixteen stations were located at Tallaboa bay and named from east to west 1, 1A, 1B, 1C; 2, 2A, 2B, 2C; 3, 3A, 3B, 3C; 4, 4A, 4B, 4C, respectively each station producing bottom cores for bacteriological isolation and water samples for chemical and physical determinations.

The physical and chemical conditions in Tallaboa bay were such that stations 1B, 2A, 2C, 3, 3A, 3B, 3C, 4 showed one or more occasions a content of DO lower than 4.5 p.p.m., this figure being chosen as a point of reference because at the time of sampling that was the lowest DO permitted by law for superficial marine waters used for industrial purposes. The pH never exceded 8.40, the maximum authorized by law at that time and for superficial industrial marine water being 6.7 to 8.7. In general, there is a marked tendency towards the alkaline level and extreme ranges fluctuating between 7.50 - 8.40. (see tables 1, 2, 3)

Carbonates and carbon dioxide content oscillated between 2.26 meq/l to 1.45 meq/l for the former and 2.32 meq/l to 1.34 meq/l for the latter. They represent the major buffer against which acid and alkali from any source can act. (see tables 4, 5, 6)

The temperature of the water at the bottom never extended beyond the

26°C - 31.5°C range, the salinity varied only from 36.404 S‰ - 30.910 S‰ and the chlorinity between 20.09 - 17.48 at 20°C.

Determination of phosphate as PO_4 - P/l, hitrates and phenol wastes were made with negative results for the presence of phenols and a maximum of 0.75 mq/l for nitrate and .20mq/l for phosphate. (see tables 7, 8, 9)

The Gram negative bacteria showed that Pseudomonas sp. group I was isolated only from station 1A (see table 12); Pseudomonas sp. group II was isolated only from sample 1 of station 1B, named 1B-1 because there was a second sample taken at that point (see table No. 15); Pseudomonas group III were isolated only from sample 1-1 of station 1 and from station 1A; Pseudomonas group IV was isolated from samples of the station 1, 1A, 1B, 1C, it is worthwhile to note it was the only group that could be recovered at stations 1B, and 1C (see tables 14, 16). No Flavobacteria sp. Vibrio sp. or Enterobacter-Coliform group was isolated from any of the 1, 1A, 1B, 1C stations which are located just above the eastern margin of the petrochemical outlet (see map Tallaboa bay station location). As for stations 2, 2A, 2B, 2C, located westward beyond the petrochemical outlet, it was possible to isolate Pseudomonas sp. group I and Pseudomonas group II from stations 2 and 2A; Pseudomonas group III from stations 2B and 2C and Pseudomonas group IV from stations 2A and 2C; no other species of gram negative aerobic bacilli grew out of the core samples from this stations (see tables 17, 18, 19, 20). It is remarkable that no gram negative bacterial sp. were obtained from a second sample taken at station 2C (see table 21).

From the core samples obtained along the transect named 3, 3A, 3B-1, 3B-2, 3B-3, 3C-1, 3C-2, the following species of Pseudomonas and Gram

negative aerobic bacilli were isolated: Pseudomonas group I were isolated from stations 3, 3B-1, 3B-3, 3C-1; Pseudomonas group II were isolated from samples 3, 3B-3, 3C-2. Pseudomonas group III was found in stations 3, 3B-3, 3C-1, 3C-2. Pseudomonas group IV was isolated from stations 3, 3B-1, 3C-2. Sample 3A taken from station 3 only produced Flavobacteria sp. and only Vibrio sp. grew out of sample 3B-2 taken at station 3B. Aerobacter sp. and Paracolon group were identified from samples of stations 3, 3B, 3C. There is a tendency in this group to harbor Vibrio sp. whereas none were isolated from samples taken at stations 2 to 2C.

The Gram negative sp. isolated from stations 4 to 4C are as follows: Pseudomonas group I were absent from all the samples obtained the same as Pseudomonas group IV and Pseudomonas group II which were not isolated from any station. Pseudomonas group III was isolated from samples of stations 4 and 4C. Only Flavobacterium sp. were isolated from station 4A and Vibrio sp. was the only isolate obtained from samples of the station 4B; it was isolated also from station 4C as well as Aerobacter sp. which were present in samples taken at station 4 and 4C.

In relation to the Micrococcaceae (40) we used anaerobic and aerobic metabolism of glucose in order to differentiate between genus Micrococcus and Staphylococcus (2). 24 test were used including mannitol and maltose, arabinose, glactose and xylose aerobic and anaerobic metabolism; sodium requirement by growth in BM broth and not in peptone broth; hemolysin production, lypolitic activity, coagulase production and the activity on casein. No work, known to us, has reported the results of the biochemical activity on blood and the production of coagulase from gram positive cocci isolated from the benthos, least in specimens taken from tropical seas.

This is true also in relation to the testing of sodium requirements for growth by studying growth in peptone medium with or without aged sea water (20). For more details see materials and methods.

Gram positive micrococci were isolated from station I at Tallaboa bay. None, out of seventeen strains under study, were able to grow in a simple nitrogen medium. None required high sodium concentration to grow and all were producing an orange pigment. All were able to hydrolyze casein and none were able to metabolize out of the twenty three were able to grow on a simple nitrogen medium and nine required high sodium concentration to grow in peptone or in any other medium. All of them were pigmented, although none were able to produce acid either aerobic or anaerobically from glucose, all were able to hydrolizate casein and none had any action on blood or were able to produce coagulase (see table 34).

Twenty three strains were isolated and studied from the sample taken at station 1B, Tallaboa bay. All of them were oxidaze and catalaze producers. All were able to metabolize glucose fermentatively, and produce acid from mannitol and maltose. They all grew in EM medium without sea water and all colonies produced a yellowish pigment. None had any action on blood or lypolitic activity (see table 35).

At station No. 2 twenty one strains were isolated and studied. One out of the twenty, one was able to metabolize glucose fermentatively and it produced acid from mannitol and maltose. Only three did not require high sodium concentration to grow and they were all able to hydrolyze casein (see table 36).

Twenty-three strains were isolated from station 2A, Tallaboa bay. All were oxidase and catalase producers. All metabolized glucose

aerobically and produced acid from mannitol, maltose and arabinose. Only two required a high sodium concentration to grow and twelve out of the colonies were either yellow pigment producers or orange pigmented and all were able to produce casein hydrolitic enzymens (see table 37).

It was possible to isolate four strains from the sample taken at station 2B, Tallaboa bay. Three had the ability to metabolize glucose fermentatively as well as mannitol and maltose. None required a high sodium concentration to grow, one was yellowish and the other three were orange pigment producers (see table 38).

At station 3, Tallaboa bay, twelve strains were isolated. Six were able to metabolize glucose fermentatively as well as mannitol, maltose and arabinose. None required a high sodium concentration to grow and they were all orange pigment producers. None had any effect on blood, lypolitic activity, coagulase production and none were able to hydrolyze casein. None were able to grow in simple nitrogen medium (see table 39).

Twenty strains were isolated from station 3A, Tallaboa bay. All of them were oxidase and catalase producers. All grew in simple nitrogen medium and none required high sodium concentration to grow. All metabolized glucose fermentatively as well as mannitol. Acid was produced from maltose and xylose by oxidative pathways. All were able to reduce nitrates to nitrites and all produced a magenta pigment. None had any activity on blood, produced coagulase or had any lypolitic activity; none was able to produce coagulase and none hydrolyzed casein (see table 40).

From the sample taken at station 3B, Tallaboa bay, fourteen strains were isolated. All were oxidase and catalase producers and none were able to grow in a simple nitrogen medium. No activity on the different carbo-

hydrates tested was shown and none required a high sodium concentration to grow. All colonies were orange pigmented and all were able to hydrolyze casein. None had any activity on blood, produced coagulase, gelatinase, hydrolyze starch or had any lypolitic activity under the conditions tested (see table 41).

It is interesting to note here that it was not possible to isolate gram positive cocci from any other samples taken at any other station at Tallaboa bay.

As published in other reports (1) the formation of loose or compact clusters showed considerable variation on different media and tetrads were formed by many strains which otherwise grew as irregular clusters. We consequently decided not to consider the genus Sarcina until more biochemical and physiological data could be obtained from the strains under study because it would have been possible to confuse the true packet formers with some of the tetrad-forming micrococci. Following this consideration, those organisms which grew as tetrads were placed in the genus Micrococcus.

Also is interesting the fact that no Staphylococcus sp. was isolated from any of the samples taken.

The percentage distribution of characteristics among 155 cultures of micrococci isolated from the benthos in tropical seas differs sharply with the reports of other investigators who studied strains isolated from temperate seas (40). While Anderson found 97.5% out of his 205 isolates to be producers of acid from glucose, we found a 49% average. He found that 90% of his strains were fermentative in contrast with 100% in our findings. We were not able to isolate strains with oxidative activity on glucose.

He reported that 53% out of his 90% of fermentative were able to produce a clot in litmus milk while our isoltates were not. In the northern seas series 37% produced acid from mannitol; in our series 100% out of the 76 glucose fermenters produced acid from mannitol, 69.7% by an oxidative pathway and 30.3% by a fermentative one. None of the 76 glucose fermenters metabolized galactose while the isolates from the northern sea turned it acid or alkaline. 84.2% out of 76 isolates which were glucose fermenters, were able to hydrolize gelatine. The 15.8% gelatine negative were all 100% mannitol oxidative and none was able to produce lypolitic enzymes under the conditions tested; again in contrast with the findings of Anderson et al. from the temperate sea series. All gelatine negative strains from our isolates were also unable to show lypolitic activity and 33% did not grow in a simple nitrogen medium.

79 isolates, or 51%, from the total strains under study did not produce acid from glucose, neither produced a clot in litmus milk, nor showed lypolitic activity. None hydrolyzed gelatin and all (110%) hydrolyzed casein. Only 2 (2.6%) were able to grow in a sinple nitrogen medium (For a summary of these results, see Table 41-A).

There were sixteen stations located at Mayaguez bay (See map Mayaguez bay station location). Station 1C,2C,3C and 4C were sampled for chemical and physical determinations only. All others were sampled for bacteriological isolation also. The DO concentration at the bottom was rather low, with readings of 2.5 ppm at the Yaguez river mouth and between 3.6 to 3.7 ppm following the transect of stations 1A, 1B, 1C, 2A, 2B, 2C. Although there were very few determinations made they do not differ from chemical and physical determinations made at superficial waters of the bay by several investigators, some reporting readings as low as 0.00 ppm in stations located very near the

area from which we sampled (25). The pH exhibited readings as high as 8.25 with a low of 7.60 at the bottom, none exceeding the maximum a minimum permitted by law at that time. The total alkalinity expanded from a high of 2.13 meq/l to 1.57meq/l at the lowest determination. Total CO_2 and CO_3 showed variations from 1.87meq/l to 1.36meq/l for the former and 2.02 meq/l to 1.48meq/l for the latter. The temperature of the water at the bottom fluctuated between a high of 29°C to a low of 26°C. Salinity was recorded from 36.108 S‰ to 30.620 S‰, all samples obtained as usual, from the bottom. Phosphates as mg PO_4-P/l were never higher than 0.75; phenols were negative and nitrates showed a maxima at 2.20 and a minima at 0.40 mg/l.(See tables No. 42, 43, 44)

No Pseudomonas group I were isolated from station I. Pseudomonas group II-III and IV grew out of this sample. Of the seven strains studied, two did not grow at a higher temperature (above 37°C) Five failed to grow in media with low concentrations of sodium and none exhibited pigment production. Three were sensitive to pencillin 2 u and four were sensitive to terramycin 10 mcg.; the absolute sodium requirement for growth not being reported in previous publications and the antibiotic sensity being in contrast with what has been previously stated. The same can be said for the effect of temperature on the inhibition of growth (5,7)(See Table 46).

At station 1A, eight strains were studied. One was not possible to identify; one showed a glucose oxidative reaction, seven were flagellated, six failed to grow in media with low concentrations of sodium and one failed to grow at 37°C. No Vibrio sp., no Paracolon group was present in this specimen. They all belonged to Psedomonas group II-III-IV (see Table 47).

Eight strains were isolated from the core taken at station 1B, Mayaguez bay. All were motile, two split urea, four failed to grow in medium with low sodium concentration and the other four showed a very weak growth. All grew at 37°C and all were penicillin resistant. All were classified as group II - III and IV. One determined to be a Pseudomonas sp. for several reasons did not fit in the sequences known and was not classified (see table No. 48

Eight strains were isolated at station 2A. Six showed an oxidative metabolism of glucose, all were flagellated and two showed a weak urease production. Two failed to grow in low sodium concentrations and all grew well at 42°C. Two were sensitive to penicillin 2 u and five were sensitive to terramycin 10 mcg. All were classified as Pseudomonas II - III and IV (see table No. 49

Station 2B produced six strains of Gram negative motile bacilli which were all none pigmented. Three failed to split the urea and one doing so in a very weak fashion. Three did not grow in medium with low sodium concentrations and all grew well at 42°C. Three were sensitive to penicillin 2 u and four were sensitive to terramycin 10 mcg. All were classified as pseudomonas group II - III - or IV (see table No. 50

Eight strains were studied from station number 3. One was not motile and five fermented glucose, sucrose and mannitol with gas. Only one grew in Marine agar plus 30% skim milk and six failed to grow in medium with low sodium concentrations. One failed to grow at 37°C, two were penicillin sensitive and four were terramycin sensitive (10 mcg). They were classified as Pseudomonas group II - IV and Aerobacter sp. no Pseudomonas group I and III or Vibrio sp. was isolated from this sample (see table No. 57

Five out of the eight strains studied from station 3A, Mayaguez bay, fermented glucose with gas and sucrose without gas production. Only one showed urease production and seven failed to grow without high sodium concentration in the medium. All grew at 42°C and none was sensitive to Penicillin, Terramycin and 0/129. They were classified as Pseudomonas group II and IV and Aerobacter sp. (see table No. 52

From station 3B, eight strains were studied. All colonies were mucoid and motile; one render the media with carbohydrates alkaline, five failed to grow in media with low sodium concentrations and all, except two, were Penicillin 2 u, Terramycin 10 mcg and 0/129 resistant. All strains were classified either as Pseudomonas group II - III or IV or Aerobacter sp. (see table No. 53

From the eight strains studied from station 4, Mayaguez bay, it was found that all were motile exhibiting urease production, none growing in media with low sodium concentration; all grew at 25°C, 37°C and 42°C, three were penicillin resistant 2 u and terramycin resistant 10 mcg. They were classified as Pseudomonas group IV (see table No. 54

Four out of eight isolates studied from station 4A sample, fermented glucose and sucrose with the production of gas. Four failed to produce urease, three failed to grow in media with low sodium concentration, all grew at 25°C, 37°C and 42°C, two were penicillin resistant 2 u, and one was terramycin resistant 10 mcg. They were classified as Pseudomonas group II and III and four as Aerobacter sp. (see table No. 55

Station 4B, Mayaguez bay, showed eight strains under study. Three of them rendered glucose, lactose, sucrose and mannitol alkaline. None had urease, while seven failed to grow with low sodium concentration in

the media but all growing well at 25°C, 37°C and 42°C. Two were penicillin and one was terramycin resistant. All were classified either as Pseudomonas group II - III and IV (see table No. 56)

Rincón Coast water samples showed 5.7 p.p.m. of D.O., a pH of 8.20, a total alkalinity of 1.92, 26° temperature and a total CO_3 of 1.82 meq/l with CO_2 being 1.63 meq/l. No nitrates, phosphate or phenol were found. (see table 57)

Eight strains were isolated from station 1, Rincón Coast, six fermented glucose with gas production, one turned glucose, lactose and sucrose alkaline, one failed to grow in Marine agar plus 30% skim milk, was oxidase negative urease megative and was classified as Alkaligenes sp. Six were polarly flagellated, one was amphitrichous. All except two were penicillin resistant and one was terramycin resistant. All grew well at 25°C, 37°C and 45°C. They were classified as Aeromonas sp. Alkaligenes sp. and one as Pseudomonas group III (see table No. 58).

Station two at Rincón Coast showed 8 strains under study whose characteristics were as follows: all were motile with polar flagella, two produced pigment and grew in Paton's media. They all failed to show urease activity, four failed to grow in media with a low sodium concentration and all grew well at 25°C, 37°C and 45°C. One was pennicillin sensitive 2 u and three were terramycin sensitive 10mcg. They were classified as Pseudomonas group I - II - IV (see table No. 59).

Table No. 60 shows the general Gram negative species distribution isolated by station and area. It is possible to see that on Rincón Coast station 1 Pseudomonas group III was the only isolate from the sample. Achromobacter-Alkaligenes sps. and Aerobacter sp. tended to predominate.

In station No. 2, the four groups of Pseudomonas were present.

Mayaguez bay presents the peculiar situation that no Pseudomonas type I could be isolated. There were Flavobacteria sp. in stations 1 and 2 and Aerobacter sp. in stations 3 and 4. There were no Vibrios sp. isolated either.

Tallaboa bay shows a rather more uniform picture in which all groups of Pseudomonas are present at one time or another; only in stations 4 to 4C Pseudomonas sp. group I and IV could not be isolated. Flavobacterium and aeromonas were present only in stations 3 to 3C and there was also a great abundance of Vibrio sp. as only Vibrio sp. could be isolated. This situation tended to duplicate in relation with other species isolated from various stations in Tallaboa bay as is the case of station 1-B where only Pseudomonas group IV was found, station 2-B where only Pseudomonas type III were isolated, although it is clear that the number of isolates is too low for drawing definite conclusions, station 2C-1 where no Pseudomonas, Vibrio, Aerobacter o Enteric was possible to isolate, station 3-A where only Flavobacteria sp. was identified and station 4-A where also Flavobacteria was the only isolate. In a very tentative form we could think that it seems that very great selective forces have been applied to that environment with the result of large benthic bacterial communities made up by one or two species. It is worth noticing that no Vibrio sp. no Enterobacter-Coliform group was isolated from the specimens taken at Mayaguez bay or Rincón Coast.

More interesting is the surprising fact that no micrococci grew out of any sample taken at Mayaguez Bay or Rincón Coast. It is necessary to do more ecological research in the three areas before anyone can put forth a definite explanation of this fact.

The requirement for sea water, or in other words of high concentration of sodium, in the medium was not reported in preceding papers. In table No. 61 can be seen that the Pseudomonas group III and IV, Aeromonas sp. and Vibrio sp. isolated from the benthos of coastal areas of Puerto Rico have shown an absolute requirement for the addition of high concentrations of salt to the medium. It has been reported also that Pseudomonas, isolated from temperate seas failed to grow at 37°C, they belonged to group III and IV, and the same happened with the genus Vibrio. In our series both grew well at 37°C, fact that we confirmed many times through our studies. In the same table we are reporting data in relation with growth in akim milk, citrate activity, urease production, and growth at 25°C, 37°C and 45°C. Also can be seen that the Flavobacterim sp. of marine origin failed to grow at 37°C and 42°C.

Flagellar characteristics were confirmed by the use of the electron microscope (Hitachi Perkin Elmer HU-11) and some abnormalities observed through the light microscope were also confirmed by the electron microscopy photography (see: Electron microphotographs of strains isolated from the benthos of tropical seas).

In relation to the media recommended for primary isolation in which peptone broth should be used, we recommend the use of aged sea water, following the procedure of Zobell (20), because many strains failed to grow in media without high salt concentrations.

The Kirsh-Bauer method (41) was followed for the sensitivity test. The Muelle Hinton media should be replaced by marine agar since several of the strains tested did not grow on the former media. Consequently our interpretation of the tests as reported do not correspond with the

Kirsh-Bauer interpretation. Several investigators have tested sensitivity to penicillin by using 2.5 u discs. In the present report we used discs with 2 u of penicillin. We considered as sensitive to penicillin only those strains which failed to grow making an inhibition halo of 25 to 30 mm.

It is important to add that after comparing the diffusion ability of penicillin in the two media named above, we found that there was a better diffusion pattern for penicillin in Muller-Hinton media than in marine agar.

Finally a table to which some minor modifications have been made is shown in order to clarify the pattern followed for the classification of Gram negative bacilli (see table No. 62).

All the above data has been presented without regard to pollution, except that two known polluted areas were studied in contrast with an area where no major source of pollution has been noticed or scientifically detected and reported.

Pollution studies have been carried out by several researchers on the areas under study and its results published elsewhere (44, 45).

IV REAGENTS

Material and Methods

BACTERIOLOGICAL METHOD
GRAM NEGATIVE BACILLI

1. Primary isolation - Inoculate on marine agar 2216 E, Difco.

Formula-Bacto Peptone	5.0	g
Bacto Yeast Extract	1.0	g
Ferric citrate	0.1	g
Sodium chloride	19.45	g
Magnesium chloride	8.8	g
Sodium sulphate	3.25	g
Calcium chloride	1.8	g
Potassium chloride	0.55	g
Sodium Bicarbonate	0.16	g
Potassium Bromide	0.08	g
Strontium chloride	0.034	g
Boric Acid	0.022	g
Sodium silicate	0.004	g
Sodium fluoride	0.0024	g
Amonium nitrate	0.0016	g
Disodium phosphate	0.008	g
Bacto agar	15.00	g

Incubate samples at 25°C, from 48hrs to 8 days.

2. Motility

a) Inoculate in semisolid Edwards media. Formula of U.S. Department of Health:

"Enterobacteriaceae, Biochemical Methods for group differentiation"

by W.H. Ewing, Center for Disease Control, Atlanta, Ga.

Formula-Motility medium

Beef Extract	3 g
Peptone	10 g
Sodium chloride	5 g
Agar	4 g
Distilled H_2O	1000 ml.

pH 7.4 Sterile 121°C, 15 min.

Stab the specimen into the top of the media column to a depth of about 5mm.

b) Inoculate in marine broth and incubate 24hr. at 25°C.

Prepare hanging drop and observe motility under microscope using high dry lens 40X and ocular 10X.

3. Gram Stain. (Bacto Gram Stain by Difco)

Loopfulls of specimen from marine broth medium are placed on a glass slide and allowed to air dry. Heat-fix through a low flame avoiding over heating. Allow to cool then flood with gram crystal violet stain for one minute. Wash. Rinse. Add bacto gram Iodine one minute. Wash. Flood off excess water with Bacto gram decolorizer until solvent runs colorlessly from the slide. Wash. Flood with bacto gram safranine one minute. Wash. Blot with paper and allow to dry. Use oil inmersion.

4. Morphology.

Observe gram stain with ocular 10X WF-24 and oil immersion lens A 355191 (Leitz microscope).

5. Colony appearance.

Inoculate marine agar from a pure culture. Incubate at 25°C for 48hr. Observe under dissecting microscope with 10X ocular, lens U.S. Polt. 2093605 Bausch & Lomb.

6. Pigment of colony.

Inoculate marine agar from a pure culture. Incubate at 25° C for 8 days.

Pick a colony and spread it on filter paper Wahtman no. 2 Observe pigment.

7. Oxidative-fermentative test.

Difco, certified MOF medium was used. To 100 ml of sterile basal medium aseptically add 10ml. of 10% Seitz filtered sterile Bacto dextrose solution.

Mix thoroughly and dispense 5ml. into sterile culture tubes. Inoculate in duplicate from the growth obtained in the marine broth. Use sterile capillary pipettes. Cover one tube with paraffin. Observe reactions in tubes for 10 days.

8. Test for carbohydrate utilization.

Prepare lactose, sucrose, and mannitol media, using MOF medium and some concentration of carbohydrate as above. Do not cover with paraffin. Observe daily for 10 days.

9. Pigment production.

Media por pigment enhancement of pseudomonas used for this purpose was that of E.O. King (1959) (14)

Medium A:

Bacto peptone	20	g.
Glycerol CP	10	ml.
$Mg\ Cl_2$ (anhyd)	1.4	g.
$K_2(SO_4)$ (anhyd)	10	g.
Agar	15	g.
Distilled water	1000	ml.

Medium B:

Proteose peptone #3	.20	g.
Glycerol C.P.	10	ml.
$KH\ (PO_4)$	1.5	g.
$Mg\ (SO_4)$	1.5	g.
Agar	15	g.
Distilled water	1000	ml.

Dispense 3 ml. in 100 mm X 125 mm tubes. Sterilize 15min. , 15lb. and slant. Inoculate slants with cultures grown in marine agar. Incubate at 25°C for 10 days. Observe daily with and without ultraviolet light for fluorescense. Use Wood's lamp. Medium A will show production of pyocyanin, medium B will show fluocescein production.

10. Casein Hydrolysis.

Use marine agar plus 30 % skim milk. Prepare and sterilize 15 ml tubes with marine agar. Prepare the skim milk and inspissate. Add 4.5ml. of skim milk to each tube containing 15ml. of marine agar (melted and kept at 42°C. Pour into a pefri dish) inoculate the plates with cultures grown from marine broth. Incubate for 24, 48 and 72 hr. at 20°C.

Observe for hydrolysis and pigment.

II. Enhancement of pigment production by Pseudomonas, and 2-Ketogluconic acid production

Dihydrogen ammonium phosphate 1.0g

Potassium chloride 0.2g

Crystalline magnesium sulfate 0.2g

Potassium or sodium gluconate 5.0g

Distilled water ..... 400 ml.

Add 2ml. of 0.5% w/v of 8-hydroxyguinoline in redistilled chloroform, allow to settle and remove. Repeat three times. Wash with 5ml. of redistilled chloroform removing excess oxime. Complete to one liter with distilled water; adjust to pH 7 and dispense into tubes and sterilize. Inoculate and incubate, for 48 hr. at 25°C; observe pigment production with U.V. light using Wood's lamp.

Impregnate a sheet of whatman no. 1 paper with a saturated solution of recrystallized aniline oxalate and spot tha with the culture, heating in an oven at 105°C for 2-3 min.

Red spoting indicates the presence of 2-Ketogluconic acid

12. Flagella determination:

Cultures grown in marine broth for 24hr. at 25°C were twice centrifuged and washed with fresh distilled water and resuspended in distilled water to a light turbidity. Let a drop to run to the other end of an acid cleaned glass slide. Air dry. Do not heat fix. Stain with flagellar stain (Leiffson's) for 8 min. Wash with strong water flow. Air dry. Examine for flagella.

13. Antibiotic sensitivity.

Discs impregnated with 2u of penicillin and 10 ug of tetracycline were used. Crystals of 0.129 were sprayed over an area of the inoculated plate. Incubate at 25°C for 24hr, interpret zones of inbition as sensitive or resistant.

14. Oxidase test

a) Kovac's method: using Whatman filter paper no.1, Lay a 6cm. square in a petri dish. Put 3 drops of 1% of tetrametyl-p-pheylenediamine dihydrochloride in the center of the paper. Pick a colony and smear it on the paper. Observe the development of a dark purple color in 5-10 sec. if the bacteria is oxidase positive

b) Use commercial test papers (Pathotex from Warner-Chilcott).

15. Urease production

The media used was Christensen's urea agar of the following formula:

Peptone	1 g.
NaCl	5 g.
Glucose	1 g.
Monobasic potassium phosphate	2 g.
Phenol red	0.012 g (6ml of 1:500 solution)
Urea	20 g
Distilled water	100 ml

Adjust pH 6.8 - 6.9, filter and - sterilize. Dissolve 15g of agar in 900 ml. of distilled water and sterilize. Cool to 50-55°C, add 100 ml urea concentrate . Mix and distribute into sterile tubes. Slant the medium with a deep butt.

The urea agar was inoculated with three drops of each culture and incubated from 48 hr. to 10 days. Daily observation developing pick color confirms the production of urease.

16. Utilization of sodium citrate and ammonium salts.

The media consisted of:

NaCl	5 g.
$MgSO_4$	0.2 g.
Ammonium dihydrogensulfate	1 g.
Dipotassium phosphate	1 g.
Sodium citrate	2 g.
Agar (washed vigorously for three days)	20 g
Distilled water	1000 ml.

Add 40 ml. of 1:500 brom thymol blue indicator.

Sterilize at 121°C for 15 min. and slant to obtain 1 inch butt and 1:5 inch slant. Inoculate the slants with 3 to 4 drops of the culture grown in marine broth. Incubate from 48 hr. to 10 days and observe for positive reaction from (green to dark blue)

17. Growth at 25; 37°and 42°C

Determine growth at different temperatures by inoculating marine agar plates with pure cultures and incubate at said temperatures for 72 hr. Record results as growth or no growth.

GRAM POSITIVE COCCI
MARINE MICROCOCCI

1. Isolation of organisms

Organisms were isolated by spreading from an inoculum (taken from the bentho's sample core) onto the surface of previously dried agar plates, and incubated at 25°C for 7 to 14 days. Figgerent colonies were selected from each plate, according to size, color and morphology.

The medium used for primary isolation and selection was one we designed as B.M. media consisting of the following ingredients.

Peptone (Difco)	2.5 g
Yeast extract (Difco)	2.5 g
$FePO_4$	0.1 g
Agar (Difco)	15.0 g
"Aged" sea water	750 ml
Distilled water	250 ml

pH adjusted to 7.5 after sterilization at 15 lb/in^2 for 14 min.

2. Characterization of isolates.

General procedure:

The B.M. media was used for the growth and maintenance of all cultures except that the agar was omitted and sterilization conditions altered in different ways. The agar and other specific ingredients were added when necessary.

3. Gram reaction

Cultures grown on B.M. agar for 48 hr. were stained by Gram's method, using "Difco" reagents consisting of gram crystal violet; gram iodine; gram decolorizer and gram safranine. Organisms were observed for staining using a Leits microscope with 100 X oil immersion objective and 10 X ocular.

4. Oxidase reaction

Using a modified Kovac method, a strip of Whatman no. 1 filter paper was impregnated with a 1% (w/v) solution of tetramethyl-p-phenylenediamine dihydrochloride (Eastman). Pick a colony and scrup it the wet paper. A positive oxidase reaction is recorded whenever a dark purple color appears within 5-10 sec.

The Patho-tec strip paper was used for comparison with the Kovac's method. This is a commercial strip paper made by Warner Chilcott Co., Laboratories. The colony is picked and scrubbed onto the paper strip. Those turning dark purple within 5-10 sec. were recorded as positive. A control of Pseudomonas sp. and E. coli were used for oxidase positive and oxidase negative organisms, respectively.

5. Catalase reaction

Allow 0.5 ml. of 3% (w/v) solution (prepared from 30% hydrogen peroxide solution) to fall on the surface growth of the organism growing on slants of B.M. agar and observe for O_2 release. Uninoculated slants and a culture of Staphilocococcus sp. and Streptococcus sp. were used for control purposes.

6. Growth in a simple nitrogen medium.

To determine which cultures were able to grow in a much simpler medium, the following was inoculated with all cultures:

Glucose ..... 5.0 g

Sodium citrate ..... 1.0 g

Sodium succinate ..... 1.0 g

Sodium gluconate ..... 1.0 g

Ammonium acetate ..... 1.0 g

Potassium nitrate ..... 1.0 g

"Aged" sea water ..... .750.0 ml

Distilled water ..... 250.0 ml

Adjust pH to 7.5 and sterilize by steaming for 20-30 min. on 3 consecutive days.

Transfer cultures from B.M. fluid media to the above medium and incubate at 25°C for 14 days. Turbidity indicates that the medium is suitable for growth. If only slight turbidity is observed after 14 days, transfer the culture to another tube of the same medium and again incubate for 14 days. If slight turbidity appears, the organism was cataloged as growing in the medium.

7. Motility and cell morphology

Use a conventional microscope (Bausch and Lomb) with a 10X W.F. 22 ocular and a 40X objective.

The hanging drop technique was used to determine motility.

8. Oxidative and fermentative metabolism of glucose.

Investigate all cultures for the ability to produce acid from glucose, both aerobic and anaerobically. Hugh & Leifson's (9) medium can not be used. With the ability to acidify glucose, we encountered many difficulties and had to use 5 different kinds of media:

1) BM liquid media ..... 1,000 ml

Glucose ..... 0.5 %

Agar (Difco) 0.25%

Phenol red 0.0015%

pH 7.5

Two tubes of the media were inoculated with the same culture; one baing covered with paraffin. The tube covered with paraffin oil showed an aeid reaction, the other tube remained unchanged. It was assumed that something was reacting with the paraffin oil.

We prepared four other media, eliminating ingredients in a trial and error fashion.

The other media were prepared as follows:

2) BM broth without peptone ..... 1,000 ml

Ammonium nitrate ..... 0.25 g

Glucose ..... 0.5 %

Agar (Difco)..... 0.25 %

Phenol red ..... 0.0015 %

3) BM broth without peptone and yeast extract...1,00 ml

Ammonium nitrate ..... 0.25 g

Glucose ..... 0.5 %

Agar (Difco) ..... 0.25 %

Phenol red 0.015%

4) BM broth without yeast extract... 1,000 ml

Glucose 0.5%

Agar (Difco) ..... 0.25 %

Phenol red 0.0015%

5) BM broth ..... 1,000 ml

Gluose 0.5%

Phenol red 0.0015%

In all these media we inoculated two tubes with the same culture, one aerobically and the other anaerobically. We arrived at the conclusion that the ingredient reacting with the paraffin oil was the agar and was acting as a reducing agent.

9. Utilization of mannitol, maltose, arabinose, galactose and xylose.

Use the following medium:

BM broth 1,000ml

The corresponding sugar 0.5%

Phenol red 0.0015%

Incubate cultures both aerobic and anaerobically. Examine daliy for acid production.

10 Nitrate reduction

Prepare media as follows:

BM broth 1,000ml

Potassium nitrate 0.5%

pH adjusted to 8.4

Inoculate and incubate for 7 to 14 days before testing for nitrate reduction. Prepare the following solution:

8g Sulphanilic acid and 6g Dimethyl-naphtylamine per liter of 5N acetic acid Add 0.5 ml of the above solution to each culture tube. Positive reduction of nitrates to nitrites is recorded when a deepred or pink color develop. Faint development of color is recorded as negative. Use a Durham tube in each tube of nitrate media to determine the ability of the organism to reduce nitrate to a gaseous end product.

Action in Litmus milk

The medium consisted of 10% (w/v) solution of skimmed litmus milk powder (Difco) in distilled water. Sterilize by steaming for 20-30 min. on three consecutive days. The inoculated media was kept for 7 days and the action on milk recorded as no change, coagulation, digestion peptonization, etc.

11. Gelatin hydrolysis

The medium used for this test consisted of:

BM broth ..... 1,000 ml

Agar (Difco) (w/v) 1.5%

Aelatin (Difco) (w/v) 0.4%

Final pH a fier sterilization 7.5

Inoculate petri dishes which containing 15 ml of the above media incubate from 3.5 days at 25°C. Flood the growth on the paltes with a solution of mercuric chloride in HCl. ($HgCl_2$, 15g; conc HCl, 20ml; distilled water, 100ml) A precipitate is observed where the gelatin is not hydrolized. A clear zone around the colonies appear when the gelatin had been hydrolized.

12. Starch hydrolysis:

Replace the gelatin by starch 0.5% (w/v) in the medium prepared for galatin hydrolysis. Inoculate, incubate for the same period at the same temperature as for gelatin hydrolysis.

Flood the plate with a 1:10 dilution of gram's iodine solution. Starch hydrolysis is present when colonies are surrounded by a clear zone.

13. Casein hydrolysis:

For this medium a 10% skimmed milk solution (Difco) was used as a source of Follows:

a) Skimmed milk (Difco) 100g

Distilled water 1,000ml

Sterilize by steaming for 20-30 min. on three consecutive days.

b) BM agar ..... 1,000ml.

Dispense the agar in 15ml portions in tubes and sterilize by autoclaving 15lb/15min.

Before using the medium add 1.5ml of skimmed milk to each 15ml of BM agar (melted and cooled to 42°C) and dispense into petri dishes. A smooth casein suspension is obtained when the agar mixture solidified.

Inoculate cultures on to the plates, incubate at room temperature (25°C) and examine at intervals for up to 14 days. Casein hydrolysis is considered positive by elearing of the medium around the bacterial growth.

14. Lypolisis:

Fresh dairy cream was used as the source of fat. Sterilize fresh dairy cream at 15lb/15min.

To BM agar, in 15ml tubes melted and cooled to 42°C, was added 1.5ml of sterile cream, mixed and poured onto petri dishes. Inoculate cultures onto the medium and incubate in day-light for 14 days.

Scrape the bacterial growth from the surface of the agar and flood with a saturated solution of aqueous copper sulfate. Let it stand for 15-20 min. Pour the excess off. An intense blue stain under the bacterial growth is taken as lypolisis.

15. Pigment

Scratch on the surface of a Whatman no.1 filter paper growth from strains which were grown for 14 days in daylight at 25°C on the cream agar described above. Examine for pigments in daylight.

Pigmentation was recorded as yellow, orange, magenta, etc.

16. Coagulase test

Inoculate all strains on both 2ml of B.M. broth and 2ml. typticase soy broth (Difco) and incubated for 7-10 days at 25°C.

Perform coagulase test as follows:

Bacto coagulase plasma 0.5ml

Bacterial growth from medium 0.5ml

Incubate the tubes at 37°C for 8 hr. observe for coagulation every 15min. Keep incubated overnight and interpret after 18 hr. Use controls for coagulase positive and negative organisms by using Staphilococcus aureus coagulase positive and Staphilococcus epidermidis coagulase negative.

17. Hemolysis:

Inoculate every strain on blood agar plates using trypticase soy agar as base and 5% defibrinated sheep blood. Incubate for 48 hr. at 25°C. Interpret as hemolysis aclear zone around the colonies. Use a culture of beta hemolytic streptococococcus and a gamma hemolytic streptococcus as positive and negative controls. The BM medium cannot be used because the high content of salt hemolizes the blood.

18. Growth in peptone broth

Prepare 1% peptone broth and dispense 2ml in tubes. Sterilize by autoclaving at 15lb/15min. Inoculate onto the broth and incubate from 7 to 14 days at 25°C.

19. Growth in B.M. broth

Inoculate tubes with B.M. broth and incubate from 7 to 14 days at 25°C.

Prepare the medium as follows:

Peptone (Difco) 2.5g

Yeast extract (Difco)	2.5g
$FePO_4$	0.1g
"Aged" sea water	750 ml
Distilled water	250 ml

Adjust to pH 7.5 after sterilization at 15lb/15min.

SEA WATER
CHEMICAL DETERMINATIONS
MATERIAL, METHODS AND REAGENTS

For water sample collection a Kemmerer-type sampler recommended for samples collected from depths grater than 150 cm. was used . A valve release was used so that only when the sampler reached the bottom was a specimen taken. This sampler permits a threefold displacement of the bottle capacity to assure a proper sample collection. It was made of brass, bronze and nickel, plated, with a cylindrical body and cover joined by an airtight thread. The cottles used were made of glass or polyethylene and were cleaned with acid, rinse with distilled water and dried.

1. Dissolved Oxygen

   Dissolved oxygen was determined by the use of a Hydrolab (R) apparatus made by ARA of Austin , Texas. The equipment was provided with a DO sensor. DO compensator and thermistor. It was calibrated once a week by reading in sea water and comparing with results of DO determinations made by the method recommended by Strickland and Parsons(42)

2. Temperature

   Temperature determinations were simultaneously taken by using the Hydrolab equipment which was provided with a separate temperature sensor. It was calibrated once a week by taking readings in sea water at different temperatures controlled by a certified laboratory thermometer.

Both probes were attached to the Kemmerer-type sampler used for the collection of liquid samples at the bottom so when the water specimen was taken simultaneous measurements of DO and temperature were recorded.

3. Salinity

For salinity determinations the Hytech Model 6220 (R) salinometer made by GM manufactures of La Jolla, Calif. was used. Salinity was obtained from standard tables by calculating from chlorinity values. The tables were obtained with the apparatus.

4. pH :

The pH of each sample was measured by a glass electrode and a Beckman Model G.S. pH meter. It was measured at laboratory temperature and pressure and the pH of the sea water "insitu" was derived by calcutions involving the temperature and pressure of the sample when taken. The pH and the temperature of the solution is recorded if the sample was initially at a temperature of tC°when taken at a depth of "d" meter, its correct pH, in situ, is given by the expression: $pHs = pH_m - a(t-tm)$. The pressure variation is negligible for samples taken above 500m, and is given in a table for temperature correction for pH measurements.

5. Total Alkalinity

The total alkalinity determination was measured by mixing 100 ml of the sea water sample with 25 ml of 0.0100 N hydrochloric acid. The pH of this solution is measured. The standard acid in excess of that required to titrate the sample to the carbon dioxide inflexion point is computed from a knowledge of this pH and an empirical factor. This excess acid is the subtrated from 2.5 milliequivalents per liter (the amount initially added by 25ml. of 0.01 N acid) and the total alkalinity of the sample is thus evaluated.

6. Carbonate Alkalinity

The calculation of the Carbonate alkalinity was made by subtracting A from the total alkalinity where A is given in milliequivalents/ liter. A is found in standard tables (42) when salinity or chlorinity is known.

7. Total CO_2

For total CO_2 content the carbonate alkalinity times F_T in millimoles/ liter will give the total CO_2. The F_T factor is found in standard tables when pHs (d) , temperature and salinity or chlorinity are known.

8. Phosphate

Phosphate determination depend upon the reaction of orthophosphate with an acidified molybdate solution to form a phosphomolybdate hetero-poly acid. It is reduced to phosphomolybdenum blue with stannous chloride. The product obeys Beer's Law under suitable conditions, up to 90ug PO_4 - P/Liter, (Deniges Method). The glassware should be cleaned and reserved only for phosphate determinations. A calibration curve is made by preparing several dilute phosphatessolutions such that each contains .25ug-of PO_4 - P/liter multiplied by the dilution factor.

Filter the sample, using a .5u membrance filter; add 2ml of a mixture of ammonium molybdate-sulfuric acid to a 100ml sample. Mix thoroughly. Record time and temperature. Make a turbidity blank, compare with distilled water at 705 mu. three minutes later add .2ml of a dilute stannous chloride solution while swirling. Measure and record the maximum absorbance. Make a reagent blank with filtered sea water.

Subtract the absorbance due the turbidity and due to the reagent blank. The concentration of the unknown sample maybe obtained by reference to the calibration curve.

The stannous chloride solution is prepared by dissolving 4.3g of $SnCl_2 \cdot 4H_2O$ in 10 ml of concentrated (12M, 37%, SP gr. 1.19) hydrochloric acid and dilute in 100 ml of cold boiled out distilled water. Dilute 5 ml of that solution to 25 ml with 5% (w/v) HCl, prepared with boiled-out distilled water.

9. Nitrate

As for nitrates determination no complete satisfactory method in sea water is presently available. However there is a simple method that is rapid, generally free of interferences, and is free of most of the difficulties inherent in other methods. The method consists in adding an equal volume of sulfuric acid to a solution which contains both nitrate and chloride and measuring the development of strong intensity band at 230 mu. At this wave-length, Beer's law is obyed up to 3mg NO_3^- N/liter.

In each of 150 X 20mm stopered test tubes, pipet 10.0ml of sample to one portion add 0.1ml of hydrazine sulfate solution (dissove 2g of $N_2 H_4 \cdot H_2 SO_4$ in 100 ml of distilled water). Add 10ml of concentrated sulfuric acid to each test tube. (H_2SO_4 reagent grade, 98%, sp. gr 1.84). Cool in running water. Put the contents into 5cm cells; measure the absorbance of each at 230mu. Make a nitrate solution by dissolving 0.101g of KNO_3 in distilled water and dilute to a liter. Dissolve X ml of standard nitrate solution previously diluted 1 to 10.

Each sample will contain X. 14ug N/liter. Plot absorbance against concentration. The concentration of an unknown sample may be determined from the calibration plot.

10- Phenol

Phenol determinations were made by the chloroform extraction method in which the steam-distillable phenols react with 4-aminoantipyrine at a pH of 10.0 ± .2 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from aqueous solution with chloroform and the absorbance is measured at 460 mu. The concentration of phenolic compounds is expresed as ug/l of phenol. This method covers the phenol concentration range of 0.0 to 1,000ug/l with a sensitivity of lug/l. (44).

TABLE No. 1

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
1	4.9	8.20	2.21	34.89	19.79	28	2.10	1.89
1 A	4.8	8.20	1.98	34.36	19.48	29	1.87	1.68
1 B	3.7	8.20	2.09	35.04	19.88	29	1.98	1.78
1 C	4.8	8.30	2.09	34.35	19.48	28	1.96	1.72
2	4.5	8.40	2.09	34.44	19.53	30	1.94	1.63
2 A	4.2	8.30	2.09	35.18	19.96	29	1.96	1.72
2 B	5.0	8.30	2.03	35.18	19.96	29	1.90	1.67
2 C	5.5	8.30	1.98	34.91	19.80	27	1.86	1.64

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 2

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S‰	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
3	3.0	7.78	2.11	34.03	19.29	29.5	2.06	1.98
3 A	3.5	8.05	1.84	34.75	19.71	29.0	1.75	1.59
3 B	3.8	8.10	1.84	34.91	19.80	28.5	1.75	1.59
3 C	4.2	7.99	2.09	———		28.0	2.02	1.88
4	3.2	7.99	2.00	34.73	19.70	28.0	1.93	1.79
4 A	4.8	8.07	2.05	34.76	19.71	28.0	1.96	1.80
4 B	5.0	8.14	1.84	34.68	19.67	28.0	1.75	1.61

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S‰: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 3

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
1	5.5	7.95	2.24	34.90	19.80	29.5	2.18	2.09
1 A	5.3	8.00	2.21	35.24	19.95	29.5	2.14	2.09
1 B	5.8	7.95	2.24	35.34	20.05	29.5	2.18	2.09
1 C	5.8	8.05	2.21	35.34	20.05	29.5	2.14	2.09
2	5.0	7.90	2.27	35.15	19.94	29.0	2.21	2.12
2 A	5.9	8.00	2.20	35.37	20.07	29.0	2.13	2.00
2 B	5.5	8.00	2.21	35.39	20.08	29.0	2.14	2.09

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 4

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
3	4.1	7.70	1.85	35.986	20.43	26	1.81	1.77
3 A	4.7	8.00	1.85	35.883	20.37	26	1.78	1.67
3 B	4.3	7.85	1.98	35.959	20.41	26	1.93	1.86
3 C	5.2	8.05	1.85	35.859	20.35	26	1.78	1.67
4	4.2	7.35	2.27	35.979	20.43	26	2.26	2.32
4 A	4.8	8.00	1.85	35.836	20.34	26	1.78	1.67
4 B	4.9	7.80	1.85	35.987	20.43	26	1.80	1.74
4 C	5.2	8.05	1.85	35.859	20.35	26	1.78	1.67

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 5

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
1	6.5	8.3	2.14	34.61	19.63	31.5	2.02	1.79
1 A	6.3	8.1	2.19	34.27	19.43	29.5	2.11	1.96
1 B	6.0	8.1	2.19	34.49	19.56	29.5	2.11	1.96
1 C	6.0	8.1	2.15	33.56	19.02	29.0	2.08	1.95
2	6.1	8.2	2.25	32.58	18.45	32.0	2.19	2.01
2 A	6.5	8.1	2.19	35.30	20.03	29.0	2.11	1.96
2 B	5.8	8.2	2.17	34.71	19.71	29.5	2.07	1.88

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 6

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alka.	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2
3	5.1	8.15	2.18	35.058	19.89	29.5	1.90	1.76
3 A	6.0	8.15	2.04	35.249	20.00	29.0	1.96	1.82
3 B	5.8	8.12	1.53	35.098	19.91	29.0	1.45	1.34
3 C	5.0	8.12	2.14	34.098	20.14	29.0	2.06	1.91
4	4.8	8.00	1.95	34.088	19.32	30.0	1.88	1.76
4 A	5.8	8.15	2.32	35.400	20.09	28.5	2.24	2.08
4 B	5.5	8.00	2.05	35.539	20.17	29.5	1.98	1.86
4 C	6.2	8.15	1.96	35.015	19.86	29.5	1.90	1.76

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk.: Total Alkalinity.

TABLE No. 7

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr μg/L	Phen mg/L
1	6.3	7.6	2.04	33.85	19.19	29.5	2.01	1.98	Less 0.1	0.30	—
1 A	5.8	7.6	1.98	34.32	19.46	29.0	1.95	1.93	Less 0.1	0.30	—
1 B	6.0	7.6	1.92	34.08	19.32	29.5	1.89	1.87	Less 0.1	0.30	—
4	4.5	7.5	1.84	30.91	17.48	29.0	1.82	1.83	Less 0.1	0.30	—
4 A	4.9	7.5	1.84	31.03	17.55	29.5	1.82	1.83	Less 0.1	Less 0.1	—
4 B	5.2	—	Specimen lost		—	29.0	—	—	Less 0.1	Less 0.1	—
4 C	5.5	7.9	1.92	31.42	17.78	29.5	1.87	1.79	Less 0.1	0.30	—

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

TABLE No. 8

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr $\mu g/L$	Phen mg/L
1	5.0	8.15	1.77	36.259	20.59	28	1.68	154	0.20	0.50	Trace
1 A	4.9	8.10	1.85	36.370	20.65	28	1.76	1.61	0.20	0.50	Trace
1 B	4.9	7.60	1.85	36.274	20.60	28	1.82	1.80	Less 0.1	0.75	——
1 C	5.0	7.85	1.92	36.269	20.59	28	1.87	1.79	Less 0.1	0.75	——
2	4.8	8.05	1.77	36.356	20.65	28	1.70	1.59	Less 0.1	0.50	Trace
2 A	5.0	7.90	1.85	36.404	20.67	28	1.79	1.70	Less 0.1	0.50	Trace
2 B	5.1	8.00	1.85	36.368	20.65	28	1.78	1.65	Less 0.1	0.30	——
2 C	4.2	7.85	1.92	36.269	20.69	28	1.87	1.79	Less 0.1	0.30	——

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

TABLE No. 9

TALLABOA BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr $\mu g/L$	Phen mg/L
3	5.2	8.10	1.76	34.48	19.55	30.0	1.65	1.50	Less 0.1	0.60	—
3 A	5.4	8.20	1.76	34.57	19.60	29.0	1.65	1.47	Less 0.1	0.50	—
3 B	5.9	8.25	1.67	34.32	19.46	29.0	1.54	1.34	Less 0.1	0.50	—
3 C	6.1	8.20	1.76	34.45	19.53	28.5	1.65	1.47	Less 0.1	0.50	—
4	5.0	7.95	2.13	34.42	19.52	29.0	2.05	1.91	0.20	0.75	—
4 A	5.0	8.05	1.98	34.36	19.48	29.0	1.89	1.72	0.20	0.75	—
4 B	5.5	8.15	1.67	34.27	19.43	29.0	1.56	1.39	0.20	0.30	—
4 C	5.4	8.20	1.67	34.53	19.58	29.0	1.56	1.39	Less 0.1	0.30	—

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

Table No. 10 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1

Strain No.	1	2	3	4	5	6	7	8
Morphology	Str Sl	Sl Str	Long Sl	Long Sl	Long Sl	Sl Str	Sl Str	Long Sl
Motility	+	+	+	+	+	+	+	+
Gram Stain	—	—	—	—	—	—	—	—
Colony appearance	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M
Pigment	—	—	—	—	—	—	—	—
Oxidative Glucose	—	—	—	—	—	—	—	—
Ferm. Glucose	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—
King's Fluorescein	—	—	—	—	—	—	—	—
King's Pyocianine	—	—	—	—	—	—	—	—
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+
Paton's Media	—	—	—	—	—	—	—	—
Flagellar Stain	P	P	Per	Per	Per	P	P	Per
Oxidase Kovacs	+	+	—	+	+	+	+	+
Oxidase Paper	+	+	—	+	+	+	+	+
Urea	—	—	—	—	—	—	—	—
Simmon's Citrate	+	+	—	—	—	+	+	—
Growth peptone H_2O	—	—	+	—	—	—	—	—
Growth marine broth	+	+	+	+	+	+	+	+
Growth 25° C	+	+	+	+	+	+	+	+
Growth 37° C	—	—	—	—	—	—	—	—
Growth 42° C	—	—	—	—	—	—	—	—
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S
Sensitivity 0/129	R	R	R	R	R	R	R	R
Tentative i.d.	Ps III	Ps III	Myco	Myco	Myco	Ps III	Ps III	Myco

M.A.: marine agar plus 30% skimmed milk

Str	: straight	R	: resistant
Sl	: slender	Per	: perithricons
Tr	: translucent	S	: sensitive
M	: mucoid	Ps	: pseudomonas
P	: polar	Myco	Mycoplana

Table No. 11 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-1

Strain No.	1		3		5		8									
Morphology	Sh		Sh St		Sh St		Sh St									
Motility	–		+		–		–									
Gram Stain	–		–		–		–									
Colony appearance	Gro		Gro		Gro		Gro									
Pigment	–		–		–		–									
Oxidative Glucose	–		–		–		–									
Ferm. Glucose	Alk		Alk		Alk		Alk									
Lactose	Alk		Alk		Alk		Alk									
Sucrose	Alk		Alk		Alk		Alk									
Mannitol	Alk		Alk		Alk		Alk									
King's Fluorescein	–		–		–		–									
King's Pyocianine	–		–		–		–									
M.A. + 30% S. Milk	–		+		–		–									
Paton's Media	–		–		–		–									
Flagellar Stain	–		P		–		–									
Oxidase Kovacs	+		+		+		+									
Oxidase Paper	+		+		+		+									
Urea	–		–		–		–									
Simmon's Citrate	–		+		–		–									
Growth peptone H_2O	+		–		+		+									
Growth marine broth	+		+		+		+									
Growth 25° C	+		+		+		+									
Growth 37° C	–		+		–		–									
Growth 42° C	–		+		–		–									
Sensitivity Penicillin 2u	S		S		S		S									
Sensitivity Terramycin 10 mcg	S		R		S		S									
Sensitivity 0/129	R		R		R		R									
Tentative i.d.	Alka		Ps III		Alka		Alka									

M.A.: marine agar plus 30% skimmed milk

Sh	:	short	P	:	polar
St	:	stout	S	:	sensitive
Gro	:	graysh, opaque	R	:	resistant
Alk	:	alkaline	Ps	:	pseudomonas
Alka	:	alkaligenes			

Table No. 12 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-A

Strain No.	1	2		4	5	6		8								
Morphology	Sh Coc	Sh Coc		Sh Coc	Long	Sh Coc		Sh St								
Motility	+	+		+	+	+		+								
Gram Stain	−	−		−	−	−		−								
Colony appearance	Gro	Gro		Gro	Ry	Gro		Gro								
Pigment	−	−		−	Y	−		−								
Oxidative Glucose	−	−		+	+	−		−								
Ferm. Glucose	Alk	−		−	Alk	−		−								
Lactose	Alk	−		−	Alk	−		−								
Sucrose	Alk	−		−	Alk	−		−								
Mannitol	Alk	−		−	Alk	−		−								
King's Fluorescein	−	−		+	−	−		−								
King's Pyocianine	−	−		−	−	−		−								
M.A. + 30% S. Milk	+	+		+	+	+		+								
Paton's Media	−	−		+	−	−		−								
Flagellar Stain	P	P		P	P	P		P								
Oxidase Kovacs	+	+		+	+	+		+								
Oxidase Paper	+	+		+	+	+		+								
Urea	−	−		±	+	−		−								
Simmon's Citrate	+	+		+	+	∓		±								
Growth peptone H_2O	−	−		+	+	−		−								
Growth marine broth	+	+		+	+	+		+								
Growth 25° C	+	+		+	+	+		+								
Growth 37° C	−	+		+	−	+		−								
Growth 42° C	−	+		+	−	+		−								
Sensitivity Penicillin 2u	SS	R		R	R	SS		R								
Sensitivity Terramycin 10 mcg	R	R		R	R	R		R								
Sensitivity 0/129	R	R		R	R	R		R								
Tentative i.d.	Ps	Ps		Ps	?	Ps		Ps								

M.A.: marine agar plus 30% skimmed milk

Sh : short
St : stout
Coc : coccoid
Gro : graysh opaque
Ry : rough-yellow
? : xantomonas

Y. : yellow
Alk : alkaline
P : polar
I : weak

R : resistant
S : sensitive
Ps : pseudomonas
Ss : slightly sensitive

Table No. I3 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-A-1

Strain No.		2	3		5	6	7	8								
Morphology		Sl Str	Sh Coc		Sl Str	Sl Str	Sh Coc	Sh Coc								
Motility		+	+		+	+	+	+								
Gram Stain		−	−		−	−	−	−								
Colony appearance		Tr M	Tr M		Tr M	Tr M	Tr M	Tr M								
Pigment		−	−		−	−	−	−								
Oxidative Glucose		−	−		−	−	−	−								
Ferm. Glucose		−	−		−	−	−	−								
Lactose		−	−		−	−	−	−								
Sucrose		−	−		−	−	−	−								
Mannitol		−	−		−	−	−	−								
King's	Fluorescein	−	−		−	−	−	−								
	Pyocianine	−	−		−	−	−	−								
M.A. + 30% S. Milk		+	+		+	+	+	+								
Paton's Media		−	−		−	−	−	−								
Flagellar Stain		P	P		P	P	P	P								
Oxidase	Kovacs	+	+		+	+	+	+								
	Paper	+	+		+	+	+	+								
Urea		−	−		−	−	−	±								
Simmon's Citrate		+	+		+	+	+	+								
Growth peptone H_2O		−	−		−	−	−	−								
Growth marine broth		+	+		+	+	+	+								
Growth	25° C	+	+		+	+	+	+								
	37° C	+	+		+	+	+	+								
	42° C	+	+		+	+	+	+								
Sensitivity	Penicillin 2u	R	R		R	R	R	R								
	Terramycin 10 mcg	R	S		S	S	S	S								
	O/129	R	R		R	R	R	R								
Tentative i.d.		Ps	Ps		Ps	Ps	Ps	Ps								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	P	: polar
Sh	: short	R	: resistant
Coc	: coccoid	S	: sensitive
Tr	: translucent	Ps	: pseudomonas
M	: mucoid		

Table No. 14 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-B

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	Cr m	Cr m	Cr m	Cr m	Cr m	Cr m	Cr m	Cr m								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	−	−	−	−	−	−								
Ferm. Glucose	−	−	−	−	−	−	−	−								
Lactose	−	−	−	−	−	−	−	−								
Sucrose	−	−	−	−	−	−	−	−								
Mannitol	−	−	−	−	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	−	−	−	−	−	−								
Simmon's Citrate	+	+	+	+	+	+	+	+								
Growth peptone H_2O	−	−	−	−	−	−	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R								
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	R I V	R II	Rs I V	Rs I V	R I V	R I V	Rs II	R I V								

M.A.: marine agar plus 30% skimmed milk

Table No. 15 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-B-1

Strain No.			2		4	5	6									
Morphology			Sh St		Sh St	Sh St	Sh Coc									
Motility			+		+	+	+									
Gram Stain			−		−	−	−									
Colony appearance			Tr M		Tr M	Tr M	Tr M									
Pigment			−		−	−	−									
Oxidative Glucose			+		+	+	−									
Ferm. Glucose			−		−	−	Alk									
Lactose			−		−	−	Alk									
Sucrose			−		−	−	Alk									
Mannitol			−		−	−	Alk									
King's	Fluorescein		−		−	−	−									
	Pyocianine		−		−	−	−									
M.A. + 30% S. Milk			+		+	+	+									
Paton's Media			−		−	−	−									
Flagellar Stain			P		P	P	P									
Oxidase	Kovacs		+		+	+	+									
	Paper		+		+	+	+									
Urea			±		±	±	+									
Simmon's Citrate			+		+	+	−									
Growth peptone H_2O			+		+	+	+									
Growth marine broth			+		+	+	+									
Growth	25° C		+		+	+	+									
	37° C		+		+	+	−									
	42° C		+		+	+	−									
Sensitivity	Penicillin 2u		R		R	R	S									
	Terramycin 10 mcg		R		R	R	SS									
	O/129		R		R	R	R									
Tentative i.d.			Ps I		Ps I	Ps I	Ps I									

M.A.: marine agar plus 30% skimmed milk

Sh	: short	Alk	: alkaline	SS	: slightly sensitive
St	: stout	P	: polar	Ps	: pseudomonas
Coc	: coccid	I	: weak	R	: resistant
Tr	: translucent	S	: sensitive		
M	: mucoid				

Table No. 16 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 1-C

Strain No.	1	2	3	4	5	6	7	8
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str
Motility	+	+	+	+	+	+	+	+
Gram Stain	−	−	−	−	−	−	−	−
Colony appearance	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M
Pigment	−	−	−	−	Y	−	−	−
Oxidative Glucose	−	−	−	−	−	−	−	−
Ferm. Glucose	−	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	−
King's Fluorescein	−	−	−	−	−	−	−	−
King's Pyocianine	−	−	−	−	−	−	−	−
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+
Paton's Media	−	−	−	−	−	−	−	−
Flagellar Stain	P	P	P	P	P	P	P	P
Oxidase Kovacs	+	+	+	+	+	+	+	+
Oxidase Paper	+	+	+	+	+	+	+	+
Urea	−	−	−	−	−	−	−	−
Simmon's Citrate	±	±	±	+	−	±	+	±
Growth peptone H_2O	−	−	−	−	−	−	−	−
Growth marine broth	+	+	+	+	+	+	+	+
Growth 25° C	+	+	+	+	+	+	+	+
Growth 37° C	+	+	+	+	+	+	+	+
Growth 42° C	+	+	+	+	+	+	+	+
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S
Sensitivity 0/129	R	R	R	R	R	R	R	R
Tentative i.d.	Ps IV	Ps IV	Ps IV	Ps IV	?	Ps IV	Ps IV	Ps IV

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	P	: polar
Str	: straight	R	: resistant
Tr	: translucent	S	: sensitive
M	: mucoid	±	: weak
Y	: yellow	Ps	: pseudomonas

Table No. 17 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 2

Strain No.	1		3		5		7									
Morphology	Sh St		Sh St		Sl Str		Sl Str									
Motility	+		+		+		+									
Gram Stain	−		−		−		−									
Colony appearance	Gr O		Gr O		Gr O		Gr O									
Pigment	−		−		−		−									
Oxidative Glucose	+		+		+		+									
Ferm. Glucose	−		−		−		−									
Lactose	−		−		−		−									
Sucrose	−	−	−		−		−									
Mannitol	−		−		−		−									
King's Fluorescein	+		+		−		−									
King's Pyocianine	+		+		−		−									
M.A. + 30% S. Milk	+		+		+		+									
Paton's Media	+		+		−		−									
Flagellar Stain	P		P		P		P									
Oxidase Kovacs	+		+		+		+									
Oxidase Paper	+		+		+		+									
Urea	−		−		−		−									
Simmon's Citrate	+		+		±		±									
Growth peptone H_2O	+		+		+		+									
Growth marine broth	+		+		+		+									
Growth 25° C	+		+		+		+									
Growth 37° C	+		+		+		+									
Growth 42° C	+		+		+		+									
Sensitivity Penicillin 2u	R		R		R		R									
Sensitivity Terramycin 10 mcg	R		R		S		S									
Sensitivity 0/129	R		R		R		R									
Tentative i.d.	R_2 I		P I		P II		Ps II									

M.A.: marine agar plus 30% skimmed milk

Sh	:	short	P	:	polar	I	: weak
St	:	stout	S	:	sensitive		
Sl	:	slender	R	:	resistant		
Str	:	straight	Ps	:	pseudomonas		

Table No. 18 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 2-A

Strain No.	1	2		4	5		7									
Morphology	Sh St	Sh St		Sh St	Sh St		Sh St									
Motility	+	+		+	+		+									
Gram Stain	−	−		−	−		−									
Colony appearance	Tr M	Tr M		Tr M	Tr M		Tr M									
Pigment	−	−		−	−		−									
Oxidative Glucose	+	−		−	+		+									
Ferm. Glucose	−	−		−	−		−									
Lactose	−	−		−	−		−									
Sucrose	−	−		−	−		−									
Mannitol	−	−		−	−		−									
King's Fluorescein	−	−		−	+		+									
King's Pyocianine	−	−		−	+		+									
M.A. + 30% S. Milk	+	+		+	+		+									
Paton's Media	−	−		−	+		+									
Flagellar Stain	P	P		P	P		P									
Oxidase Kovacs	+	+		+	+		+									
Oxidase Paper	+	+		+	+		+									
Urea	±	±		−	−		±									
Simmon's Citrate	+	−		−	+		+									
Growth peptone H_2O	+	−		−	+		+									
Growth marine broth	+	+		+	+		+									
Growth 25° C	+	+		+	+		+									
Growth 37° C	+	+		+	+		+									
Growth 42° C	+	+		+	+		+									
Penicillin 2u	R	R		R	R		R									
Sensitivity Terramycin 10 mcg	S	S		S	S		S									
Sensitivity 0/129	R	R		R	R		R									
Tentative i.d.	Ps II	Ps IV		Ps II	Ps I		Ps I									

M.A.: marine agar plus 30% skimmed milk

Sh : short
St : stout
Tr : translucent
M : mucoid
P : polar
R : resistant
S ; sensitive
Ps : pseudomonas

Table No. 19 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 2-B

Strain No.		3			6										
Morphology		Sh Coc			Sh Coc										
Motility		+			+										
Gram Stain		−			−										
Colony appearance		Gr O			Gr O										
Pigment		−			−										
Oxidative Glucose		−			−										
Ferm. Glucose		Alk			Alk										
Lactose		Alk			Alk										
Sucrose		Alk			Alk										
Mannitol		Alk			Alk										
King's	Fluorescein	−			−										
	Pyocianine	−			−										
M.A. + 30% S. Milk		+			+										
Paton's Media		−			−										
Flagellar Stain		P			P										
Oxidase	Kovacs	+			+										
	Paper	+			+										
Urea		−			−										
Simmon's Citrate		+			+										
Growth peptone H_2O		∓			∓										
Growth marine broth		+			+										
Growth	25° C	+			+										
	37° C	−			−										
	42° C	−			−										
Sensitivity	Penicillin 2u	SS			SS										
	Terramycin 10 mcg	R			R										
	0/129	R			R										
Tentative i.d.		Ps III			Ps III										

M.A.: marine agar plus 30% skimmed milk

Sh : short
Coc : coccoid
Gro : graysh opaque
Alk : alkaline
P : polar
∓- : weak
SS : slightly sensitive
Ps : pseodomonas

Table No. 20 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 2-C

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sh Coc.	Sh cox	Sh cox	Se cur	Se cur	Se cur	Sh cox.	Sh cox								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	tr m	tr m	tr m	tr m	tr m	tr m	tr m	tr m								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	−	−	−	−	−	−								
Ferm. Glucose	−	Alk	−	+	+	+	Alk	Alk								
Lactose	−	Alk	−	−	−	−	Alk	Alk								
Sucrose	−	Alk	−	−	−	+	Alk	Alk								
Mannitol	−	Alk	−	−	−	−	Alk	Alk								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	−	−	−	−	−	−								
Simmon's Citrate	+ −	+ −	−	+	+	−	−	−								
Growth peptone H_2O	−	−	−	−	−	−	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R								
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S								
Sensitivity O/129	R	R	R	S	S	S	R	R								
Tentative i.d.	B IV	B III	B IV	Vib	Vib	Vib	B III	B III								

M A.: marine agar plus 30% skimmed milk

Sh : short
Coc : coccoid
Trm : translucent moist
Alk : alkaline
P : polar
R : resistant
S : sensitive
Vib: vibrio
Ps : pseudomonas

Table No. 21 Biochemical and morphological characteristics of Gram Negative bacilli at Tallaboa bay. Station 2-C-1

Strain No.															
Morphology															
Motility															
Gram Stain															
Colony appearance															
Pigment															
Oxidative Glucose															
Ferm. Glucose															
Lactose															
Sucrose															
Mannitol															
King's Fluorescein															
Pyocianine															
M.A. + 30% S. Milk															
Paton's Media															
Flagellar Stain															
Oxidase Kovacs															
Oxidase Paper															
Urea															
Simmon's Citrate															
Growth peptone H_2O															
Growth marine broth															
Growth 25° C															
Growth 37° C															
Growth 42° C															
Sensitivity Penicillin 2u															
Sensitivity Terramycin 10 mcg															
Sensitivity 0/129															
Tentative i.d.															

M.A.: marine agar plus 30% skimmed milk

No gram negative flora present.
All isolates were Gram + bacilli.

Table No. 22 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3

Strain No.	1	2	3	4		6	7	8	9	10	11	12	13	14	15	
Morphology	Coc	Sh Coc	Sh Coc	Sh Coc		Sl Cur	Sl cur	Sh coc	Sh Coc	Sh coc	Sh Coc	Sl Sh	Sl SO	Sl Sl	Sh Coc	
Motility	+	+	+	+		+	+	+	+	+	+	−	+	−	+	
Gram Stain	−	−	−	−		−	−	−	−	−	−	−	−	−	−	
Colony appearance	tr m	tr m	tr m	Gr O		tr m	Gr O	Gr O	tr m	tr m	tr m	tr m	tr m	tr m	tr m	
Pigment	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−
Oxidative Glucose	−	+	+	+		−	−	+	−	−	+	−	−	−	−	
Ferm. Glucose	Alk	−	−	−		Alk	Alk	−	−	−	−	+	+	Alk	+	
Lactose	Alk	−	−	−		Alk	Alk	−	−	−	−	−	+	Alk	−	
Sucrose	Alk	−	−	−		Alk	Alk	−	−	−	−	−	−	Alk	−	
Mannitol	Alk	−	−	−		Alk	Alk	−	−	−	−	−	−	Alk	−	
King's Fluorescein	−	−	−	+		−	−	+	−	−	−	−	−	−	−	
King's Pyocianine	−	−	−	+		−	−	+	−	−	−	−	−	−	−	
M.A. + 30% S. Milk	+	+	+	+		+	+	+	+	+	+	Y	−	Y	−	
Paton's Media	−	−	−	+		−	−	+	−	−	−	−	−	−	−	
Flagellar Stain	P	P	P	P		P	P	P	P	P	P	−	Per	−	P	
Oxidase Kovacs	+	+	+	+		+	+	+	+	+	+	+	−	+	+	
Oxidase Paper	+	+	+	+		+	+	+	+	+	+	+	−	+	+	
Urea	−	−	−	±		−	−	−	+	−	±	+	−	−	−	
Simmon's Citrate	−	+	+	+		−	−	+	+	+	+	−	−	−	+	
Growth peptone H_2O	−	+	+	+		−	−	+	−	−	+	+	+	+	+	
Growth marine broth	+	+	+	+		+	+	+	+	+	+	+	+	+	+	
Growth 25° C	+	+	+	+		+	+	+	+	+	+	+	+	+	+	
Growth 37° C	+	+	+	+		+	+	+	+	+	+	+	+	+	+	
Growth 42° C	+	+	+	+		+	+	+	+	+	+	+	+	+	+	
Penicillin 2u	R	R	R	R		R	R	R	R	R	R	R	R	R	R	
Sensitivity Terramycin 10 mcg	S	S	S	S		R	S	S	S	S	S	S	S	S	S	
Sensitivity 0/129	R	R	R	R		S	S	R	R	R	R	R	R	R	R	
Tentative i.d.	Ps II	Ps II	Ps II	Ps I		Vib	Vib	Ps I	Ps II	Ps II	Ps I	Flu	Par	Fla	?	

M.A.: marine agar plus 30% skimmed milk

Sh	: short	Gr	: grayish	R	: resistant
Coc	: coccoid	O	: opaque	S	: sensitive
Sl	: slender	Alk	: alkaline	Vib	: vibrio
Cur	: curved	Y	: yellowish	Ps	: pseudomonas
Tr	: translucent	P	: polar	Fla	: flavobacterium
M	: mucoid	Per	: perithrichous	Par	: paracolor
?	: unknown				

Table No. 23 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-A

Strain No.	1	2	3	4	5	6	7	8
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str
Motility	—	—	—	—	—	—	—	—
Gram Stain	—	—	—	—	—	—	—	—
Colony appearance	Gr O	Gr O	Gr O	Gr O	Gr O	Gr O	Gr O	Gr O
Pigment	Y	Y	Y	Y	Y	Y	Y	Y
Oxidative Glucose	—	—	—	—	—	—	—	—
Ferm. Glucose	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—
Sucrose	—	—	—	—	—	—	—	—
Mannitol	—	—	—	—	—	—	—	—
King's Fluorescein	—	—	—	—	—	—	—	—
Pyocianine	—	—	—	—	—	—	—	—
M.A. + 30% S. Milk	Y	Y	Y	Y	Y	Y	Y	Y
Paton's Media	—	—	—	—	—	—	—	—
Flagellar Stain	—	—	—	—	—	—	—	—
Oxidase Kovacs	+	+	+	+	+	+	+	+
Oxidase Paper	+	+	+	+	+	+	+	+
Urea	—	—	—	—	—	—	—	—
Simmon's Citrate	—	—	—	—	—	—	—	—
Growth peptone H_2O	+	+	+	+	+	+	+	+
Growth marine broth	+	+	+	+	+	+	+	+
Growth 25° C	+	+	+	+	+	+	+	+
Growth 37° C	—	—	—	—	—	—	—	—
Growth 42° C	—	—	—	—	—	—	—	—
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S
Sensitivity O/129	R	R	R	R	R	R	R	R
Tentative i.d.	Fla	Fla	Fla	Fla	Fla	Fla	Fla	Fla

. marine agar plus 30% skimmed milk

Sl : slender
Str : straight
Gr : grayish
O : opaque
Y : yellowish
Fla : flavobacterium
R : resistant
S : sensitive
+ : positive

Table No. 24 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-B-1

Strain No.	1	2	3	4		6	7	8	9	10	11	12				
Morphology	Sl Cur	Sh St	Sh St	Sh St		Sh Coc	Sh St	Sh Coc	Sl Cur	Sl Phl	Sl cur	St cur				
Motility	+	−	−	−		+	−	+	+	−	+	+				
Gram Stain	−	−	−	−		−	−	−	−	−	−	−				
Colony appearance	Tr M	Tr M	Tr M	Tr M		Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M				
Pigment	−	−	−	−		−	−	−	−	−	−	−				
Oxidative Glucose	−	−	−	−		+	−	−	−	−	−	−				
Ferm. Glucose	+	Alk	Alk	Alk		−	Alk	−	+	+	⊕g	⊕g				
Lactose	−	Alk	Alk	Alk		−	Alk	−	−	−	⊕g	⊕g				
Sucrose	−	Alk	Alk	Alk		−	Alk	−	−	−	−	−				
Mannitol	−	Alk	Alk	Alk		=	Alk	−	−	−	⊕g	⊕g				
King's Fluorescein	−	−	−	−		+	−	−	−	−	−	−				
King's Pyocianine	−	−	−	−		+	−	−	−	−	−	−				
M.A. + 30% S. Milk	+	+	+	+		+	+	+	+	+Y	−	−				
Paton's Media	−	−	−	−		+	−	−	−	−	−	−				
Flagellar Stain	P	−	−	−		P	−	P	P	P	Per	Per				
Oxidase Kovacs	+	+	+	+		+	+	+	+	+	−	−				
Oxidase Paper	+	+	+	+		+	+	+	+	+	−	−				
Urea	−	−	−	−		−	−	−	−	−	−	−				
Simmon's Citrate	−	−	−	−		+	−	−	−	−	+	+				
Growth peptone H_2O	−	+	+	+		+	+	−	−	+	+	+				
Growth marine broth	+	+	+	+		+	+	+	+	+	+	+				
Growth 25°C	+	+	+	+		+	+	+	+	+	+	+				
Growth 37°C	+	+	+	+		+	+	+	+	+	+	+				
Growth 42°C	+	+	+	+		+	+	+	+	+	+	+				
Sensitivity Penicillin 2u	R	R	R	R		R	R	R	R	R	R	R				
Sensitivity Terramycin 10 mcg	S	S	S	S		S	S	S	S	R	R	R				
Sensitivity O/129	S	R	R	R		R	R	R	S	R	R	R				
Tentative i.d.	Vib	Alka	Alka	Alka		BT	Alka	Vib	Vib	Flav	Para	Para				

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	Tr	: translucent	S	: sensitive
Cur	: curved	M	: mucoid	Fla	: flavobacterium
Sh	: short	Alk	: alkaline	Para	: paracolon
St	: stout	+	: positive with gas	Ps	: pseudomonas
Y	: yellowish	P	: polar	Alka	: alkaligenes
P	: perithrichous	R	: resistant		

Table No. 25 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-B-2

Strain No.	1	2	3C	4	5	6	7	8	9	10	11	12	13	14	15	16
Morphology	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur	Sl Cur
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram Stain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Colony appearance	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Oxidative Glucose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ferm. Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
King's Fluorescein	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pyocianine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Paton's Media	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Flagellar Stain	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Oxidase Kovacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase Paper	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Simmon's Citrate	−	−	−	±	−	−	−	−	−	−	±	−	−	−	−	−
Growth peptone H_2O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth marine broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth 25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth 42°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sensitivity 0/129	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Tentative i.d.	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib	Vib

M.A. marine agar plus 30% skimmed milk

Sl : slender
Cur : curved
Mo : moist, opaque
P : polar
R : resistant
S : sensitive
Vib: vibrio
± : weak

Table No. 26 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-B-3

Strain No.	1		3	4	5	6	7	8	9	10	11	12	13	14		
Morphology	Sh coc.		Sl Fil	Sl Cur	Sl Cur	Sh coc	Sh coc	Sh coc	Sh St	Sh St	Sh St	Sh St	Sh St	Sh St		
Motility	+		−	+	+	+	+	+	+	+	+	+	+	+		
Gram Stain	−		−	−	−	−	−	−	−	−	−	−	−	−		
Colony appearance	Tr m		Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m		
Pigment	−		−	−	−	−	−	−	−	−	−	−	−	−		
Oxidative Glucose	−		−	−	−	−	−	+	−	−	−	−	+	−		
Ferm. Glucose	Alk		Alk	Alk	+	Alk	+	−	+	+	+	+	−	Alk		
Lactose	Alk		Alk	Alk	Alk	Alk	−	−	−	−	−	−	−	Alk		
Sucrose	Alk		Alk	Alk	Alk	Alk	−	−	−	−	−	−	−	Alk		
Mannitol	Alk		Alk	Alk	Alk	Alk	−	−	−	−	−	−	−	Alk		
King's Fluorescein	−		−	−	−	−	−	+	−	−	−	−	−	−		
King's Pyocianine	−		−	−	−	−	−	+	−	−	−	−	−	−		
M.A. + 30% S. Milk	+		−	+	+	+	+	+	−	−	−	−	+	+		
Paton's Media	−		−	−	−	−	−	+	−	−	−	−	−	−		
Flagellar Stain	P		−	P	P	P	P	P	Per	Per	Per	Per	P	P		
Oxidase Kovacs	+		+	+	+	+	+	+	+	+	+	+	+	+		
Oxidase Paper	+		+	+	+	+	+	+	+	+	+	+	+	+		
Urea	−		−	+	−	−	−	−	−	−	−	−	−	−		
Simmon's Citrate	+		−	−	−	+	+	+	+	+	+	+	+	+		
Growth peptone H_2O	+		+	−	−	−	−	+	+	+	+	+	+	+		
Growth marine broth	+		+	+	+	+	+	+	+	+	+	+	+	+		
Growth 25° C	+		+	+	+	+	+	+	+	+	+	+	+	+		
Growth 37° C	+		+	+	+	+	+	+	+	+	+	+	+	+		
Growth 42° C	+		+	+	+	+	+	+	+	+	+	+	+	+		
Sensitivity Penicillin 2u	R		R	R	R	R	R	R	R	R	R	R	R	R		
Sensitivity Terramycin 10 mcg	S		S	S	S	S	S	R	R	R	R	R	R	R		
Sensitivity 0/129	R		R	R	R	R	R	R	R	R	R	R	R	R		
Tentative i.d.	R Vib		Ps	Vib	Vib	R Vib	Ae	R Ps	Ae	Ae	Ae	Ae	R Vib	R Vib		

M.A.: marine agar plus 30% skimmed milk

Sh	: short	Tr m	: translucent moist
Coc	: coccoid	Alk	: alkaline
Sl	: slender	P	: polar
Fil	: philamentous	Per	: perithrichous
Cur	: curved	R	: resistant
St	: stout	S	: sensitive
Ps	: pseudomonas	Ae	: aeromonas
Vib	: vibrio		

Table No. 27 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-C-1

Strain No.	1	2	3	4		6	7	8	9	10	11	12	13	14		
Morphology	Sh Coc	Sl St	Sl Sl	Long Cur		long Cur	Sl Str	Sl Sl	long Cur	long Cur	long Cur	long Cur	long Cur	long Cur		
Motility	+	+	+	+		+	+	+	+	+	+	+	+	+		
Gram Stain	−	−	−	−		−	−	−	−	−	−	−	−	−		
Colony appearance	Tr m	Tr m	Tr m	Tr m		Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m	Tr m		
Pigment	−	−	−	Py		Py	−	−	−	−	−	−	−	−		
Oxidative Glucose	+	−	−	−		−	−	+	−	−	−	−	−	−		
Ferm. Glucose	−	Alk	Alk	+		+	Alk	−	+	+	+	+	+	+		
Lactose	−	Alk	Alk	−		−	Alk	−	−	−	−	−	−	−		
Sucrose	−	Alk	Alk	−		−	Alk	−	−	−	−	−	−	−		
Mannitol	−	Alk	Alk	−		−	Alk	−	−	−	−	−	−	−		
King's Fluorescein	+	−	−	−		−	−	+	−	−	−	−	−	−		
King's Pyocianine	+	−	−	−		−	−	+	−	−	−	−	−	−		
M.A. + 30% S. Milk	+	+	+	+		+	+	+	+	+	+	+	+	+		
Paton's Media	+	−	−	−		−	−	+	−	−	−	−	−	−		
Flagellar Stain	P	P	P	P		P	P	P	P	P	P	P	P	P		
Oxidase Kovacs	+	+	+	+		+	+	+	+	+	+	+	+	+		
Oxidase Paper	+	+	+	+		+	+	+	+	+	+	+	+	+		
Urea	−	−	−	−		−	−	−	−	−	−	−	−	−		
Simmon's Citrate	+	+	+	−		+	+	+	+	−	−	−	+	+		
Growth peptone H_2O	+	−	−	−		−	−	+	−	−	−	−	−	−		
Growth marine broth	+	+	+	+		+	+	+	+	+	+	+	+	+		
Growth 25° C	+	+	+	+		+	+	+	+	+	+	+	+	+		
Growth 37° C	+	+	+	+		+	+	+	+	+	+	+	+	+		
Growth 42° C	+	+	+	+		+	+	+	+	+	+	+	+	+		
Sensitivity Penicillin 2u	R	R	R	R		R	R	R	R	R	R	R	R	R		
Sensitivity Terramycin 10 mcg	S	S	S	R		R	S	S	S	R	R	R	R	R		
Sensitivity 0/129	R	R	R	S		S	R	R	S	S	S	S	S	S		
Tentative i.d.	Vib	Vib	Vib	Vib		Vib	Vib	Ps	Vib	Vib	Vib	Vib	Vib	Vib		

M.A.: marine agar plus 30% skimmed milk

Sh	: short	Coc	: coccoid
Sl	: slender	Str	: straight
Cur	: curved	Tr m	: translucent, moist
Py	: yellowish	Alk	: alkaline
P	: polar	R	: resistant
S	: sensitive	Ps	: pseudomonas
Vib	: vibrio		

Table No. 28 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 3-C-2

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Morphology	Coc	Sl Str	Sl Str	Coc	Coc	Coc	Coc	Coc	Coc	Sl Str	Fil Bro	Long Fil	Long Fil	Long Fil	Long Fil	
Motility	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	
Gram Stain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Colony appearance	Brown-ish Opa	Tr M	Tr M	Tr M	Tr M	Brown-ish Opa	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	
Pigment	Brown	−	−	−	−	Brown	−	−	−	−	−	−	−	−	−	
Oxidative Glucose	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Ferm. Glucose	−	⊕	⊕	−	Alk	−	Alk	Alk	⊕	⊕	−	⊕	⊕	+	+	
Lactose	−	−	−	−	Alk	−	Alk	Alk	−	−	−	−	−	−	−	
Sucrose	−	−	+	−	Alk	−	Alk	Alk	−	+	+	−	−	+	+	
Mannitol	−	−	−	−	Alk	−	Alk	Alk	−	−	−	−	−	−	−	
King's Fluorescein	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
King's Pyocianine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
M.A. + 30% S. Milk	+	+	Y	+	Y	+	+	+	+	Y	Y	+	+	Y	Y	
Paton's Media	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Flagellar Stain	P	P	P	P	−	P	P	−	P	P	P	P	P	P	P	
Oxidase Kovacs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Oxidase Paper	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urea	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
Simmon's Citrate	+	+	+	+	−	+	+	−	±	±	±	±	±	±	±	
Growth peptone H_2O	+	+	+	−	+	−	−	+	−	−	+	−	−	+	+	
Growth marine broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth 25° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth 37° C	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	
Growth 42° C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Sensitivity O/129	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Tentative i.d.	Sl Bro	Ae	Ae	Fla	Alk	Fla	Fla	Alk	Ae	Ae	Fla	Ae	Ae	Fla	Fla	

M.A.: marine agar plus 30% skimmed milk

Coc	: coccoid	Tr	: translucent	R	: resistant
Sl	: slender	M	: moist	S	: sensitive
Str	: straight	+		Ae	: aeromonas
Fil	: philamentous	Alk	: alkaline	Fla	: flavobacteri[illegible]a
Bro	: brownich	Y	: yellowish	Ps	: pseudomonas
Opa	: opaque	P	: polar	Alk	: alkaligenes

Table No. 30 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 4-A

Strain No.	1	2	3	4	5	6									
Morphology	L fil	L fil	L fil	L fil	L fil	L fil									
Motility	+	+	+	+	+	+									
Gram Stain	—	—	—	—	—	—									
Colony appearance	Sm Y	Sm Y	Sm Y	Sm Y	Sm Y	Sm Y									
Pigment	Y	Y	Y	Y	Y	Y									
Oxidative Glucose	—	—	—	—	—	—									
Ferm. Glucose	+	—	—	+	—	+									
Lactose	—	—	—	—	—	—									
Sucrose	+	—	—	+	—	+									
Mannitol	+	—	—	—	—	—									
King's Fluorescein	—	—	—	—	—	—									
Pyocianine	—	—	—	—	—	—									
M.A. + 30% S. Milk	P+	P+	P+	P+	P+	P+									
Paton's Media	—	—	—	—	—	—									
Flagellar Stain	P	P	P	P	P	P									
Oxidase Kovacs	+	+	+	+	+	+									
Oxidase Paper	+	+	+	+	+	+									
Urea	—	—	—	—	—	—									
Simmon's Citrate	±	—	—	+	+	—									
Growth peptone H_2O	+	+	+	+	+	+									
Growth marine broth	+	+	+	+	+	+									
Growth 25°C	+	+	+	+	+	+									
Growth 37°C	+	+	+	+	+	+									
Growth 42°C	+	+	+	+	+	+									
Sensitivity Penicillin 2u	R	R	R	R	R	R									
Sensitivity Terramycin 10 mcg	R	R	R	R	R	R									
Sensitivity 0/129	R	R	R	R	R	R									
Tentative i.d.	Fla	Fla	Fla	Fla	Fla	Fla									

M.A.: marine agar plus 30% skimmed milk

L : long
fil : filamentous
Sm : smooth
M : mucoid
Y : yellow

P+ : hydrolized with pigment production
± : late positive

Fla : flarobacterium
R : resistant

Table No. 31 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 4-B

Strain No.	1	2	3	4												
Morphology	Sl Cu	Sl Cu	Sl Cu	Sl Cu												
Motility	+	+	+	+												
Gram Stain	−	−	−	−												
Colony appearance	Tr Co	Tr Co	Tr Co	Tr Co												
Pigment	−	−	−	−												
Oxidative Glucose	−	−	−	−												
Ferm. Glucose	+	+	+	+												
Lactose	−	−	−	−												
Sucrose	−	−	−	−												
Mannitol	−	−	−	−												
King's Fluorescein	−	−	−	−												
King's Pyocianine	−	−	−	−												
M.A. + 30% S. Milk	−	−	−	−												
Paton's Media	−	−	−	−												
Flagellar Stain	P	P	P	P												
Oxidase Kovacs	+	+	+	+												
Oxidase Paper	+	+	+	+												
Urea	−	−	−	−												
Simmon's Citrate	−	−	±	−												
Growth peptone H_2O	−	−	−	−												
Growth marine broth	+	+	+	+												
Growth 25° C	+	+	+	+												
Growth 37° C	+	+	+	+												
Growth 42° C	+	+	+	+												
Sensitivity Penicillin 2u	R	R	R	R												
Sensitivity Terramycin 10 mcg	S	S	S	S												
Sensitivity 0/129	S	S	S	S												
Tentative i.d.	Vib	Vib	Vib	Vib												

M.A.: marine agar plus 30% skimmed milk

Sl : slender
Cu : curved
Tr : translucent
Co : colorless
S : sensitive
Vib : vibrio
+ : late positive
R : resistant

Table No. 32 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 4-C

Strain No.	1	2	3		5	6	7	8	9	10	11	12	13			
Morphology	Sh St	Sh St	Sh St		Sh St	Sh St	Sh St	Sh St	Sh St	Sh St	Sh St	Sh St	Long Cur			
Motility	+	+	+		+	+	+	+	+	+	+	+	+			
Gram Stain	—	—	—		—	—	—	—	—	—	—	—	—			
Colony appearance	Tr M	Tr M	Tr M		Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M			
Pigment	—	—	—		—	—	—	—	—	—	—	—	—			
Oxidative Glucose	—	—	—		—	—	—	—	—	—	—	—	—			
Ferm. Glucose	Alk	Alk	Alk		Alk	Alk	Alk	Alk	⊕	Alk	Alk	Alk	⊕			
Lactose	Alk	Alk	Alk		Alk	Alk	Alk	Alk	—	Alk	Alk	Alk	—			
Sucrose	Alk	Alk	Alk		Alk	Alk	Alk	Alk	—	Alk	Alk	Alk	+			
Mannitol	Alk	Alk	Alk		Alk	Alk	Alk	Alk	—	Alk	Alk	Alk	+			
King's Fluorescein	—	—	—		—	—	—	—	—	—	—	—	—			
King's Pyocianine	—	—	—		—	—	—	—	—	—	—	—	—			
M.A. + 30% S. Milk	+	+	+		+	+	+	+	+	+	+	+	+			
Paton's Media	—	—	—		—	—	—	—	—	—	—	—	—			
Flagellar Stain	P	P	P		P	P	P	P	P	?	P	P	P			
Oxidase Kovacs	+	+	+		+	+	+	+	+	+	+	+	+			
Oxidase Paper	+	+	+		+	+	+	+	—	+	+	+	+			
Urea	—	—	+		±	±	+	—	—	—	—	—	—			
Simmon's Citrate	+	+	±		+	+	±	±	—	+	+	+	+			
Growth peptone H_2O	—	—	—		—	—	—	—	—	—	—	—	—			
Growth marine broth	+	+	+		+	+	+	+	+	+	+	+	+			
Growth 25° C	+	+	+		+	+	+	+	+	+	+	+	+			
Growth 37° C	+	+	+		+	+	+	+	+	+	+	+	+			
Growth 42° C	+	+	+		+	+	+	+	+	+	+	+	+			
Penicillin 2u	R	R	R		R	R	R	R	R	R	R	R	R			
Sensitivity Terramycin 10 mcg	S	S	S		S	S	S	S	S	S	S	S	S			
Sensitivity 0/129	R	R	R		R	R	R	R	R	R	R	R	S			
Tentative i.d.	Ps	Ps	Ps		Ps	Ps	Ps	Ps	Ae	Ps	Ps	Ps	Vib			

M.A.: marine agar plus 30% skimmed milk

Sh	: short	+	: positive with gas	Ps	: pseudomonas
St	: stout	P	: polar	Ae	: aerobacter
Cur	. curved	±	: weak	Vib	: vibri
Tr	: translucent	R	: resistant		
M	: moist	S	: sensitive		
Alk	: alkaline				

Table No. 33 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa Bay. Station No. I

Strain No.	1	2	3		5	6		8	9	10	11				15	16		18	19	20				24	25
Gram Stain	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Oxidase	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Catalase	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Simple Nitrogen Media	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Mobility & Morph	Bct	Bct	Bct		Bct	Bct		Bct	Bct	Bct	Bct				Bct	Bct		Bct	Bct	Bct				Bct	Bct
Ox. Ferm. Glucose	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Glucose aerobic	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Glucose anaerobic	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Mannitol	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Maltose	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Arabinose	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Galactose	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Xylase	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Growth BM broth	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Growth peptone broth	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Nitrate reduction	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Litmus milk	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Gelatin hydrolisis	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Starch hydrolisis	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Pigmentation	Or	Or	Or		Or	Or		Or	Or	Or	Or				Or	Or		Or	Or	Or				Or	Or
Action in blood	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
lipolysis	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Coagulase	—	—	—		—	—		—	—	—	—				—	—		—	—	—				—	—
Casein hydrolisis	+	+	+		+	+		+	+	+	+				+	+		+	+	+				+	+
Tentative i. d.	Mic	Mic	Mic		Mic	Mic		Mic	Mic	Mic	Mic				Mic	Mic		Mic	Mic	Mic				Mic	Mic

Ox: oxidative

Ferm: fermentative

— : negative

Or: orange

Bct: big coccoid tetrads

+ : positive

Table No. 33 - Cont.

Atrain No.	27	29	30	31	32	33	35	36
Gram Stain	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Simple Nitrogen Media	−	−	−	−	−	−	−	−
Mobility & Morph	Bct	Bct	Bct	Bct	Bct	Bct	Bct	Bct
Ox. Ferm. Glucose	−	−	−	−	−	−	−	−
Glucose aerobia	−	−	−	−	−	−	−	−
Glucose anaerobia	−	−	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	−
Maltose	−	−	−	−	−	−	−	−
Arabinase	−	−	−	−	−	−	−	−
Galactose	−	−	−	−	−	−	−	−
Xylase	−	−	−	−	−	−	−	−
Growth BM broth	+	+	+	+	+	+	+	+
Grwth peptone broth	+	+	+	+	+	+	+	+
Nitrate reduction	−	−	−	−	−	−	−	−
Litmus milk	−	−	−	−	−	−	−	−
Gelatin hydrolisis	−	−	−	−	−	−	−	−
Starch hydrolisis	−	−	−	−	−	−	−	−
Pigmentation	Or	Or	Or	Or	Or	Or	Or	Or
Action in blood	−	−	−	−	−	−	−	−
Lipolysis	−	−	−	−	−	−	−	−
Coagulase	−	−	−	−	−	−	−	−
Casein hydrolisis	+	+	+	+	+	+	+	+
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic

Ox: oxidative

Ferm: fermentative

\+ : positive

Bct: big coccoid tetrade

Or: orange

− : negative

Table No. 29 Biochemical and morphological characteristics of Gram negative bacilli at Tallaboa bay. Station 4

Strain No.	1	2	3	4	5	6	7	8	9						
Morphology	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc	Sh Coc						
Motility	+	+	+	+	+	+	+	+	+						
Gram Stain	−	−	−	−	−	−	−	−	−						
Colony appearance	Tr M	Br M	Tr M	Gr O	Gr O	Br O	Br O	Br O	Br O						
Pigment	−	Br	−	−	−	Br	Br	Br	Br						
Oxidative Glucose	−	−	−	−	−	−	−	−	−						
Ferm. Glucose	Alk	+	+	Alk	Alk	Alk	Alk	Alk	Alk						
Lactose	Alk	−	−	Alk	Alk	Alk	Alk	Alk	Alk						
Sucrose	Alk	+	−	Alk	Alk	Alk	Alk	Alk	Alk						
Mannitol	Alk	−	−	Alk	Alk	Alk	Alk	Alk	Alk						
King's Fluorescein	−	−	−	−	−	−	−	−	−						
Pyocianine	−	−	−	−	−	−	−	−	−						
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+	+						
Paton's Media	−	−	−	−	−	−	−	−	−						
Flagellar Stain	P	P	P	P	P	P	P	P	P						
Oxidase Kovacs	+	+	+	+	+	+	+	+	+						
Oxidase Paper	+	+	+	+	+	+	+	+	+						
Urea	−	+	+	−	±	−	−	±	−						
Simmon's Citrate	+	+	+	+	±	±	±	±	±						
Growth peptone H_2O	−	−	−	−	−	−	−	−	−						
Growth marine broth	+	+	+	+	+	+	+	+	+						
Growth 25° C	+	+	+	+	+	+	+	+	+						
Growth 37° C	+	+	+	+	+	+	+	+	+						
Growth 42° C	+	+	+	+	+	+	+	+	+						
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R	R						
Sensitivity Terramycin 10 mcg	S	S	S	S	S	S	S	S	S						
Sensitivity O/129	R	R	R	R	R	R	R	R	R						
Tentative i.d.	Ae	Ae	Ae	Ps III	Ps III	Ps III	Ps III	Ps III	Ps III						

M.A.: marine agar plus 30% skimmed milk

Sh	: short	Gr O	: grayish	R	: resistant
Coc	: coccoid	Alk	: alkaline	S	: sensitive
Tr M	: translucent moist	+	: positive with gas	Ae	: aeromonas
Br M	: brownish moist	P	: polar	Ps	: pseudomonas
+	: weak				
-					

Table No. 34 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa Bay. Sation I-A

Strain No.	1	2	3	4	5	6	7	8	9					14	15	16	17	18	19						
Gram Stain	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+						
Oxidase	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+						
Catalase	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+						
Simple Nitrogen Media	−	−	−	−	+	−	−	−	−					−	−	−	−	−	+						
Mobility & Morph.	$\overline{Sh.ch}$	$\overline{St}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$	$\overline{St}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$	$\overline{St}$					$\overline{St}$	$\overline{St}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$	$\overline{Sh.ch}$						
Ox. Ferm Glucose	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Glucose aerobic	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Glucose anaerobic	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Mannitol	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Maltose	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Arabinase	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Galactose	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Xylase	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Growth BM broth	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+						
Grwth peptone broth	−	+	−	−	−	+	−	−	−					+	+	−	+	+	−						
Nitrate reduction	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Litmus milk	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Gelatin hydrolisis	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Starch hydrolisis	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Pigmentation	Y	Or	Y	Y	Or	Y	Y	Y	Y					Or	Or	Y	Or	Or	Y						
Action in blood	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Lipolysis	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Coagulase	−	−	−	−	−	−	−	−	−					−	−	−	−	−	−						
Casein hydrolisis	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+						
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic					Mic	Mic	Mic	Mic	Mic	Mic						

Ox: oxidative

Ferm: fermentative

Or: orange

− : negative

Sh.ch.: short and long chains

Y: yellowish

St: small tetrads

+ : positive

Table No. 35 Biochemical and Morphological characteristics of Gram positive micrococci at Tallabos Bay. Station 1-B

Strain No.	1	2	3	4	5	6	7		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Gram Stain	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Oxidase	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Catalase	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Simple Nitrogen Media	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mobility & Morph.	Sb	Sb	Sb	Sb	Sb	Sb	Sb		Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	
Ox. Ferm. Glucose	F	F	F	F	F	F	F		F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	
Glucose aerobic	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Glucose anaerobic	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mannitol	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Maltose	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Arabinase	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Galactose	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Xylase	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Growth BM broth	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth peptone broth	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Nitrate reduction	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Litmus milk	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Gelatin hydrolisis	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Starch hydrolisis	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Pigmentation	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	
Action in blood	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Lipolysis	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Coagulase	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Casein hydrolisis	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic		Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	

Ox: oxidative

Ferm: fermentative

F: fermentative

-: negative

Sb: spherical bunches

Y: yellowish

+: positive

Table No. 36 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa bay. Station No. 2

Strain No.	1	2	3	4	5	6	7	8	9	10			13	14	15				19	20	21	22	23	24	25
Gram Stain	+	+	+	+	+	+	+	+	+	+			+	+	+				+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+			+	+	+				+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+			+	+	+				+	+	+	+	+	+	+
Simple Nitrogen Media	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Mobility & Morph.	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb	Sb			Sb	Sb	Sb				Sb	Sb	Sb	Sb	Sb	Sb	Sb
Ox. Ferm. Glucose	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	F	−	−	−	−
Glucose aerobic	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	+	−	−	−	−
Glucose anaerobic	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	+	−	−	−	−
Maltose	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	+	−	−	−	−
Arabinase	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	±	−	−	−	−
Galactose	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	±	−	−	−	−
Xylase	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	±	−	−	−	−
Growth BM broth	+	+	+	+	+	+	+	+	+	+			+	+	+				+	+	+	+	+	+	+
Grwth peptone broth	+	−	−	−	−	−	−	−	−	+			−	−	−				−	−	±	−	−	−	−
Nitrate reduction	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Litmus milk	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Gelatin hydrolisis	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Starch hydrolisis	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Pigmentation	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y			Y	Y	Y				Y	Y	Y	Y	Y	Y	Y
Action in blood	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Lipolysis	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Coagulase	−	−	−	−	−	−	−	−	−	−			−	−	−				−	−	−	−	−	−	−
Casein hydrolisis	+	+	+	+	+	+	+	+	+	+			+	+	+				+	+	+	+	+	+	+
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic			Mic	Mic	Mic				Mic	Mic	Mic	Mic	Mic	Mic	Mic

Ox : oxidative

Ferm : fermentative

Y : yellowish

Sb: small bunches

F: fermentative

Sh. ch.: short chains

Table No. 37. Biochemical and morphological characteristics of Gram positive cocci at Tallaboa Bay. Station No. 2-A

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Simple nitrogen Media	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Mobility & Morph.	−	−	B	B	T	T	B	−	B	B	−	−	−	−	−	−	−	B	B	B	B	B	B		
Ox. Ferm. Glucose	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		
Glucose aerobic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Glucose anaerobic	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Arabinase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Galactose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Xylose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Growth BM broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Growth peptone brouth	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Nitrate reduction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Litmus milk	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Gelatin hydrolisis	+	+	+	−	+	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−		
Starch hydrolisis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Pigmentation	Y	Y	Y	Or	Y	Or	Or	Or	Y	Y	Y	Y	Y	Y	Y	Y	Or	Or	Or	Or	Or	Or	Or		
Action in blood	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Lipolysis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Coagulase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		
Casein hydrolisis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic		

Ox: oxidative

Ferm: fermentative

Sh. ch.: short chains

B: bunches

Y: yellowish

Or: orange

T: tetrads

F: fermentative

Table No. 38 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa bay. Station No. 2 B

Strain No.	5	6	8	10
Gram Stain	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Simple Nitrogen Media	−	−	−	−
Mobility & Morph.	Cu	bu	bu	bu
Ox. Ferm. Glucose	−	F	F	F
Glucose aerobic	−	F	F	F
Glucose anaerobic	−	−	−	−
Mannitol	−	F	F	F
Maltose	−	F	F	F
Arabinase	−	−	−	−
Galactose	−	−	−	−
Xylase	−	−	−	−
Growth BM broth	+	+	+	+
Growth peptone broth	+	+	+	+
Nitrate reduction	−	−	−	−
Litmus milk	−	−	−	−
Gelatin hydrolisis	−	−	−	−
Starch hydrolisis	−	−	−	−
Pigmentation	Y	Or	Or	Or
Action in blood	−	−	−	−
Lipolysis	−	−	−	−
Coagulase	−	−	−	−
Casein hydrolisis	+	+	+	+
Tentative i. d.	Mic	Mic	Mic	Mic

Ox : oxidative

Ferm: fermentative

F : fermentative

Y : yelowish

Or: orange

bu: bunches

Table No. 39 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa bay. Station No. 3

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Simple Nitrogen Media	–	–	–	–	–	–	–	–	–	–	–	–
Mobility & Morph.	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –	Bct –
Ox. Ferm. Glucose	–	–	–	–	–	–	F	F	F	F	F	F
Glucose aerobic	–	–	–	–	–	–	+	+	+	+	+	+
Glucose anaerobic	–	–	–	–	–	–	–	–	–	–	–	–
Mannitol	–	–	–	–	–	–	+	+	+	+	+	+
Maltose	–	–	–	–	–	–	+	+	+	+	+	+
Arabinose	–	–	–	–	–	–	+	+	+	+	+	+
Galactose	–	–	–	–	–	–	–	–	–	–	–	–
Xylase	–	–	–	–	–	–	–	–	–	–	–	–
Growth BM broth	+	+	+	+	+	+	+	+	+	+	+	+
Growth peptone broth	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	–	–	–	–	–	–	–	–	–	–	–	–
Litmus milk	–	–	–	–	–	–	–	–	–	–	–	–
Gelatin Hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–
Starch Hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–
Pigmentation	Or	Or	Or	Or	Or	Or	Or	Or	Or	Or	Or	Or
Action in blood	–	–	–	–	–	–	–	–	–	–	–	–
Lipolysis	–	–	–	–	–	–	–	–	–	–	–	–
Coagulase	–	–	–	–	–	–	–	–	–	–	–	–
Casein hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic

Ox : oxidative

Ferm: fermentative

Bct: big coccoid tetrads

F : fermentative

Or : orange

Table No. 40 - Biochemical and morphological charateristics of Gram positive micrococci at Tallaboa bay. Station No. 3 A

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simple Nitrogen Media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mobility & Morph	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.	S.b.
Ox. Ferm. Glucase	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Glucase aerobic	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Glucase anaerobic	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mannitol	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Galactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Xylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth BM broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth peptone broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Litmus milk	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gelatin hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Starch hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pigmentation	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Action in blood	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lipolysis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Coagulase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Casein hydrolisis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tentative i. d.	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic	Mic

Ox : oxidative

Ferm: fermentative

S.b.: short bunches

F : fermentative

M: magenta - orange

Table No. 41 - Biochemical and morphological characteristics of Gram positive micrococci at Tallaboa bay. Station No. 3 B

Strain No.	1	2	3	4	5		7	8		10	11	12	13	14	15	16
Gram Stain	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Oxidase	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Catalase	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Simple Nitrogen Media	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Mobility & Morph.	±	±	±	±	±		±	±		±	±	±	±	±	±	±
Ox. Ferm. Glucase	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Glucase aerobic	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Glucase anaerobic	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Mannitol	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Maltose	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Arabinase	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Galactose	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Xylase	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Growth BM broth	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Growth peptone broth	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Nitrate reduction	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Litmus milk	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Gelatin hydrolisis	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Starch hydrolisis	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Pigmentation	Or	Or	Or	Or	Or		Or	Or		Or	Or	Or	Or	Or	Or	Or
Action in blood	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Lipolysis	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Coagulase	−	−	−	−	−		−	−		−	−	−	−	−	−	−
Casein hydrolisis	+	+	+	+	+		+	+		+	+	+	+	+	+	+
Tentative i. d.	Mic	Mic	Mic	Mic	Mic		Mic	Mic		Mic	Mic	Mic	Mic	Mic	Mic	Mic

Ox : oxidative
Ferm: fermentative

Or: orange
t : tetrads

TABLE No. 42

MAYAGUEZ BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr μg/L	Phen mg/L
1	5.0	8.12	1.71	34.13	19.35	27.0	1.62	1.49	0.50	0.60	Trace
1 A	5.0	8.15	2.00	34.76	19.71	26.5	1.91	1.76	0.20	0.50	———
1 B	5.5	8.21	1.91	34.21	19.39	27.0	1.80	1.62	Less 0.1	0.50	———
1 C	5.8	8.16	1.76	34.51	19.57	27.0	1.66	1.49	Less 0.1	0.50	———
2	4.0	8.07	1.87	30.62	19.31	29.0	1.78	1.62	0.50	1.20	Trace
2 A	4.5	8.16	2.13	34.47	19.55	29.0	2.02	1.80	0.50	0.60	———
2 B	4.0	8.15	1.57	34.85	19.77	28.0	1.48	1.36	Less 0.1	0.50	———
2 C	5.3	8.13	1.76	35.00	19.85	28.5	1.66	1.49	Less 0.1	0.50	———

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

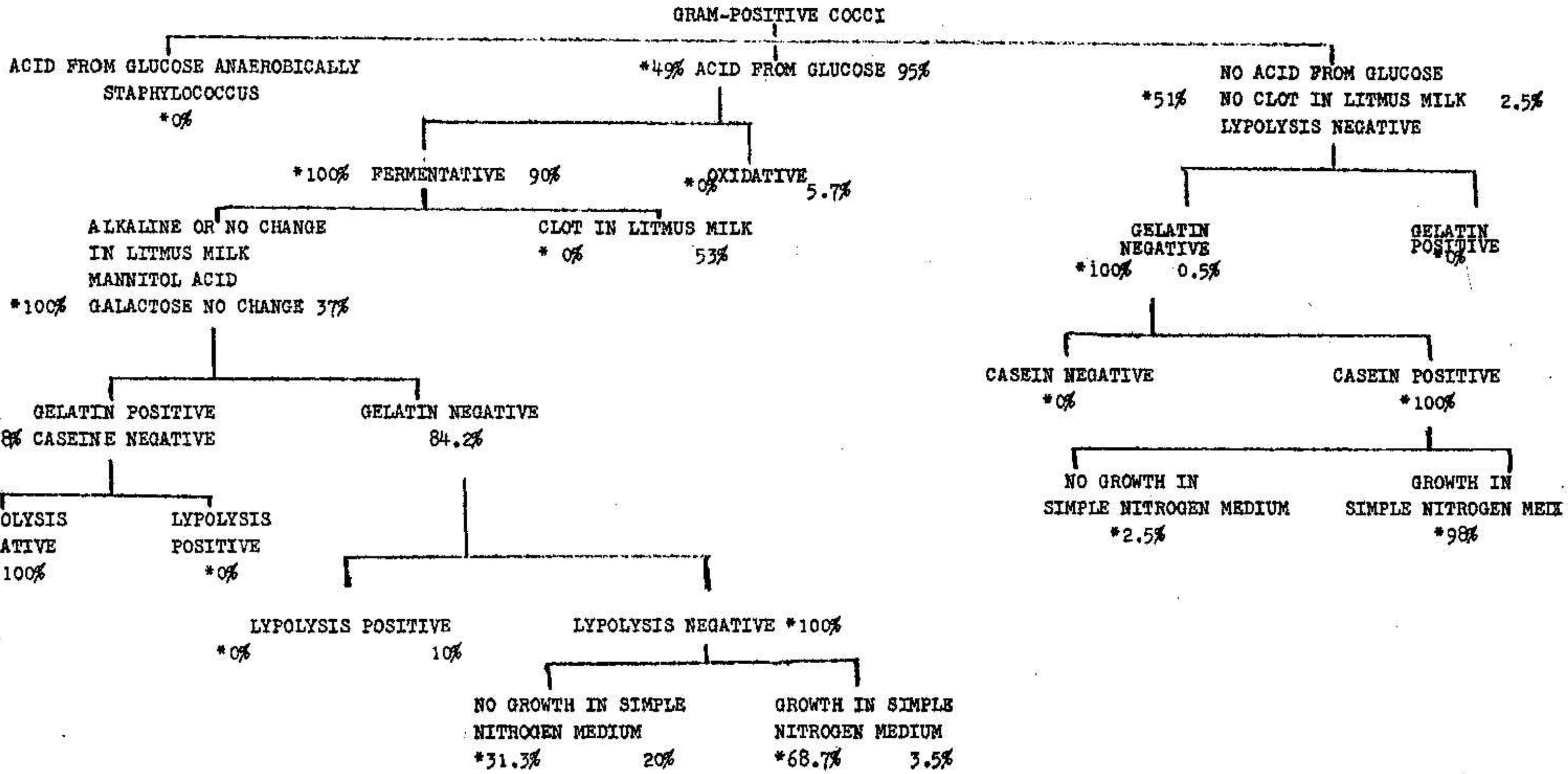


TABLE 41-A

COMPARATIVE PERCENTAGE DISTRIBUTION OF CHARACTERISTICS AMONG 205 CULTURES OF MICROCOCCI ISOLATED FROM THE NORTH SEA AND 155 STRAINS ISOLATED FROM THE BENTHOS IN THE CARIBBEAN SEA. MODIFIED FROM ANDERSON, J.I.W. (40)

* OUR RESULTS

TABLE No. 43

MAYAGUEZ BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr $\mu g/L$	Phen mg/L
3	5.6	8.15	1.85	35.910	20.38	26	1.76	1.61	0.35	0.85	
3 A	5.1	7.90	1.85	36.108	20.50	26	1.79	1.70	0.30	0.70	
3 B	5.0	8.00	1.77	36.023	20.45	26	1.70	1.59	0.30	0.50	
3 C	5.2	8.00	1.92	35.935	20.40	26	1.85	1.70	0.35	0.40	
4	4.5	8.20	1.85	35.978	20.42	26	1.75	1.57	0.75	0.90	
4 A	4.9	8.15	1.92	36.072	20.48	26	1.83	1.68	0.30	0.50	
4 B	5.2	8.10	1.85	35.917	20.39	26	1.70	1.61	0.35	0.50	
4 C	5.2	8.15	1.77	35.973	20.42	26	1.68	1.54	0.35	0.50	

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

TABLE No. 44

MAYAGUEZ BAY

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr $\mu g/L$	Phen mg/L
1	5.2	8.00	1.85	35.788	20.31	26	1.78	1.67	0.30	1.90	—
1 A	3.7	8.25	1.85	35.780	20.31	26	1.75	1.57	0.20	0.90	—
1 B	3.6	8.22	1.85	35.768	20.31	26	1.75	1.57	0.10	0.70	—
1 C	3.7	8.22	1.92	35.555	20.18	26	1.82	1.63	0.10	0.70	—
2	2.5	7.60	1.92	35.351	20.06	26	1.89	1.87	0.09	2.20	—
2 A	3.7	8.25	1.85	35.781	20.31	26	1.55	1.57	0.09	1.10	—
2 B	3.6	8.25	1.85	35.762	20.30	26	1.75	1.57	0.10	0.70	—
2 C	3.7	8.20	1.85	35.791	20.32	26	1.75	1.57	0.30	0.70	—

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20): Chlorinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

Table No. 45 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 1

Strain No.	1	2	3	4	5	6	7									
Morphology	Sl Str	Sl Str	Sh St	Sh St	Sh St	Sh St	Sl Str									
Motility	+	+	+	+	+	+	+									
Gram Stain	—	—	—	—	—	—	—									
Colony appearance	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M									
Pigment	—	—	—	—	—	—	—									
Oxidative Glucose	+	Alk	Alk	Alk	+	Alk	—									
Ferm. Glucose	—	Alk	Alk	Alk	—	Alk	—									
Lactose	—	Alk	Alk	Alk	—	Alk	—									
Sucrose	—	Alk	Alk	Alk	—	Alk	—									
Mannitol	—	Alk	Alk	Alk	—	Alk	—									
King's Fluorescein	—	—	—	—	—	—	—									
King's Pyocianine	—	—	—	—	—	—	—									
M.A. + 30% S. Milk	+	+	+	+	+	+	+									
Paton's Media	—	—	—	—	—	—	—									
Flagellar Stain	P	P	P	P	P	P	P									
Oxidase Kovacs	+	+	+	+	+	+	+									
Oxidase Paper	+	+	+	+	+	+	+									
Urea	—	—	—	—	+	—	—									
Simmon's Citrate	+	+	+	+	+/—	+/—	+									
Growth peptone H_2O	+	—	—	—	+	—	—									
Growth marine broth	+	+	+	+	+	+	+									
Growth 25° C	+	+	+	+	+	+	+									
Growth 37° C	+	+	+	+	+	+	+									
Growth 42° C	+	+	—	+	—	+	+									
Sensitivity Penicillin 2u	R	S	R	R	S	S	R									
Sensitivity Terramycin 10 mcg	R	S	S	R	S	S	R									
Sensitivity 0/129	R	R	R	R	R	R	R									
Tentative i.d.	R.II	Ps.III	Ps.III	Ps.III	R.II	R.III	Ps.IV									

M.A.: marine agar plus 30% skimmed milk

Sl	: Slender	R	: resistant	St	: stout
Str	: straight	Ps	: pseudomonas	(+)	: late
Tr	: translucent	alk	: alkaline	-	positive
M	: mucoid moist	S	: sensitive		
P	: polar	Sh.	: short		

Table No.46 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 1-A

	1	2	3	4	5	6	7	8
Strain No.								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str
Motility	+	+	+	+	—	+	+	+
Gram Stain	—	—	—	—	—	—	—	—
Colony appearance	Tr M	M gr	M gr	M gr	M	M gr	M gr	M gr
Pigment	—	—	—	—	Y	—	—	—
Oxidative Glucose	—	—	—	—	—	—	—	+
Ferm. Glucose	—	Alk	Alk	—	—	—	—	—
Lactose	—	Alk	Alk	—	—	—	—	—
Sucrose	—	Alk	Alk	—	—	—	—	—
Mannitol	—	Alk	Alk	—	—	—	—	—
King's Fluorescein	—	—	—	—	—	—	—	—
King's Pyocianine	—	—	—	—	—	—	—	—
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+
Paton's Media	—	—	—	—	—	—	—	—
Flagellar Stain	P	P	P	P	—	P	P	P
Oxidase Kovacs	+	+	+	+	+	+	+	+
Oxidase Paper	+	+	+	+	+	+	+	+
Urea	—	—	—	—	—	+	—	—
Simmon's Citrate	±	—	±	+	—	±	±	+
Growth peptone H_2O	—	—	—	—	+	—	—	+
Growth marine broth	+	+	+	+	+	+	+	+
Growth 25°C	+	+	+	+	+	+	+	+
Growth 37°C	+	+	+	+	—	+	+	+
Growth 42°C	+	+	+	+	—	+	+	+
Sensitivity Penicillin 2u	R	R	S	R	R	R	R	R
Sensitivity Terramycin 10 mcg	R	S	S	S	S	S	R	R
Sensitivity 0/129	R	R	R	R	R	R	R	R
Tentative i.d.	R_3 IV	R_3 III	R_3	R_3 IV	?	R_3 IV	P_3 IV	R_3 IV

M.A.: marine agar plus 30% skimmed milk

Sl : slender
Str: straight
Tr : translucent
M : mucoid
M : moist, mucoid
Y : yellow
Alk: alkaline
P : polar
± : weak
R : resistant
S : sensitive
Ps: pseudomonas
? : xanthomonas

Table No. 47 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 1-B

Strain No.		1	2	3	4	5	6	7	8								
Morphology		Sh St	Sh St	Sh Str	Sh Sh	Sh St	Sh St	Sh Str	Sh St								
Motility		+	+	+	+	+	+	+	+								
Gram Stain		−	−	−	−	−	−	−	−								
Colony appearance		M	M	M	Mgr	Mgr	M	M	M								
Pigment		−	−	−	−	−	−	−	−								
Oxidative Glucose		−	−	−	−	+	−	−	−								
Ferm. Glucose		−	Alk	+	−	−	Alk	+	−								
Lactose		−	Alk	−	−	−	Alk	−	−								
Sucrose		−	Alk	−	−	−	Alk	−	−								
Mannitol		−	Alk	−	−	−	Alk	−	−								
King's	Fluorescein	−	−	−	−	−	−	−	−								
	Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk		+	+	+	+	+	+	+	+								
Paton's Media		−	−	−	−	−	−	−	−								
Flagellar Stain		P	P	P	P	P	P	P	P								
Oxidase	Kovacs	+	+	+	+	+	+	+	+								
	Paper	+	+	+	+	+	+	+	+								
Urea		−	−	−	−	+	+	−	−								
Simmon's Citrate		+	+	−	±	+	+	−	+								
Growth peptone H_2O		−	±	+	−	+	±	+	−								
Growth marine broth		+	+	+	+	+	+	+	+								
Growth	25° C	+	+	+	+	+	+	+	+								
	37° C	+	+	+	+	+	+	+	+								
	42° C	+	+	+	+	+	+	+	+								
Sensitivity	Penicillin 2u	R	R	R	R	R	R	R	R								
	Terramycin 10 mcg	R	R	S	R	S	S	S	R								
	O/129	R	R	R	R	R	R	R	R								
Tentative i.d.		Ps	Ps	Ps	Ps	Ps	Ps	P?	Ps								

M.A.: marine agar plus 30% skimmed milk

Sh : short
St : stout
M : moist mucoid
Alk: alkaline
(+): weak
P : polar
R : resistant
S : sensitive
Ps : Pseudomonas
Mgr: moist, mucoid graysh

Table No. 48 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 2

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str								
Motility	+	−	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	M gr	M	Fr m	M gr	M br	M	M	M								
Pigment	−	Py	−	−	Br	−	−	−								
Oxidative Glucose	+	−	−	+	+	+	−	Alk								
Ferm. Glucose	−	−	−	−	−	−	−	Alk								
Lactose	−	−	−	−	−	−	−	Alk								
Sucrose	−	−	−	−	−	−	−	Alk								
Mannitol	−	−	−	−	−	−	−	Alk								
King's Fluoresceir	−	−	−	−	-	−	-	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	−	+	+	+	+	+	+								
Paton's Media	−	-	−	−	−	−	−	-								
Flagellar Stain	P	-	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	+−	+	+	+−	−	+−	+	−								
Simmon's Citrate	+	+	+	+	+	+	+	+								
Growth peptone H_2O	+	+	−	+	+	−	—	+								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	−	−	—	−	−	−	−	−								
Growth 37° C	+	−	+	+	+	+	+	+								
Growth 42° C	+	−	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	S	R	R	R	R	R	S								
Sensitivity Terramycin 10 mcg	R	S	R	R	R	R	R	S								
Sensitivity O/129	R	R	R	R	R	R	R	R								
Tentative i.d.	Fla	Ps	Fla	Fla	Fla	Fla	Fla	Fla								

M.A.: marine agar plus 30% skimmed milk

M : moist, mucoid
M : moist
Gr : graysh
Br : brownish
Alk: alkaline
P : polar
(±): weak
R : resistant
S : sensitive
Ps : pseudomonas
Fla : flavobacterium

Table No.49 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 2-A

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Sl	Sl Sl	Sl Sl	Sl Sl								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	+	+	+	−	−	+	+	+								
Ferm. Glucose	−	−	−	Alk	−	−	−	−								
Lactose	−	−	−	Alk	−	−	−	−								
Sucrose	−	−	−	Alk	−	−	−	−								
Mannitol	−	−	−	Alk	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	−	−	−	−	+−	+−								
Simmon's Citrate	+	+−	+−	+	+	+−	+	+								
Growth peptone H_2O	+	+	+	−	−	+	+	+								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25°C	+	+	+	+	+	+	+	+								
Growth 37°C	+	+	+	+	+	+	+	+								
Growth 42°C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	S	R	R	S	R								
Sensitivity Terramycin 10 mcg	S	S	S	S	R	R	S	R								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	P Sl	P Sl	P Sl	P Sl	P Sl	P Sl	P Sl	P Sl								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	Alk	: alkaline
Str	: straight	P	: polar
Tr	: translucent	±	: weak
M	: mucoid	S	: sensitive
R	: resistant		

Table No. 50 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 2-B

Strain No.		2	3		5	6	7	8								
Morphology		Sl Str	Sl Str		Sl Str	Sl Str	Sl Str	Sl Str								
Motility		+	+		+	+	+	+								
Gram Stain		−	−		−	−	−	−								
Colony appearance		Tr M	Tr M		Tr M	Tr M	Tr M	Tr M								
Pigment		−	−		−	−	−	−								
Oxidative Glucose		+	Alk		−	+	+	Alk								
Ferm. Glucose		−	Alk		−	−	−	Alk								
Lactose		−	Alk		−	−	−	Alk								
Sucrose		−	Alk		−	−	−	Alk								
Mannitol		−	Alk		−	−	−	Alk								
King's	Fluorescein	−	−		−	−	−	−								
	Pyocianine	−	−		−	−	−	−								
M.A. + 30% S. Milk		+	+		+	+	+	+								
Paton's Media		−	−		−	−	−	−								
Flagellar Stain		P	P		P	P	P	P								
Oxidase	Kovacs	+	+		+	+	+	+								
	Paper	+	+		+	+	+	+								
Urea		+	+		−	−	±	−								
Simmon's Citrate		+	+		+	+	+	+								
Growth peptone H_2O		+	−		−	+	+	−								
Growth marine broth		+	+		+	+	+	+								
Growth	25° C	+	+		+	+	+	+								
	37° C	+	+		+	+	+	+								
	42° C	+	+		+	+	+	+								
Sensitivity	Penicillin 2u	S	S		R	R	R	S								
	Terramycin 10 mcg	S	S		R	R	S	S								
	0/129	R	R		R	R	R	R								
Tentative i.d.		Ps II	Ps III		Ps IV	Ps II	Ps II	Ps III								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	R	: resistant	St	: stout
Str	; straight	Ps	: Pseudomonas	±	: late positive
Tr	: Translucent	alk	: alkaline		
M	: mucoid moist	S	: sensitive		
P	: Polar	Sh	: short		

Table No. 51 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 3

	1	2	3	4	5	6	7	8
Strain No.								
Morphology	Sh St.	Sl Str	Sh Sc	Sh Sc	Sl Str	Sh St	Sh St	Sh Coc
Motility	+	+	+	+	+	+	+	—
Gram Stain	—	—	—	—	—	—	—	—
Colony appearance	tr	tr M	tr M	tr	tr M	tr	tr	tr
Pigment	—	—	—	—	—	—	—	—
Oxidative Glucose	—	+	—	—	—	—	—	+
Ferm. Glucose	⊕	—	⊕	⊕	—	⊕	⊕	—
Lactose	—	—	—	—	—	—	—	—
Sucrose	⊕	—	⊕	⊕	—	⊕	⊕	—
Mannitol	⊕	—	⊕	⊕	—	⊕	⊕	—
King's Fluorescein	—	—	—	—	—	—	—	—
Pyocianine	—	—	—	—	—	—	—	—
M.A. + 30% S. Milk	—	+	—	—	—	—	—	—
Paton's Media	—	—	—	—	—	—	—	—
Flagellar Stain	P	P	P	P	P	P	P	—
Oxidase Kovacs	+	+	+	+	+	+	+	—
Oxidase Paper	+	+	+	+	+	+	+	—
Urea	+	—	+	+	±	+	+	—
Simmon's Citrate	+	—	+	+	+	+	+	+
Growth peptone H_2O	—	+	—	—	—	—	—	+
Growth marine broth	+	+	+	+	+	+	+	+
Growth 25° C	+	+	+	+	+	+	+	+
Growth 37° C	+	+	+	+	+	+	+	—
Growth 42° C	+	+	+	+	+	+	+	—
Sensitivity Penicillin 2u	R	R	R	R	R	S	R	S
Sensitivity Terramycin 10 mcg	S	R	R	R	R	S	S	S
Sensitivity 0/129	R	R	R	R	R	R	R	R
Tentative i.d.	Aer	Ps II	Aer	Aer	Ps IV	Aer	Aer	Achr coc

M.A.: marine agar plus 30% skimmed milk

Sl : slender
Str : straight
Tr : translucent
M : mucoid moist
P : polar
Aer : aeromonas
R : resistant
Ps : pseudomonas
alk: alkaline
S : sensitive
Sh : short
\+ : positive with gas
St : stout
+ : late positive
coc : coccoid
Achr: achromobacter alkaligenes

Table No. 52 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 3-A

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sh St	Sh Sc	Sl Str	Sl Str	Sl Sc	Sl Sc	Sl Sc								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	tr m	tr m	tr m	tr m	tr m	tr m	tr m	tr m								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	+	−	−	−	−	−	−								
Ferm. Glucose	−	−	−	⊕	⊕	⊕	⊕	⊕								
Lactose	−	−	−	−	−	−	−	−								
Sucrose	−	−	−	+	+	+	+	+								
Mannitol	−	−	−	−	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	+	−	−	−	−	−	−								
Simmon's Citrate	−	+	−	−	+	+	−	−								
Growth peptone H_2O	−	+	−	−	−	−	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	R	R	R	R	R								
Sensitivity Terramycin 10 mcg	R	R	R	R	R	R	R	R								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	Ps	Ps	Ps	Ae	Ae	Ae	Ae	Ae								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	±	: weak
Str	: straight	P	: polar
St	: stout	R	: resistant
Sh	: short	Ps	: Pseudomonas
⊕g	: positive with gas	Ae	: Aeromonas

Table No.53 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 3-B

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sh St	Sh St	Sh St	Sh Cc	Sh St	St Sc	Sh St	St Sc								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	M	M	M	M	M	M	M	M								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	+	+	−	+	−	−								
Ferm. Glucose	Alk	⊕	−	−	−	−	−	−								
Lactose	Alk	−	−	−	+	−	−	−								
Sucrose	Alk	+	−	−	−	−	−	−								
Mannitol	Alk	−	−	−	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	−	+	−	−	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	+	+	±	+	−	−								
Simmon's Citrate	−	−	+	+	±	+	−	−								
Growth peptone H_2O	−	−	+	+	−	+	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	S	R	R	R	R								
Sensitivity Terramycin 10 mcg	R	R	S	R	R	R	R	R								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	R III	Ae	P I	P II	P IV	P II	P IV	P IV								

M.A.: marine agar plus 30% skimmed milk

Sh : short
St : stout
M : moist, mucoid
Alk : alkaline
⊕g : positive gas

P : polar
S : sensitive
Ps : pseudomonas
Ae : Aeromonas

Table No. 54 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 4

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	−	−	−	−	−	−								
Ferm. Glucose	−	−	−	−	−	−	−	−								
Lactose	−	−	−	−	−	−	−	−								
Sucrose	−	−	−	−	−	−	−	−								
Mannitol	−	−	−	−	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	+	+	+	+	+	+	+	+−								
Simmon's Citrate	+	+	+	+	+	+	+	+−								
Growth peptone H_2O	−	−	−	−	−	−	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	S	R	R	R	R	S	S								
Sensitivity Terramycin 10 mcg	R	S	R	R	R	R	S	S								
Sensitivity O/129	R	R	R	R	R	R	R	R								
Tentative i.d.	Ps I N	Ps I N	Ps I N	Ps I N	Ps I N	Ps I N	Ps I N	Ps I N								

M.A.: marine agar plus 30% skimmed milk

Sl	:	slender	R	:	resistant
Str	:	straight	S	:	sensitive
Tr	:	translucent	I	:	weak
M	:	mucoid	Ps	:	pseudomonas
P	:	polar			

Table No. 55 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 4-A

Strain No.		1	2	3	4	5	6	7	8							
Morphology		Sh Coc	Sl Str	Sl Str	Sl Str	Sh Coc	Sl Str	Sl Str	Sl Str							
Motility		+	+	+	+	+	+	+	+							
Gram Stain		−	−	−	−	−	−	−	−							
Colony appearance		Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M	Tr M							
Pigment		−	−	−	−	−	−	−	−							
Oxidative Glucose		−	−	−	−	−	−	−	+							
Ferm. Glucose		Alk	⊕	−	⊕	Alk	⊕	⊕	−							
Lactose		Alk	−	−	−	Alk	−	−	−							
Sucrose		Alk	⊕	−	⊕	Alk	⊕	⊕	−							
Mannitol		Alk	−	−	−	Alk	−	−	−							
King's	Fluorescein	−	−	−	−	−	−	−	−							
	Pyocianine	−	−	−	−	−	−	−	−							
M.A. + 30% S. Milk		+	+	+	+	+	+	+	+							
Paton's Media		−	−	−	−	−	−	−	−							
Flagellar Stain		P	P	P	P	P	P	P	P							
Oxidase	Kovacs	+	+	+	+	+	+	+	+							
	Paper	+	+	+	+	+	+	+	+							
Urea		+	−	−	−	−	+	±	+							
Simmon's Citrate		+	+	−	−	+	+	+	+							
Growth peptone H_2O		±	−	−	−	±	+	+	+							
Growth marine broth		+	+	+	+	+	+	+	+							
Growth	25° C	+	+	+	+	+	+	+	+							
	37° C	+	+	+	+	+	+	+	+							
	42° C	+	+	+	+	+	+	+	+							
Sensitivity	Penicillin 2u	S	R	R	R	S	R	R	R							
	Terramycin 10 mcg	R	R	R	S	R	R	R	R							
	O/129	R	R	R	R	R	R	R	R							
Tentative i.d.		Ps III	Ae	Ps II	Ae	Ps III	Ae	Ae	Ps II							

M.A.: marine agar plus 30% skimmed milk

Sl : slender
Coc : coccoid
Str : straight
Tr : translucent
M : moist

⊕g : positive with gas
Alk : alkaline
P : polar
I : weak

R : resistant
S : sensitive
Ps: pseudomonas
Ae: aeromonas

Table No. 56 Biochemical and morphological characteristics of Gram negative bacilli at Mayaguez bay. Station 4-B

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl St.	Sl Str	Sh St	Sl Str	Sl Str								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	tr m	tr m	tr m	tr m	tr m	tr m	tr m	tr m								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	−	+	−	Alk	Alk	Alk								
Ferm. Glucose	−	−	−	−	−	Alk	Alk	Alk								
Lactose	−	−	−	−	−	Alk	Alk	Alk								
Sucrose	−	−	−	−	−	Alk	Alk	Alk								
Mannitol	−	−	−	−	−	Alk	Alk	Alk								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	−	−	−	−	−	−								
Simmon's Citrate	−	−	−	+	−	−	−	−								
Growth peptone H_2O	−	−	−	+	−	−	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	S	R	S	R	R								
Sensitivity Terramycin 10 mcg	R	R	R	S	R	R	R	R								
Sensitivity O/129	R	R	R	R	R	R	R	R								
Tentative i.d.	P_3 III	P_3 III	P_3 III	P_3 III	P_3 III	P_3 III	P_3 III	P_3 III								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	R	: resistant	St	: stout
Str	: straight	Ps	: pseudomonas	±	: late positive
Tr	: translucent	alk	: alkaline		
M	: mucoid moist	S	: sensitive		
P	: polar	Sh	: short		

TABLE No. 57

RINCON COAST

CHEMICAL AND PHYSICAL DETERMINATIONS OF THE SEA WATER AT THE BOTTOM

Station	DO	pH	Total Alk	S%	Cl/liter (20)	Temp °C	Total CO_3	Total CO_2	Phosph μgPO_4-P/L	Nitr $\mu g/L$	Phen mg/L
1	5.7	8.25	1.92	35.935	20.52	26	1.82	1.63	Less 0.1	Less 0.1	——
2	5.7	8.25	1.92	35.973	20.42	26	1.82	1.63	Less 0.1	Less 0.1	——
3	5.5	8.20	1.85	35.791	20.40	25	1.75	1.57	Less 0.1	Less 0.1	——

DO: Dissolved oxygen in p.p.m.; pH: Hydrogen ion concentration of bottom sample, direct reading; S%: Salinity; Cl/liter (20) Chlordinity at 20 °C; Temp: Temperature in °C; Total Alk: Total Alkalinity; Phosph: Phosphates; Nitr: Nitrates; Phen: Phenols.

Table No. 58 Biochemical and morphological characteristics of Gram negative bacilli at Rincón Coast. Station No. 1

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sm St	Sl Str	Sl Str								
Motility	+	+	+	+	+	−	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	tr M	tr M	tr M	tr M	tr M	tr M	tr M	M								
Pigment	−	−	−	−	−	−	−	−								
Oxidative Glucose	−	−	−	−	−	+	−	−								
Ferm. Glucose	⊕	⊕	⊕	⊕	⊕	−	⊕	Alk								
Lactose	−	−	−	−	−	+	−	alk								
Sucrose	−	−	+	+	+	−	+	alk								
Mannitol	−	−	−	−	−	−	−	−								
King's Fluorescein	−	−	−	−	−	−	−	−								
King's Pyocianine	−	−	−	−	−	−	−	−								
M.A. + 30% S. Milk	+	+	+	+	+	−	+	+								
Paton's Media	−	−	−	−	−	−	−	−								
Flagellar Stain	P	AM	P	P	P	−	P	P								
Oxidase Kovacs	+	+	+	+	+	−	+	+								
Oxidase Paper	+	+	+	+	+	−	+	+								
Urea	−	−	−	−	−	−	−	−								
Simmon's Citrate	−	−	−	−	−	−	−	+								
Growth peptone H_2O	+	+	−	−	−	+	−	−								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25°C	+	+	+	+	+	+	+	+								
Growth 37°C	+	+	+	+	+	+	+	+								
Growth 42°C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	R	R	R	R	S	R	S								
Sensitivity Terramycin 10 mcg	R	R	R	R	R	S	R	R								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	Ae	Ae	Ae	Ae	Ae	Alk	Ae	Ps								

M.A.: marine agar plus 30% skimmed milk

Sl	: slender	R	: resistant	Sh	: short
Str	: straight	Ps	: pseudomonas	St	: stout
Tr	: translucent	Ae	: aeromonas	⊕g	: positive with gas
M	: mucoid	Alk	: alkaligenes		
P	: polar	alk	: alkaline		
AM	: amphitricous	S	: sensitive		

Table No. 59 Biochemical and morphological characteristics of Gram negative bacilli at Rincón Coast. Station No. 2

Strain No.	1	2	3	4	5	6	7	8								
Morphology	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str	Sl Str								
Motility	+	+	+	+	+	+	+	+								
Gram Stain	−	−	−	−	−	−	−	−								
Colony appearance	tr M	tr M	tr M	brow M	tr M	tr M	tr M	tr M								
Pigment	−	−	−	brw	−	−	−	←								
Oxidative Glucose	−	−	+	+	+	−	+	+								
Ferm. Glucose	−	−	−	−	−	−	−	−								
Lactose	−	−	−	−	−	−	−	−								
Sucrose	−	−	−	−	−	−	−	−								
Mannitol	−	−	−	−	−	−	−	−								
King's Fluorescein	−	−	+	−	−	−	−	+								
King's Pyocianine	−	−	+	−	−	−	−	+								
M.A. + 30% S. Milk	+	+	+	+	+	+	+	+								
Paton's Media	−	−	+	−	−	−	−	+								
Flagellar Stain	P	P	P	P	P	P	P	P								
Oxidase Kovacs	+	+	+	+	+	+	+	+								
Oxidase Paper	+	+	+	+	+	+	+	+								
Urea	−	−	−	−	−	−	−	−								
Simmon's Citrate	+	+	+	+	+	+	+	+								
Growth peptone H_2O	−	−	+	+	−	−	+	+								
Growth marine broth	+	+	+	+	+	+	+	+								
Growth 25° C	+	+	+	+	+	+	+	+								
Growth 37° C	+	+	+	+	+	+	+	+								
Growth 42° C	+	+	+	+	+	+	+	+								
Sensitivity Penicillin 2u	R	S	R	R	R	R	R	R								
Sensitivity Terramycin 10 mcg	R	S	R	R	R	R	S	S								
Sensitivity 0/129	R	R	R	R	R	R	R	R								
Tentative i.d.	R I V	R V	P_s I	R II	P_s II	R IV	P_s II	P_s I								

M.A.: marine agar plus 30% skimmed milk

Sl : slender
Str : straight
Tr : translucent
M : moist
Brow: brownish
P : polar

R : resistant
S : sensitive
- : negative
+ : positive
Ps : pseudomonas

TABLE No. 60

GROUPING OF SOME HETEROTROPHIC BACTERIA FOUND IN COASTAL AREAS OF PUERTO RICO

AREA	Pseud I	Pseud II	Pseud III	Pseud IV	Flavo	?	Aer.	Achr-Alk.	Myco	Vib	Enterob-Colif.	Cyto	Total
RINCON													
Station 1	0	0	1	0	0		6	1					8
2	2	3	1	3	0		0	0					9
MAYAGUEZ													
Station 1-A-B	0	4	9	9	1	1	0	0					24
2-A-B	0	13	4	4	1		0	0					22
3-A-B	0	5	1	6	0		11	0					23
4-A-B	0	2	6	13	0		4	1					26
TALLABOA													
Station 1-A-B-C	1	3	2	29	0	2	0	3	4	3	0	0	47
2-A-B-C	4	3	5	4	0	0	0	0	0	0	0	0	16
3-A-B-C	6	5	8	5	14	1	7	6	0	30	1	5	88
4-A-B-C	0	7	10	0	6	0	3	0	0	5	0	0	31
	13	45	47	73	22	4	31	11	4	38	1	5	294

Pseud: pseudomonas. Flavo: flavobacterium. Aer: aeromonas. Achr-Alk: Achromobacter-alkaligenes.
Myco: mycoplana. Vib: vibrio. Enterob-Colif: Enterobacter-coliforme. Cyto: Cytophaga. ? unknown.

TABLE No. 61

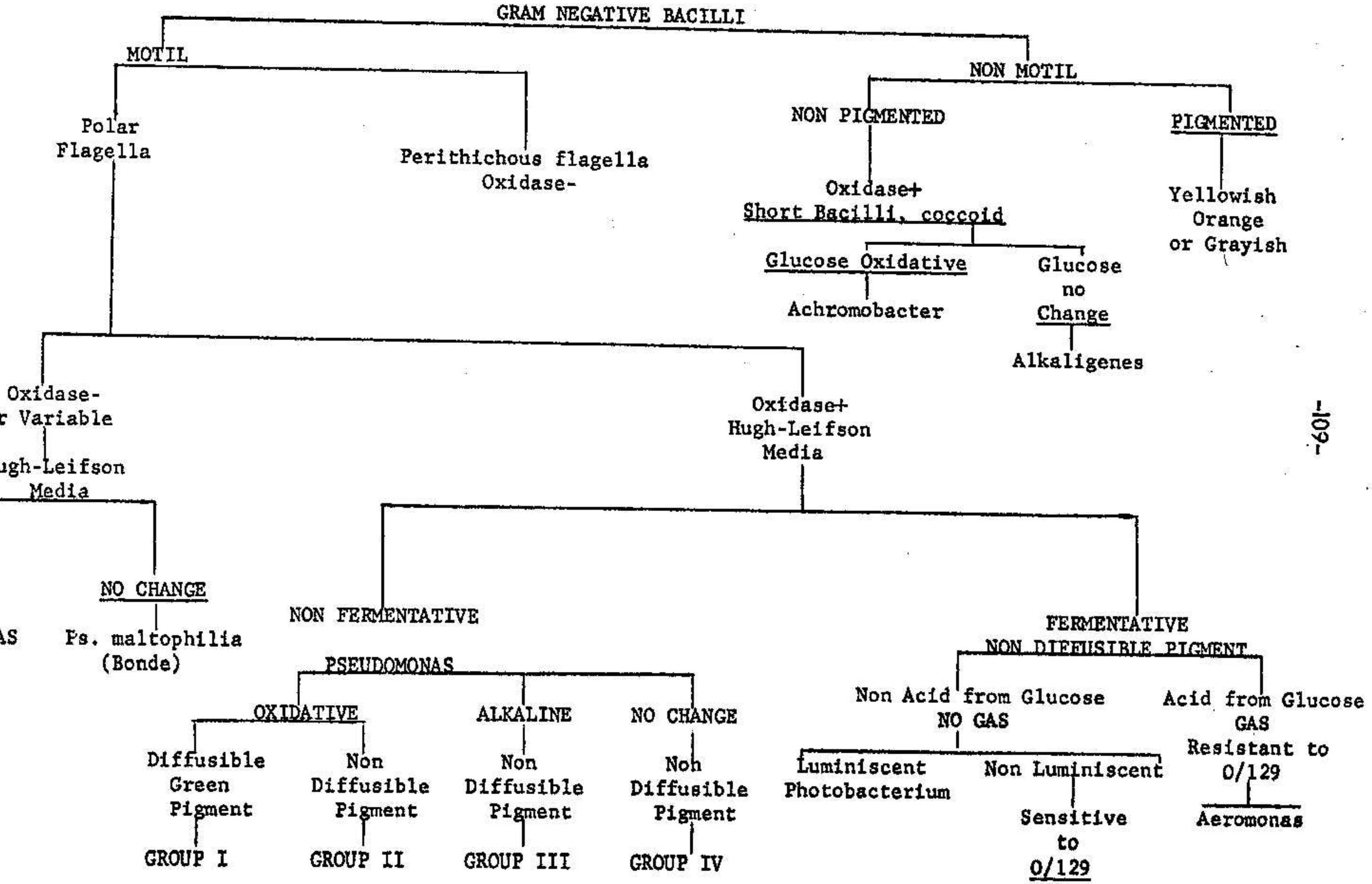
COMPARISON OF SOME DETERMINATIVE TEST FOR GRAM-NEGATIVE BACTERIA ISOLATED FROM TROPICAL SEAS (RIGHT COLUMNS) AND GRAM-NEGATIVE BACTERIA ISOLATED FROM TEMPERATE SEAS (LEFT COLUMNS)

SPECIES	MOTILITY		PEN. SEN.		HUGH-L		KOV-OX		GROWTH 37 °C		UREA		CITRATE		SKIM MILK		SEA WATER REQUIRED		GROWTH °6 250•37•42•		
Achromobacter	-	idem	+	idem	Alk	idem	N.A.	+	-	+	N.A.	-	N.A.	+	N.A.	+	N.A.	-	+	+	+
Alkaligenes	-	idem	++	+						-										-	-
Flavobacterium	-	idem	-	idem	Ox N.A.	-	N.A.	+	-	idem	N.A.	-	N.A.	+	N.A.	+	N.A.	-	+	-	-
Pseudomonas I-II	+	idem	-	idem	Ox	idem	+	idem	+	+	N.A.	+	N.A.	+	N.A.	+	N.A.	-	+	+	+
Pseudomonas III-IV	+	idem	-	+	Alk	idem	+	Idem	-	+	N.A.	+	N.A.	+	N.A.	+	N.A.	+	+	+	+
Aeromonas	+	idem	-	idem	N.C. Ferm gas	idem	+	idem	+	idem	+	idem	N.A.	+	N.A.	+	N.A.	+	+	+	+
Vibrio	+	idem	-	idem	Ferm	idem	+	idem	-	+	N.A.	-	N.A.	-	N.A.	+	N.A.	+	+	-	-
Enterobacter-Coliforms	+	idem	-	idem	Ferm	idem	-	idem	+	idem	N.A.	+	N.A.	+	N.A.	+	N.A.	-	+	+	+

Pen. Sen. penicillin sensitivity.: Hugh-L: Hugh-L media.: Kov-Ox. Kovacs-oxidase test.: N.A. not made.: N.C. no change.: Ox. Oxidative.: Alk. alkaline.

TABLE No. 62

ISOLATED CULTURE



SCHEME FOR GROUPING THE MARINE GRAM NEGATIVE BACTERIA
Modified from: Shewan-HodKiss, 1960. Simidu y Aiso, 1962. Bonde, 1966.

V. CONCLUSIONS

Predominant growth of single species of Gram negative bacteria was observed in specimens taken at several stations either at Tallaboa or Mayaguez bay.

Some stations did not produce any growth either of gram negative bacilli of the species studied or of gram positive cocci or of both.

It was not possible to isolate gram positive aerobic cocci from the Mayaguez bay or Rincón coast samples in contrast with samples from Tallaboa bay which produced abundant gram positive cocci flora.

Some physiological characteristics of the Pseudomonas sp. isolated showed different patterns in relation with the ones reported from isolates taken from temperature seas.

Pseudomonas group III and IV, Aeromonas sp. and Vibrio sp. showed a definite requirement for high sodium concentration to grow in the media tested.

There is a sharp contrast in relation to the biochemical and physiological characteristics of the gram positive benthic cocci isolated from tropical seas and the characterictics of the strains reportedly isolated from temperature seas.

Due to the general lack of knowledge in relation to the ecology and physiology of bacteria from benthic coastal areas in tropical seas and the forseeable utilization of those areas as possible sites of discharge outlets of industrial or thermoelectrical effluents, it is highly recommended that this type of research be promoted and performed prior to the industrial and/or thermoelectrical developments. Also it would be convenient to measure and compare findings after the incorporation of new elements in the coastal environment.

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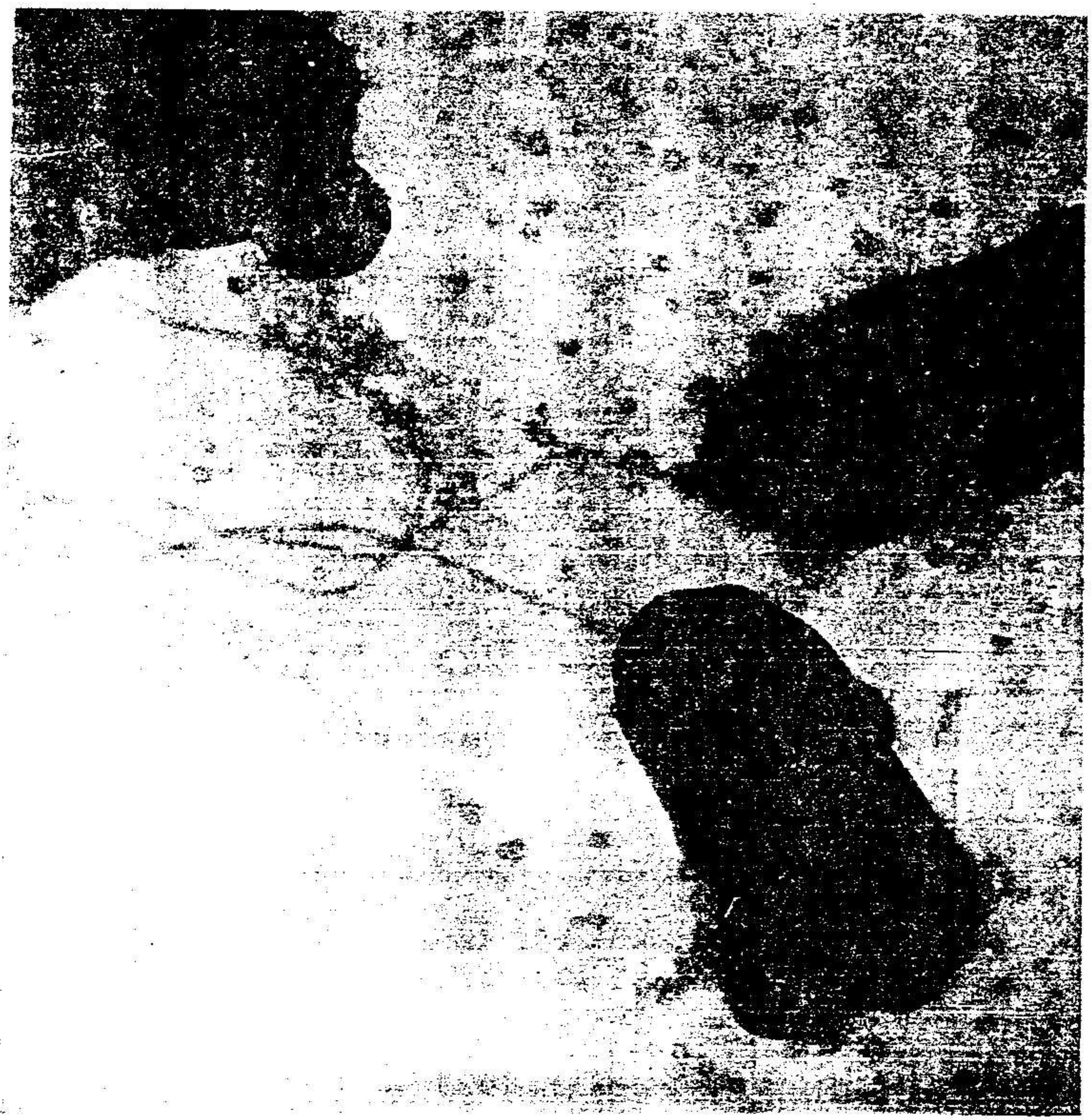
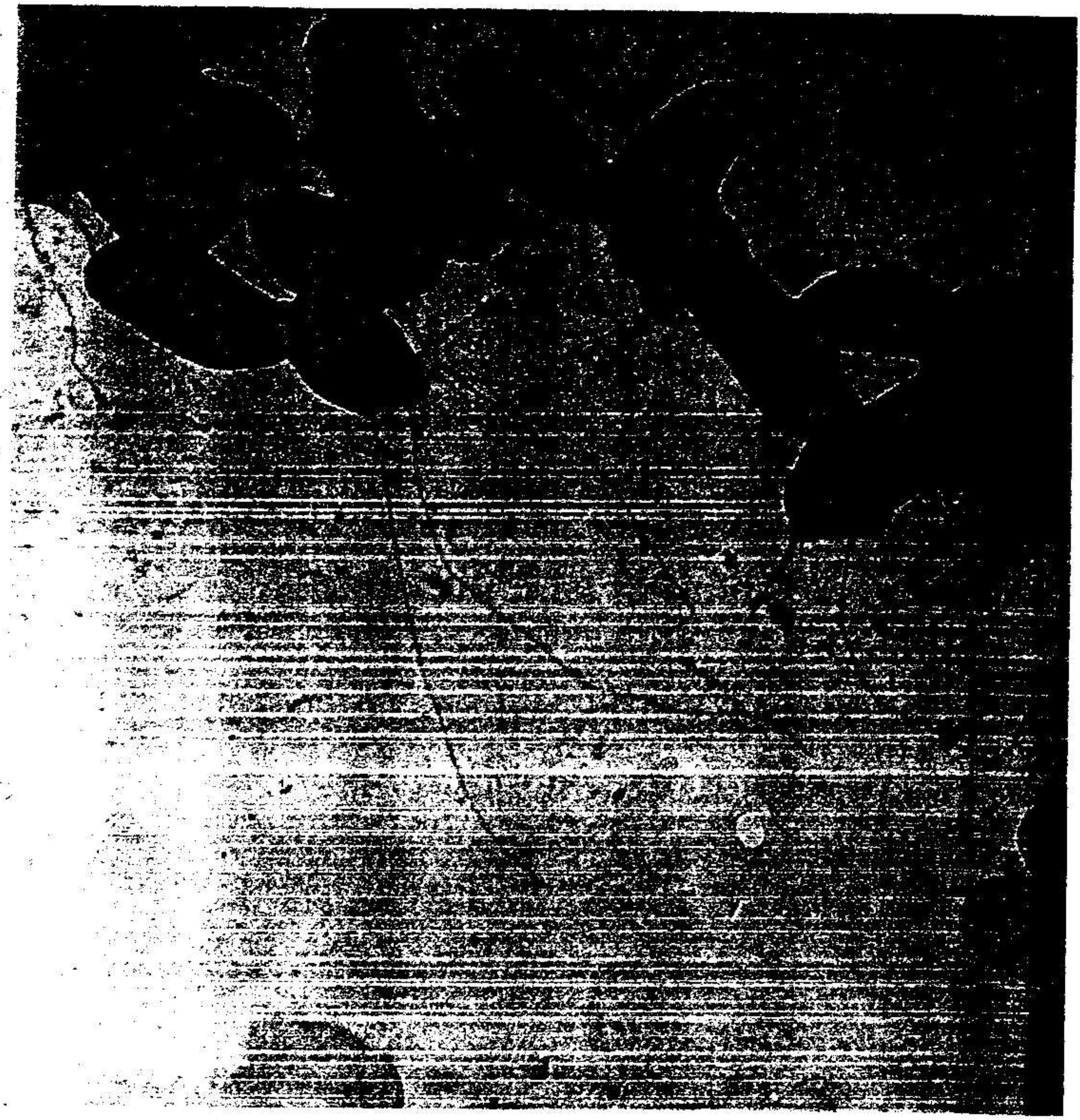


ELECTRON MICROPHOTOGRAPHS OF STRAINS ISOLATED FROM THE BENTHOS OF TROPICAL SEAS

ELECTRON MICROPHOTOGRAPHS OF STRAINS ISOLATED FROM THE BENTHOS OF TROPICAL SEAS.

ELECTRON MICROPHOTOGRAPHS OF STRAINS ISOLATED FROM THE BENTHOS OF TROPICAL SEAS.



DINOFLAGELLATES STRAINS ISOLATED FROM THE BENTHOS OF TROPICAL SEAS.

MAP I

MAP II

MAP III.