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by

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INTRODUCTION

The bacterial organismus occurring in the Adriatic have comparatively little been investigated. Some data on two new species have been given by Hansgirg (1890). Additional sixteen species have been described by the same author (1892), five of them being new ones, and one of them a variety. This author's investigations involved the regions of Trst (Trieste), Pula, Rovinj, Split, Solin, Dubrovnik, etc. Several species, new for the Adriatic, have been described by Schaudin (1903). Four new species of luminescent bacteria, found on fish caught in Trst harbour, have been given by Molisch (1904). Further new species were found by the same author in the same region and described, one in 1907, and six in 1912. A new species of luminescent bacteria was isolated and described by Vouk V., Klas Z., and Škorić V. (1931). Seventeen species, reported to be new for the Adriatic or for Yugoslavia as a whole, were found by Klas Z. (1938) during her examination of the bacterial flora contained in the sulfuric springs at Split. This author has given a description for five of species.

In consideration of the fact that the bacterial flora of the Adriatic is scantily known, investigations into this subject were started in Kaštela Bay. These investigations were carried out as a part of the research program of the Institute of Biology and Fisheries at Split.

The author is greatly indebted to Dr. Tonko Šoljan, Director of the Institute, who made these investigations possible, and to Dr. Ante Ercegović, Chief of the Biological Division, for valuable criticism and suggestions. Her specials thanks are due to Professor Vlaho Cviić, Chief of the Microbiological Laboratory, under whose guidance she developed her work in the field of marine microbiology; his technical skill and suggestions have been of assistance in the preparation of the manuscript. The author, finally, expresses her thanks to lab technicians, Miss Ojdana Marović and Mr. Petar Bilić for their technical help in the course of the investigations.

MATERIAL AND METHODS

Samples for the determination of bacteriological species were taken from Kaštela Bay, by means of a bacteriological pump of the Z-J type. In order to obtain a larger number of different species, samples were taken at three points, at each of them going to a depth of 15 metres.

The samples were taken into the Laboratory and inoculated into a broth consisting of 75% sea water and 25% distilled water. (Z o B e l l, 1946). Into 10 ml. liquid medium sea water from each of the samples was inoculated in quantities amounting to 1 ml. pro sample. But besides inoculating the samples into broth, we tried to inoculate them into solid media also, by taking the same quantity of sea water from each of the samples, and inoculating it into 10 ml. agar nutrient. This inoculation into a solid media performed in three Petri dishes pro sample at the same time, in order to obtain a wider range of possibilities for the selection of species.

The incubation of the inoculated broth and of the agar nutrient as well was performed in the dark, at temperatures measuring 20 to 22° C.

It took 48 hours to incubate the broth cultures. Streaks were then made according to D r y g a l s k i mehod. The Petri dishes containing these streaks were held under the same conditions as the previous cultures, and the period of their incubation expanded then to three days.

The purity of the isolated strains having been checked, they were inoculated both into broth and agar slant, in order to make the examination of cultures and their properties possible. We carefully observed the instructions contained in the Manual of Methods for Pure Culture Study of Bacteria, published by the Society of American Bacteriologists (1949), introducing a few insignificant alterations, which will be mentioned when dealing with method concerned.

The morphological characters of the examined strains were observed under the microscope by means of an immersion objective. The streaks were stained with gentian violet for 1 to 2 minutes.

The sizes were determined by means of an immersion objective and were calculated by means of a computing table.

In order to find out in what way the strains were related to dyes, the streaks were stained by gentian violet, fuchsin, methylene blue safranine, and acid fuchsin.

The Gram stain system was performed by H u c h e r' s method.

The motility was examined by the hanging-drop method, and Bailey's method (1929) — as modified by Fisher and Conn (1942) — was employed for the staining of flagella. It is recommended by this method, among others, to keep the streaks, on which Ziehl's carbol fuchsin is being poured, on a hot metal sheet for a minute. As no results were ever obtained by heating the streaks in this way, a gradual heating of glass slides was employed by putting them over a gas-flame and keeping them first, for some 20-30 seconds, at a distance of 15 to 20 cm from the flame cone. This distance was then gradually and slowly made shorter and shorter during a period of 1—1,5 minutes, not allowing it to drop below 2—3 cm. A clear picture of flagella not dissociated from bacterial cells was thus obtained.

Anthony's method was applied for the examination of capsules and Dorner and Klein's one for the examination of spores. We have, moreover, watched the occurrence of spores in the age function of cultures. Cultures were bred on standard liquid and solid media and stained with gentian violet and fuchsin. Daily examination took place for the first fortnight which subseqently turned to every second day survey. The maximum age of cultures, used for this purpose, was 50 days.

The relation to oxygen was examined by the method of stabbing the agar which was covered with 4 ml. of sterile paraffin oil. The aerobic growth was thus preevnted which, at the same time, was watched on agar slant too.

The kind of sugar used to determine the relation to carbohydrates were the following: glucose, lactose, sucrose, xylose, mannite, dextrose, inulin, galactose, arabinose, and maltose, but beside these dulcitol, sorbitol, glycerin and starch were also applied. To demonstrate the hydrolysis of the carbohydrates bromthymol blue and brmcresol purple reagents were used, the tests being repeated twice. In case of uncertain results the test was repeated once again, applying the reagent concerned. These tests lasted for a fortnight and the examination of cultures was performed daily.

Special tests took also place for the determination of hydrolysis of starch for which peptonic water (with 1% soluble starch) was used as in the case of other carbohydrates. The hydrolysis was demonstrated by the iodine reaction, and for the determination of sugar Fehling's reagent was used.

The ammonification was demonstrated by means of Nessler's reagent, using a medium composed as follows (each liter of liquid consisting of 75% sea water and 25% distilled water):

Nutrient Broth	8 gr.
Peptone	4 gr.
K_2HPO_4	in traces
Fe PO_4	in traces
pH 7,6	

The nitrification was demonstrated by means of sulfodiphenylamin acid. The broth used for this test consisting of 75% sea water and 25% distilled water, contained:

$(NH_4)_2$ SO_4	2 gr.
K_2HPO_4	1 gr.
$MgSO_4$	1,5 gr.
Fe PO_4	1,5 gr.
pH 7,6	

The reduction of nitrate was demonstrated by means of Griess's reagent in a broth containing 1 gr. KNO_3 pro 1 liter of water, beef extract and peptone.

The relation to NO_2, i.e. the destruction of nitrite was established as in the case of the reduction of nitrate to nitrite by means of Griess's reagent. Since the selective broth for the destruction of nitrite contained some NO_2 itself, it was by means of Griess's reagent that we discovered in what measure the colours of the reagent differed, by applyng every time a checking droplet of broth with the same amount of liquid from the cultures under examination. This difference served as an indicator of the intensity of destruction. One liter of the selective broth, consisting of 750 ml. of sea water and 250 ml. of distilled water contained:

Nutrient Broth	8 gr.
Na NO_2	1 gr.
K_2HPO_4	in traces
Fe PO_4	in traces
pH adjusted to 7,6	

The production of hydrogen sulfide was demonstrated by means of lead acetate reagent paper. Ordinary broth was used as medium for proteolysis containing, in 750 ml. of sea water and 250 ml. distilled water:

Nutrient Broth	8 gr.
Glucose	1 gr.
Peptone	4 gr.
K_2HPO_4	0,1 gr.
pH 7,6	

Apart from the above media we also used an ordinary solid medium of the same composition to which 1,5% of bactoagar »Difco« had been added.

For the reduction of SO_4 to H_2S a selective broth was used (for heterotrophic bacteria) containing, in 750 ml. of sea water and 250 ml. of distilled water:

K_2HPO_4	0,2 gr.
$Mg\ SO_4$	0,2 gr.
$(NH_4)_2\ SO_4$	1 gr.
Calcium Lactate	3,5 gr.
Ascorbic Acid	0,1 gr.
$Fe\ (NH_4)_2\ SO_4$	0,1 gr.
Peptone	2 gr.
Yeast Extract	2 gr.
pH 7,6	

But beside this selective broth for heterotrophic bacteria, we also used a selective broth for autotrophic ones. The composition of the latter included, in 750 ml. of sae water and 250 ml. of distilled water:

$Mg\ SO_4$	5 gr.
NH_4Cl	1 gr.
$Fe\ (NH_4)_2\ SO_4$	0,1 gr.

The following specially sterilized solutions were added to this broth under sterile conditions after its sterilisation and the adjustment of pH in order to amount it to 7,6:

1/M K_2HPO_4	5 ml.
1/M $Na\ HCO_3$	20 ml.
1/M Na_2S and Na_2CO_3	1 ml.

All these tests, involving various media, and starting with the ammonification, to close with the production of H_2S, lasted for three weeks. The examinations of inoculated cultures took place daily.

A broth prepared with standard »Bacto-Trypthone Difco« was used to demonstrate the production of indole, availing ourselves of Kovacs's test.

The relation to the concentration of H-ions was examined by means of a standard broth (as above), adjusting the pH to range between 11,52 to 3,51. The optimum pH and the extreme concentrations of H-ions still allowing the growth were established in this way.

To examine the growth of our strains in the anorganic midst we applied an anorganic broth containing, pro 750 ml. of sea water and 250 ml. of distilled water:

K_2HPO_4	1 gr.
Ca Cl_2	1 gr.
Mg SO_4	0,4 gr.
KNO_3	0,4 gr.
pH 7,6	

The adjustment of the pH was obtained in all cases by a normal solution of Na OH.

Cylindric slices of potatoes were kept immersed in sea water for a period of 60 to 90 minutes to examine the growth on potatoes. The sterilisation took place in a Koch's kettle for 15 minutes on each of the successive three days.

We must point out that all the tests were repeated twice or three times at least to ensure a higher accuracy of the obtained results.

Having been described and isolated, the strains were determined by means of Bergey's classification handbook. The families of gnera being thus fixed we tried to compare our strains to the most closely related marine strains from the genus concerned. When making these comparisons we also availed ourselves of the works by Bergey (1948) and Zo Bell-Upham (1944) as well, which give the description of sixty new species of marine bacteria. The scope of our comparison has been to establish whether our species have already been described and — in the negative case — whether they are varieties of some already known species.

Owing to the incomplete descriptions frequently found in Bergey's work, we made better use of Zo Bell-Upham's work. We had many reasons to consider the results obtained by Zo Bell as most suitable for comparison with our results, since Zo Bell examined the marine bacteria by breeding them on media containing the same percentage of sea water as practised by us, and since we took the same characteristics as he did.

DESCRIPTION OF NEW STRAINS AND DISCUSSION

The examinations have shown that five out of these 13 strains belong to the species from the *Micrococcaceae* family, four of them being from genus *Micrococcus,* i.e. 241 — *M. aurantio-luteus* -- 249 *M. aeroroseus*

-- 250 — *M. inactivus* -- 261 — *M. leukos* and one from genus *Sarcina* -- 260 (1) *S. ocros.*

Seven of the remaining strains from the *Pseudomonadaceae family,* all of them belonging to genus *Pseudomonas;* 242 — *Ps. indolgena* -- 243 — *Ps. variabilis* -- 245 — *Ps. variabilis* var. a -- 247 — *Ps. variabilis* var. b -- 257 — *Ps. adriatica* -- 259 — *Ps. adhaerens* -- 260 (2) — *Ps. adhaerens* var. a.

One of the strains has been determined as genus *Bacillus* -- 258 — *Bacillus uniflagellaris.*

MICROCOCCACEAE

A) DESCRIPTIONS OF STRAINS

260 (1) — *Sarcina ocros* n.sp.

Morphological properties: Coccae averaging 1,43 microns in diameter. Occurring as sarcinae, tetrads, in pairs and singly. Gram positive. Well stained with gentian violet, methylene blue and fuchsin. Failing to retain acid fuchsin.

Growth on agar slant: Moderate growth, glistening lustre, filiform, crenulate edge, flat, rough surface, butyrous consistency.

Gelatin stab: No liquefaction, abundant, filiform, crenulate growth in the depth.

Growth in broth: No clouding, scanty, yellow, sandy sediment.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Scanty growth, colony of yellow colour.

Growth on potato medium (with distilled water added): Scanty growth, yellow colony.

Agar colony (48 hours): Punctiform, minimum elevation, uneven edges, opaque, yellow.

Gelatin colony: Punctiform, irregular under the microscope, minimum elevation, yellow.

Relation to temperature: Opt. 25 to 27° C, max. 45° C, min. 13° C.

H-ion concentration: Opt. 7,28, min. 6,11; growth still occurring at 11,52.

Chromogenesis in media: Agar and potato — yellow; gelatin — creamy-yellow.

Production of indole: None.

Production of hydrogen sulfide: Negative.
Hydrolysis of starch: None.
Relation to oxygen: Facultative anaerobic.
Relation to NO_3: No production out of NH_4; reduction of NO_3 to NO_2.
Relation to NH_3: No production of NH_3 out of peptone.
Relation to NO_2: No destruction of NO_2.
Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, dulcitol, and dextrose with the formation of acid but not gas. Lactose, glycerin, inuline, sarch, galactose, arabinose, maltose and sorbitol are not attacked (neither acid nor gas formation).
Habitat: Sea water.

261 — *Micrococcus leukos* n. sp.

Morplogocal properties: Coccae, singly, in pairs and masses, measuring 0,71 to 1,43 microns in diameter. Garm positive. Able to be stained with gentian violet and methylene blue, but not so well with fuchsin. Failing to retain acid fuchsin.
Growth on agar slant: Moderate growth, glistening lustre, filiform, elevated, opaque, butyrous consistency.
Gelatin stab: No liquefaction; filiform, crenulate growth along the stab line.
Growth in broth: Slight clouding; scanty, slimy, flocculent sediment.
Growth in anorganic broth: Absent.
Growth on potato medium (with sea water added): Moderate growth, colony of white colour.
Growth on potato medium (with distilled water added): Absent.
Agar colony (48 hours): Punctiform, crenulate edges, fine granular surface, translucent.
Gelatin colony: Circular, flat, entire edges, fine granular surface.
Relation to temperature: Opt. 25 to 27° C, max. 45° C, min. 13° C.
H-ion concentration: Opt. 6,11, max. 10,05, min. 3,51.
Chromogenesis in media: Agar, potato, and gelatin — white.
Production of indole: None.
Production of hydrogen sulfide: Negative.
Hydrolysis of starch: None.
Relation to oxygen: Facultative anaerobic.
Relation to NO_3: No production out of NH_4; reduction of NO_3 to NO_2.
Relation to NH_3: Production out of peptone, but scanty.
Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, lactose, sucrose, mannite and maltose with the formation of acid, but not gas. Xylose, dulcitol, glycerin, dextrose, inulin, starch, galactose, arabinose and sorbitol are not attacked (neither acid nor gas formation.).

Habitat: Sea water.

250 — *Micrococcus inactivus* n. sp.

Morphological properties: Coccae, singly, diplococcae, and in masses. Average diameter 1,06 micron. Gram-positive. Well stained with gentian violet, fuchsin, and methylene blue. Failing to retain acid fuchsin.

Growth on agar slant: Moderate growth, filiform, elevated, glistening, butyrous consistency.

Gelatin stab: No liquefaction. Filiform, crenulate growth in the depth.

Growth in broth: Clouding of medium duration; scently, slimy, yellowish sediment.

Growth in anorganic broth: Showing a scanty growth.

Growth on potato medium (with sea water added): From scanty to moderate; colony of yellow colour.

Growth on potato medium (with distilled water added): Absent.

Agar colony (48 hours): Uneven edges, circular, convex, fine granular surface.

Gelatin colony: Not completely even edges, circular, convex, fine granular surface.

Relation to temperature: Opt. 25 to 27° C, max. 55° C, min. 5—10° C.

H-ion concentration: Opt. 8,07 to 7,28, max. 10,05; no growth at 5,13.

Chromogenesis in media: Agar, potato, and gelatin — yellow.

Production of idole: None.

Production of hydrogen sulfide: Positive (after 10 to 15 days).

Hydrolysis of starch: None.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4; reduction of NO_3 to NO_2.

Relation to NH_3: No production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose and dextrose with the formation of acid, but not gas. Lactose, sucrose, xylose, mannite, dulcitol, glycerin, inulin, starch, galactose, arabinose, maltose, and sorbitol are not attacked (neither acid nor gas formation).

Habitat: Sea water.

249 — *Micrococcus aëreroseus* n. sp.

Morphological properties: Coccae singly, in pairs, in tetrads, and in masses. From 0,71 to 1,06 microns in diameter. Gram-positive. Well stained with gentian violet, but not so well with fuchsin and methylene blue. Failing to retain acid fuchsin.

Growth in agar slant: Abundant, filiform, glistening, raised, smooth surface, opaque, brittle consistency. Showing unmistakably rugose surface when bred for a longer period under laboratory conditions. Pink coloured.

Gelatin stab: No liquefaction; filiform, crenulate growth in the depth.

Growth in broth: Transient clouding (of short duration); slimy, scanty sediment, of pink colour.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Scanty growth, of pink colour.

Growth on potato medium (with distilled water added): Scanty growth, pink colony.

Agar colony (48 hours): Circular, convex, opaque, uneven edges, fine granular surface, pink colour.

Gelatin colony: Circular, convex, entire edges, fine granular surface, pink coloured.

Relation to temperature: Opt. 20 to 27° C, max. 40° C, min. 10° C.

H-ion concentration: Opt. 8,07 to 7,28, max. 11,52.

Chromogenesis in media: Agar — pink at aerobic growth; none at anaerobic growth; gelatin — pink on the surface; none in the depth; potato — pink.

Production of indole: None.

Production of hydrogen sulfide: Negative.

Hydrolysis of starch: None.

Relation to oxygen: Facultative anaerobic.

Relation to NO_2*:* No production out of NH_4; reduction of NO_3 to NO_2.

Relation to NH_3*:* No production out of peptone.

Relation to NO_2*:* No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, and dextrose with the formation of acid, bout not gas. Lactose, dulcitol, glycerin, inulin, starch, galactose, arabinose, maltose, and sorbitol are not attacked (neither acid nor gas formation).

Habitat: Sea water.

241 — *Micrococcus aurantio — luteus* n. sp.

Morphological properties: Coccae 1,11 to 0,71 micron in diameter, singly, diplococcae, less frequently tetrads and masses. Gram-positive. Well stained with gentian violet and methylene blue, but not so well with fuchsin. Failing to retain acid fuchsin.

Growth on agar slant: Moderate, filiform, convex, smooth, opaque, butyrous consistency, orange-yellow colour, frequently besprinkled with white.

Gelatin stab: No liquefaction; filiform, crenulate, uniform growth both on the surface and in the depth.

Growth in broth: Strong, transient clouding; scanty and slimy sediment.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Scanty growth, of orange-yellow colour.

Growth on potato medium (with distilled water added): Scanty growth, of orange-yellow colour.

Agar colony (48 hours): Punctiform, convex, entire edges, orange, fine granular surface, opaque.

Gelatin colony: Circular, fine, convex, crenulate edges, fine granular surface.

Relation to temperature: Opt. 25° C, max. 52° C, min. about 10° C.

H-ion concentration: Opt. 7,28 to 8,7, min. 5,13.

Chromogenesis in media: Agar — orange-yellow, frequently besprinkled with white; potato — orange-yellow; gelatin — orange-yellow on the surface, cream in the depth.

Production of indole: None.

Production of hydrogen sulfide: Negative.

Hydrolysis of starch: None.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_3; no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone, but scanty.

Relation to NO_2: No destruction.

Relation to carbohydrates: Able to attack glucose, sucrose, dextrose, and maltose with the formation of acid, but not gas. Lactose, xylose, mannite, dulcitol, glycerin, inulin, starch, galactose, arabinose, and sorbitol are not attacked (neither acid nor gas formation).

Habitat: Sea water.

Discussion

The strains, whose cells have a coccoid form, have shown a considerable amount of common properties. They are not spore-forming, and are Gram-positive. They occur singly, in pairs, in tetrads, in irregular masses, or in regular packets (sarcinae). All of them have an abundant growth in standard media. No growth occurs in anorganic media i.e. they are heterotrophic. They are facultative anaerobic, but their growth is more abundant under aerobic conditions. Most of them are pigment-producing (yellow, pink, or orange-yellow) and one of them is white in colour.

All these properties, among others, are characteristic for the *Micrococcaceae* family (B e r g e y, 1948). It is also characteristic for the members of this family that they frequently liquefy gelatin. However, none of the five strains under examination has shown that property.

As far as the genus *Micrococcus* is concerned, we could add to the above family characteristics that the glucose broth is turned acid and the lactose one remains generally neutral.

A peculiar feature of the genus *Sarcina* (which has all the characteristics of the family and of the genus *Micrococcus)* consists in its occurrence in packets of a regular form.

The presence of characteristics like these justify the classifications of our strains not only in the family *Micrococcaceae* but also in the genera *Micrococcus* and *Sarcina.*

It has already been pointed out that the examined genera were compared, after their classification, with the most closely related marine species from the genus concerned. By this way we have been able to notice the following:

1) That the genus 241 ist most closely related to *Micrococcus infimus* (Z o B e l l - U p h a m, 1948). Beside a series of common properties it possesses some particular ones, manifesting themselves in the pigment which is orange-yellow in the genus 241, and pink in *M. infimus;* in the reduction of nitrate to nitrite which is present in the genus 241, but much less in *M. infimus;* in the fermentation of mannite, as the genus 241 fails to attack it, whilst *M. infimus* is capable of fermenting it; in the growth in broth, as the genus 2441 produces strog clouding with a slimy sediment and unperceivable pigment of sediment whilst *M. infimus* produces only slight clouding and a flaky sediment of a pink colour. Since these differences are not insignificant, the genus 241, in our opinion, cannot be considered a variety of *M. infimus,* but a new species which has been defined by us as *Micrococcus aurantio-luteus.*

2) That the genus 249 in most closely related to *Micrococcus agilis* Ali — Cohen (Bergey, 1948).

These two genera share a large number of common properties. There are, however, some unmistakably differential characters also. They differ in motility, — while *Micrococcus agilis* moves by means of a single flagellum, the genus 249 does not exhibit any motion. Their Gram stain reaction is different — *M. agilis* is Gram-variable, while the genus 249 is Gram-positive. There is also a difference between the two compared genera as to their production of NH_3 on peptone; while *M. agilis* is able to reduce nitrates to nitrites, the genus 249 is not capable to attack nitrates. These two genera disclose also dissimilar actions in inulin broth: while *M. agilis* is responsible for fermentation, the genus 249 produces none.

It is, however, necessary to note that the kinds of sugar used during the examination of these two genera were not the same. It should also be noted that the author, dealing with morphological properties, dwelt longer upon the pigmentation and its variations. The forms of colonies and the growth on agar slant are not described in details. We still considered the perceived differences distinguishable enough to enable us to take the genus 249 as a separate species, which we have named *M. aëreroseus*. This hypothesis is supported to some extent by the fact that we find *M. agilis* to have the same properties in sea water, in fresh water, and in sea fishes, and as such, it would seem that it has not the tendency to vary.

3) That the genus 250 is most closely related to *Micrococcus infimus* (Zo Bell-Upham, 1944). Beside some common properties, the majority of which belong to the characteristics of the genus, there are evident differences between some of their physiological and morphological characters. Whilst *M. infimus is able to produce* NH_3 *out of peptone,* the genus 250 is not capable to do that. While *M. infimus* attacke maltose and mannite broths with the formation of acids, no fermentation is performed by the genus 250. While a scanty growth of *M. infimus* is produced on potato medium, beginning with a bright-pink colour which turns to orange with the aging, there is no growth of the genus 250. The pigmentation of *M. infimus* is pink, and that of the genus 250 is yellow. There is a flaky sediment in the ordinary broth inoculated with *M. infimus* and a slimy one in the case of the genus 250. An abundant growth is exibited by *M. infimus* in fresh water broth, but a scanty one by the genus 250. Whilst *M. infimus* produces a granular growth on agar slant, the genus 250 has a smooth one. The edges are entire in agar colonies of *M. infimus* and crenulate in those of the genus 250.

All these differnces evidently support the opinion that the genus 250 is a species, which we have named *Micrococcus inactivus*.

4) That the genus 261 is most closely related to *Micrococcus euryhalis* (Z o B e l l - U p h a m, 1944). Here also considerable departures go side by side with a series of accordances. Whilst *M. euryhalis* moves by means of one flagellum, the genus 261 is neither capable of motion nor possesses any flagella. The liquefaction of gelatin takes place slowly and incompletely in 50 days in the tormer genus, and it is quite absent in the latter one. While growth is present on potato medium inoculated with *M. euryhalis* and the medium is turned dark, it is absent in the genus 261. While no fermentation of sucrose in performed by *M. euryhalis,* that broth is attacked by the genus 261 with the formation of acid. These strains also depart in their sizes — *M. euryhalis* measuring 0,6 to 0,8 micron and the 261 from 0,71 to 1,43 microns in diameter.

Although the above differences are not numerous, we consider them distinctive enough to determine the genus 261 as a separate species: *Micrococcus leukos.*

5) That the genus 260 (1) belongs to the genus *Sarcina* and that it ist most closely related to *Sarcina pelagia* (Z o B e l l - U p h a m, 1944). Here again, a considerable amount of dissimilar properties goes side by side with a series of accordances. The difference is evident from their ability to liquefy gelatin: while it is stratiform in *S. pelagia,* no liquefaction at all is produced by genus 260 (1). These two genera behave quite differently on xylose, mannite, and sucrose media ,as neither of them is fermented bö *S. pelagia,* while the genus 260 (1) is able to attack them all with the formation of acid, but not gas. There is some difference also between the growths of these two genera on potato media, but growth was, however, noticed in both cases. The medium is turned dark by *S. pelagia,* but no alteration is caused by the genus 260 (1). While the gelatin colony is diffuse and concave in *S. pelagia* it is irregular and convex in the genus 265 (1). There is a marked difference between their respective growths in broth:

S. pelagia is ring-forming, exibits strong clouding with abundant viscid sediment, while the genus 260 (1) produces slight clouding after eight days' growth, which comes from the sediment attached to the sides of the tube, the bottom sediment being scanty and fine granular, like sand. They differ in diameter also, *S. pelagia* measuring 0,8 micron and the genus 260 (1) averaging 1,43 microns in diameter. All these differences

are sufficient, in our opinion, to consider the compared genera two separate species. We have named our genus *Sarcina ochros*.

Apart from the comparisons made in our laboratory between the examined genera and the most closely related among the described ones, we have also compared our genera from this family with each other and have noticed differences which justify their placing into separate species.

FAM. *PSEUDOMONADACEAE* — GENUS *PSEUDOMONAS*

A) DESCRIPTIONS OF GENERA

242 — *Pseudomonas indolgena* n. sp.

Morphological properties: Short rods with rounded ends, measuring 0,35 to 0,71 by 0,71 to 2,14 microns. Occurring singly, in pairs, in short chains, and in masses. The cells from older cultures (15 days) exhibit long, filiform form. Gram-negative. Well stained with gentian violet, not so well with fuchsin and methylene blue. Failing to retain acid fuchsin.

Growth on agar slant: Abundant growth, fine, hardly noticeably crenulate, flat, smooth, opaque, cream, butyrous consistency.

Gelatin stab: Total liquefaction of gelatin in fifteen days.

Growth in broth: Moderate and transient clouding; scanty, flocculent sediment; pellicle appearing later.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Absent.

Growth on potato medium (with distilled water added): Absent.

Agar colony: (48 hours) Circular, slightly convex, opaque, even margins, coarse granular surface.

Gelatin colony: Circular, even margins, concave, fine granular.

Relation to temperature: Opt. 25 to 26° C, max. 40° C, min. 13° C.

H-ion concentration: Opt. 7,28, min. 5,13; growth still occurring above 11,52.

Chromogenesis in media: Agar and gelatin — cream.

Production of indole: Indole-producing in broth with tryptone added.

Production of Hydrogen sulfide: No production in ordinary broth; production in agar after six days. Production in selective broth in very small quantities after ten days.

Hydrolysis od starch: Capable for hydrolizing starch, but not for forming sugars.

Relation to Oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4; reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, lactose, sucrose, xylose, mannite, glycerin, galactose, arabinose, and starch with the formation of acid, but not gas. Dextrose, inulin, maltose, and sorbital are not attacked (neither acid nor gas formation).

Habitat: Sea water.

243 — *Pseudomonas variabilis* n. sp.

Morphological properties: Short rods with rounded ends measuring 0,35 to 0,71 micron by 0,71 to 4,27 microns. Occurring singly and in pairs. Gram-negative. Well stained with gentian violet, but not so well with fuchsin and methylene blue. Failing to retain acid fuchsin. Motile by means of a single polar flagellum.

Growth on agar slant: Abundant growth, dull, diffuse, rugose surface, echinulate margins, brittle consistency, cream in colour. The denticulation of margins varies from a slightly undulated to a more or less crenulate one.

Gelatin stab: Total liquefaction of gelatin in fifteen days.

Growth in broth: Pellicle and ring on the surface. Clouding of medium duration; scanty, flocculent sediment.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Absent.

Growth on potato medium (with destilled water added): Absent.

Agar colony: (48 hours) Circular, even margins, rough surface, convex, with radial rays. The edges of agar colonies vary between even and uneven ones.

Gelatin colony: Irregular form, rough surface, concave, diffuse edges.

Relation to temperature: Opt. 25 to 30° C, max. 44° C, min. about 10° C.

H-ion concentration: Opt. 6,11, max. 10,5; no growth at 5,13.

Chromogenesis in media: Agar and gelatin — cream.

Production of indole: None.

Production of hydrogen sulfide: Negative.

Hydrolysis of starch: Capable of hydrolyzing starch; scanty production of sugar.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4; no reduction of NO_3 to NO_2.

Relation to NH_3: Scanty production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, dulcitol, dextrose, inulin, starch, galactose, arabinose, maltose, and sorbitol, with the formation of acid, but not gas. Lactose and glycerin are not attacked.

Habitat: Sea water.

245 — *Pseudomonas variabilis* var. a

Morphological properties: Short single rods, occurring also in pairs; rounded ends measuring 0,35 to 0,71 micron by 0,71 to 2,86 microns. Gram-positive. Well stained with gentian violet, not so well with fuchsin and methylene blue. Failing to retain acid fuchsin. Motile by means of a single polar flagellum.

Growth on agar slant: Abundant growth, diffuse, glistening, convex, smooth, opaque, opalescent, slimy consistency. The denticulation of margins varies from a crenulate to an undulated one.

Gelatin stab: Liquefies gelatin, cylindrically in the beginning, and totally in eighteen days.

Growth in broth: Strong and persistent clouding, ring and pellicle; abundant, flocculent sediment, yellow in colour.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Scanty growth, light brown colony.

Growth on potato medium (with distilled water added): Absent.

Agar colony: (48 hours) Circular, convex, opaque, even edges, coarse granular suface.

Gelatin colony: Circular, concave, diffuse edges, fine granular surface.

Relation to temperature: Opt. 25 to 30° C, max. 46 to 47° C, min. about 10° C.

H-ion concentration: Opt. 6,11, max. 10,05; no growth at 5,13.

Chromogenesis in media: Agar and gelatin — cream; potato — light brown.

Production of indole: None.

Production of hydrogen sulfide: None.

Hydrolysis of starch: Hydrolyzing starch; scanty production of sugar.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4; no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, dulcitol, glycerin, dextrose, inulin, starch, galactose, arabinose, maltose, and sorbitol with the formation of acid, but not gas. Lactose is not attacked (no acid formation).

Habitat: Sea water.

247 — *Pseudomonas variabilis* var. b.

Morphological properties: Short rods, occurring singly and in pairs, rounded ends, measuring 0,35 to 0,71 micron by 0,71 to 1,78 microns. Gram-negative. Well stained with gentian violet, not so well with fuchsin and methylene blue. Failing to retain acid fuchsin. Motile by means of a single polar flagellum.

Growth on agar slant: Moderate growth, crenulate, diffuse, glistening, opaque, smooth suface, brittle consistency. The denticulation of margins varies from a fine crenulate to a more undulated one.

Gelatin stab: Liquefying gelatin cylindrically in the beginning and totally in fifteen days.

Growth in broth: Slight, presistent clouding. Pellicle on the surface; scanty flocculent sediment.

Growth in anorganic broth: Absent.

Growth on potato medium (with sea water added): Absent.

Growth on potato medium (with distilled water added): Absent.

Agar colony: (48 hours) Circular, convex, fine crenulate edges, fine granular and opaque surface.

Gelatin colony: Circular, concave, diffuse edges, fine granular surface.

Relation to temperature: Opt. 25 to 27° C, max. 43° C, min. below 10° C.

H-ion concentration: Optimum 6,11, max. 10,05; no growth at 5,13.

Chromogenesis in media: Agar — light brown; gelatin — creamy light brown.

Production of indole: None.

Production of hydrogen sulfide: None.

Hydrolysis of starch: Hydrolyzes starch, scanty production of sugar.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4; no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, dulcitol, glycerin, dextrose, inulin, starch, galactose, and maltose, with the formation of acid, but not gas. Lactose and arabinose not attacked (no acid formation).

Habitat: Sea water.

257 — *Pseudomonas adriatica* n. sp.

Morphological properties: Short rods with rounded ends measuring 0,30 to 0,71 micron by 0,71 to 1,06 microns. Occurring singly. Gram-negative. Well stained with gentian violet, moderately with fuchsin, and slightly with methylene blue. Failing to retain acid fuchsin. Motile by means of a single polar flagellum.

Growth on agar slant: Abundant growth, glistening, opaque, light brown, diffuse, convex, smooth surface, butyrous consistency, beaded structure in the central part.

Gelatin stab: No liquefaction of gelatin; scanty growth, uniform both on the surface and in the depth.

Growth in broth: Ring and pellicle on the surface. Strong and persistent clouding; scanty and flocculent sediment.

Growth in anorganic broth: Scanty growth, scanty sediment within 22 days.

Growth on potato medium (with sea water added): Abundant growth, light brown — brown in colour.

Growth on potato medium (with distilled water added): Absent.

Agar colony: (48 hours) Punctiform, circular, slightly convex, even edges, fine granular surface.

Gelatin colony: Circular, convex, translucent, even edges, fine granular surface.

Relation to temperature: Opt. 25° C, max. 35 to 36° C, min. 13° C.

H-ion concentration: Opt. 8,07 to 6,11 max. 11,52.

Chromogenesis in media: Agar and gelatin — light brown; potato — brown.

Production of indole: None.

Production of hydrogen sulfide: None.

Hydrolysis of starch: Hydrolyzing starch slowly, not starting before the tenth day.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3*:* No production out of NH_4; no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: able to attack glucose, sucrose, xylose, dulcitol, dextrose, and sorbitol, with the production of acid, but not gas. Not attacking lactose, mannite, glycerin, inulin, starch, galactose, arabinose and maltose (no acid production).

Habitat: Sea water.

259 — *Pseudomonas adhaerens* var. a.

Morphological properties: Long rods with rounded ends, measuring 0,28 to 0,71 micron by 0,71 to 2,86 microns. Some are slightly curved. Occurring singly and in pairs. Gram-positive. Well stained with gentian violet and methylen blue, not so well with fuchsin. Failing to retain acid fuchsin. Motile by means of a single polar flagellum.

Growth on agar slant: Moderate growth, filiform, crenulate edges, glistening, opaque, opalescent, butyrous consistency. When bred for some time under laboratory conditions, the margins exhibit a rhizoid — flocculent growth.

Gelatin stab: Slow, cylindrical liquefaction — 0,6 to 0,7 cm in 15 days, 3,5 cm in 30 days.

Growth in broth: Slight, transient clouding; scanty sediment, sandlike, beaded.

Growth in anorganic broth: Scanty growth, scanty sediment in 22 days.

Growth on potato (with sea water added): Abundant growth, colony of a light brown — brown colour. Medium turned dark.

Growth on potato (with destilled water added): Abundant growth, colony of a cream colour, darker in the middle.

Agar colony (48 hours): Circular, concave, diffuse edges, curled and granular surface.

Relation to temperature: Opt. 25 to 27° C, max. 54° C, min. about 13° C.

H-ion concentration: Opt. 8,07 to 7,28, max. 11,25, no growth at 5.

Chromogenesis in media: Agar and gelatin — cream; potato with sea water — light brown — brown; potato with distilled water — darker cream.

Production of indole: None.

Production of hydrogen — sulfide: No production in ordinary broth; in selective broth (for the reduction of sulfite) production starting on the sixth day.

Hydrolysis of starch: Hydrolyzes starch with a slight production of sugar.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NH_4, no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone, but slight.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, mannite, glycerin, and dextrose with the production of acid, but not gas. Not attacking lactose, xylose, dulcitol, inulin, starch, galactose, arabinose, maltose, and sorbitol (no acid formation).

Habitat: Sea water.

260 (2) — *Pseudomonas adhaerens* n. sp.

Morphological properties: Short rods with even ends, some slightly curved. Measuring 0,28 to 0,71 micron in width and 1,43 to 6,40 microns in length. Occurring singly and in pairs. Gram-positive. Well stained with gentian violet and methylene blue, not so well with fuchsin. Failing to retain acid fuchsin. Motile by means of a single flagellum.

Growth on agar slant: Moderate growth, diffuse in the lower part, filiform, glistening, crenulate, surface mildly rugose, opalescent, slimy consistency. When bred for a longer period under laboratory conditions, rhizoid-flocculent growth appearing on edges.

Gelatin stab: Slow liquefaction of gelatin, 1 cm in 30 days.

Growth in broth: Slight, persistent clouding, abundant sediment, slimy ragged.

Growth in anorganic broth: Scanty growth, poor sediment in 22 days.

Growth on potato medium (with sea water added): Abundant growth, colony of a light brown — brown colour, darkening the medium.

Growth on potato medium (with distilled water added): Abundant growth, colony of a light brown — brown colour.

Agar colony: (48 hours) Circular, slightly convex, diffuse edges, fine granular surface. When attached to each other, they tend to curl.

Gelatin colony: Circular, concave, fine granular surface, diffuse edges.

Relation to temperature: Opt. 23 to 25° C, max. 57 to 58° C, min. about 10° C.

H-ion concentration: Opt. 8,07 to 7,28, max. 10,05, min. 6,11.

Chromogenesis in media: Agar and gelatin — cream; potato with sea water added — light brown-brown; potato with distilled water added — creamy-brown.

Production of indole: None.

Production of hydrogen sulfide: No production in ordinary broth; production starting after 10 days in selective broth (for the reduction of sulfates).

Hydrolysis of starch: Not hydrolyzing starch.

Relation to oxygen: Facultative anaerobic.

Relation to NO_3: No production out of NO_3; no reduction of NO_3 to NO_2.

Relation to NH_3: Production out of peptone, but scanty.

Relation to NO_2: No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, mannite, glycerin, and dextrose with the production of acid, but not gas. Not attacking lactose, xylose, dulcitol, inulin, starch, galactose, arabinose sorbitol, and maltose (no acid formation).

Habitat: Sea water.

DISCUSSION

None of the seven strains from the *Pseudomonadaceae* family, i.e. from the genus *Pseudomonas,* has shown the ability of spore formation. All of them are motile by means of a single polar flagellum. All of them ferment glucose with the production of acid, but not gas. They are mostly inactive in lactose broth. They are Gram-negative with rare exceptions. Beside these properties, which constitute the characteristics of the genus *Pseudomonas,* it should be added that this genus frequently reduces nitrates in a rapid way (Bergey, 1948). However, there was no reduction present in most of our cases.

A comparison between our strains and the most closely related species was made in the same way as already exposed on the foregoing pages.

The strain 242 is most closely related to the species *Pseudomonas ichtyodermis* (Zo Bell-Upham, 1944). Beside a number of common properties, which are often identical to the minutest details, these two species show some important differing characters. The difference between the strains consists in their ability to produce capsules. Whilst *Ps. ichtyodermis* is capsule producing, the strain 242 is unable to form capsules. On potato medium with sea water added, *Ps. ichtyodermis* exhibits

nonpigmented growth, while no growth at all is present in the strain 242. Fermentation of maltose broth with the production of acid is caused by the former, but not by the latter.

Lactose broth is not attacked by *Ps. ichtyodermis,* but the strain 242 utilizes it with the formation of acid. There are further differences with regard to their sizes: *Ps. ichtyodermis* varies from 0,9 to 1,3 microns in width and from 0,71 to 3,5 microns in length and the strain 242 measures 0,25 to 0,71 micron in width and 0,71 to 2,14 microns in length.

Although their common properties are in the majority, we still consider them outstanding and characteristic enough to abstain from calling the strain 242 a variety of *Pseudomonas ichtyodermis,* and to place it as a separate species, calling it *Pseudomonas indolgena.*

The strain 243 is most closely related to the species *Pseudomonas membranula* (Z o B e l l - U p h a m, 1944). The physiological properties of these two strains agree almost completely. Their behaviour in mannite broth is different: *Ps. membranula* does not attack it, but the strain 243 ferments it with the formation of acid. There is a difference between these two strains in production of pigments in gelatin medium: *Ps. membranula* produces a yellowish pigment, but no pigment is present in the strain 243. Different growths are also exhibited in ordinary broth: whilst no pellicle if formed by *Ps. membranula,* the strain 243 is both pellicle and ringforming. Their growths on agar slant disclose further dissimilarities: *Ps. membranula* has a poor, filiform growth, exhibiting butyrous consistency, and the strain 243 has an abundant filiform growth, crenulate edges and brittle consistency. Agar colonies are entire and smooth in *Ps. membranula* and with radial rays in the strain 243. The strains differ also in their sizes. *Ps. membranula* varies from 0,4 to 0,5 micron in width and from 1,2 to 2,4 microns in length ,and the strain 243 measures 0,35 to 0,71 micron in width and 0,71 to 4,27 microns in length. Differences are also evident in the optimal temperatures of growth of these strains.

The dissimilarities of the compared strains, though mostly of morphological nature, are unmistakable and numerous. There are, besides, physiological differences also. On the ground of these facts we suppose that the strain 243 represents a separate species which has been called *Ps. variabilis.*

The strain 245 is also most closely related to *Pseudomonas membranula* (Z o B e l l - U p h a m, 1944). As in the foregoing cases, the strains differ in their ability to ferment mannite broth. Unlike *Ps. membranula,* the

strain *245* utilizes mannite broth with the formation of acid. While no noticeable growth is exhibited by *Ps. membranula* in fresh water broth, there is a scanty growth in the strain 245. In ordinary broth, moreover, *Ps. membranula* produces moderate clouding without pellicle and with a poor sediment, while the strain 245 exhibits strong clouding, forming ring and pellicle, as well as abundant sediment. The growth on agar slant is scanty, filiform and butyrous in the former strain, and abundant, filiform and slimy in the latter. The strains differ with regard to their sizes also, but not so much as in the foregoing case. *Ps. membranula* measures 0,4 to 0,5 micron in width and 1,2 to 2,4 microns in length, while the strain 245 varies from 0,35 to 0,71 micron in width and from 0,71 to 2,85 microns in length.

As to optimal temperatures, the differences are the same as in the case of the strain 243. The optimal temperature in *Ps. membranula* ranges, namely, between 20 and 25°C, and in the *strains 245* it vaires from 25 to 30° C. Here also we find that the strain 245 differs from *Ps. membranula* to an extent which does not allow to consider it as a variety of the latter.

The strain 247, like the two foregoing ones, is most closely related to *Pseudomonas membranula* (Zo Bell-Upham, 1944). Yet they differ in their behaviour in mannite broth. As in the foregoing cases, *Ps. membranula* does not attack mannite, but the *strain 247* ferments it with the producion of acid. While there is no growth of *Ps. membranula* in fresh water broth, the strain 247 exhibits scanty growth. They also vary with regard pellicle-forming in ordinary broth, as no pellicle is formed by Ps. membranula while the strain 247 is pellicle-forming. There is a distinct difference in their lengths also, *Ps. membranula* measuring 1,2 to 2,4 microns and the strain 247 having a considerably smaller length, i. e. 0,71 to 1,78 microns. The optimal temperature ranges between 20 and 25° C for *Ps. membranula* and between 25 and 27° C for the strain 247. Owing to these differing characters, we do not consider the strain 247 a variety of the species Ps. membranula.

The strain 257 is most closely related to *Pseudomonas xanthochrus* (Zo Bell-Upham 1944). These two strains differ in their behaviour in glucose broth. While no bermentation is produced by *Ps. xanthochrus,* the strain 257 utilizes it with the formation of acid. Neither sucrose broth is attacked by the former, whereas the strain 257 is able to ferment it with the formation of acid. Xylose broth is not fermented by *Ps. xanthochrus,* but it is attacked by the strain 257 with a slight formation of acid. While

the former has 1 to 2 flagella on each pole, unfrequently also more than that, our strain has but a single polar flagellum. There is a difference in their chromogeneses also: *Ps. xanthochrus* produces a yellow pigment, but the strain 257 develops no pigment at all. Neither do their agar colonies agree between themselves, as the former produces an entire and smooth one, whereas that produced by the latter exhibits a darker center with radial rays, not reaching to the edges of the colony. They produce different growths on agar slant: *Ps. xanthochrus* has a scanty, dark, filiform growth, pigmented waxy-yellow, and the strain 257 has an abundant growth with undulate edges, exhibiting granular structure in the central part along the smear; it is filiform, glistening, butyrous, bright brown in colour. Their sizes are different, varying from 0,3 to 0,7 micron in width and from 1,6 to 3,2 micron in length in *Ps. xanthochrus* and from strain 257.

0,4 to 0,5 micron in width and from 0,71 to 1,6 microns in length in the

The differences stated above unmistakably confirm that the strain 257 should be considered as a separate species which we have called *Pseudomonas adriatica.*

The strain 259 is most closely related to *Pseudomonas periphyita* (Z o Bell-Upham, 1944).

In spite of their close relationship these two marine strains differ from each other considerably. While *Ps. periphyta* ferments maltose and lactose broths alike, with the formation of acid, the strain 259 does not utilize them. But their behaviour is reverse in mannite and sucrose broths: here the former does not attack them, but the latter does, with the formation of acid. While no growth is exhibited by *Ps. periphyta* on potato medium, the strain 259, on the contrary, has an abundant growth. The former is Gram-negative, and the latter Gram-positive. The growth of *Ps. preiphyta* in ordinary broth exhibits strong clouding, flocculent sediment and pellicle, whereas slight clouding, sandy sediment and no pellicle are present in the growth of the starin 259. The former has 1 to 2 flagella on each pole and the latter has a single polar flagellum. The growth of *Ps. periphyta* is profuse on agar slant and brown pigment is developed in agar, whereas moderate, nonpigmented growth is produced by the strain 259 on that medium. While *Ps. periphyta* does not occur in chains, the strain 259 occurs in shorter ones. Their sizes differ likewise: while *Ps. periphyta* measures 0,6 to 0,7 micron by 1,9 to 4,0 microns, the strain 259 measures 0,28 to 0,71 micron by 0,71 to 2,86 microns. Optimum temperature for *Ps. periphyta* is 20 to 25° C and for the strain 259 it is betwen 23 and 27° C.

Considering all these differences we cannot say that the strain 259 is a variety of *Ps. periphyta,* but a separate species which we have named *Pseudomonas adhaerens* var. a.

The strain 260 (2) is also most closely related to *Pseudomonas periphyta* (ZoBell-Upham, 1944). There are, however, evident differences in this case too. Thus, *Ps. periphyta* is able to ferment lactose broth with the formation of acid, but the strain 260 (2) does not attack it. While *Ps. periphyta* utilises mannite and sucrose broths, the strain 260 (2) fails to ferment them. No growth is exhibited on potato medium by the former, but an abundant growth, creamy-brown in colour, is produced by the strain 260 (2) tend to curl. The agar slant growth of *Ps. periphyta* is is produced by *Ps. periphyta* in ordinary broth, whereas the strain 260 (2) produces slight clouding with abundant, slimy-flocculent sediment and pellicle. Their Gram reactions are different: the former is Gram-negative and the latter Gram-positive. Agar colonies have crenulate edges in *Ps. periphyta* and the edges are undulate in the strain 260 (2), tending to curl, and unfrequently exhibiting rhizoid outgrowth. Connected colonies of the strain 260 (2) tend to curl. The agar slant growth ef *Ps. periphyta* is profuse, of butyrous consistency, and developing brown pigment in agar. The strain 260 (2) exhibits, on the contrary, a moderate growth, widening in the lower part and filiform in the upper one, with crenulate margins. Their sizes are different, *Ps. periphyta* measuring 0,28 to 0,36 micron by 1,43 to 6,0 microns, and the strain 260 (2) measuring 0,6 to 0,7 micron by 1,43 to 2,86 microns.

Here also, as in the foregoing case, the characters of the compared strains differ to such an extent that we are allowed to suggest a separate species which we have named *Pseudomonas adhaerens.*

By comparing our strains one to another we have found similar properties in strains 243, 245 and 247. These three strains have more common characters than any of our strains in comparison with the most closely related marine ones. This is evident from the complete agreement of both the common and the differing characters with those of the next described marine species which is one and the same for all the three strains, i. e. *Pseudomonas periphyita.* The differences exhibited by these three strains are secudary ones and are mildly expressed as shown in the following table containing the characters of dissimilarity only (tabl. I.)

It has been noticed in the course of these researches that there are individual variations of crenulate margins on agar slant in all the three cases. The margins were either undulate, or slightly crenulate, or expressly

Tabl. I.

Dissimilar Characters in Strains 243, 245, and 247.

Dissimilar Characters	Strain 243	Strain 245	Strain 247
Gelatin colony	Uneven, rough surface, diffuse margins	Circular, smooth	Circular, crenulate margins with darker center
Agar colony	Even margius, with radial rays	Even margins, coarse granular surface	Fine crenulate margins with dark center
Agar slant	Abundant growth, brittle consistency	Abundant growth, slimy consistency	Moderate growth, slimy consistency
Ordinary broth	Moderate clouding, ring and pellicle, poor sediment	Strong clouding, ring, pellicle, abundant sediment	Slight clouding, pellicle, poor sediment
Freshwater broth	No growth	Scanty growth	Scanty growth
Potato with sea water	No growth	Scanty growth, yellowish-brown in colour	No growth
Maximal temperature	44° C	46—47° C	43° C
Length	0,71 to 4,27 microns	0,71 to 2,86 microns	0,71 to 1,78 microns

crenulate. The agar colonies of the strain 243, however, exhibited both even and crenulate margins.

All the three strains were isolated from the same sample. They differ only inconsiderably and disclose no definite dividing line indispensable in case of separate species Variable edges as noticed on agar slant suggest that these three strains are varieties. It is rather difficult to say which of the three is the species of which the other two are varieties, as no definite stability was noticed in one of them to contrast with the instability of the other two. They always exhibited the same properties, with the exception of the already mentioned variability on agar slant, which occured in all the three alike. The variation from even to crenulate margins in the agar colonies of the strain 243, can really be of little help in making our decision

on thise subject. Are there here not, perhaps, three varieties of a species which we have not yet encountered?

In the course of comparing the examined strains with each other we have also noticed an evident likeness between the strains 259 and 260 (2). The characters in which these two strains differ are shown in the following table.

Tabl. II.

Dissimilar Characters Batween the Strains 259 and 260 (2).

Dessimilar Characters	Strain 260 (2)	Strain 259
Hydrolysis of starch	Not hydrolyzing	Hydrolyzing, but feebly; scanty production of glucose
Ordinary broth	Rich sediment, slimy, flocculent	Poor sediment, sandy-granular
Extreme pH still allowing growth	10,05	11,52
Temperature of growth	Opt. 23 to 25° C max. 57 to 58° C min. about 10° C	Opt. 23 to 27° C max. 54° C min. about 13° C
Occurring	Singly, in pairs, and in shorter chains.	Singly and in pairs

There is a conspicuous morphological likeness between these two strains and a number of their physiological character also completely coincide with each other. Most of their differences, however, are of physiological nature, but they are not very distinct. Their ability to ferment starch is particularly different. We must add here that the strain 259 which hydrolyzes starch only feebly, was twice found inactive in this broth (twice exhibiting a feeble hydrolysis and twice failing to hydrolyze at all), whereas the stain 260 (2) always remained inactive in this broth.

Yet neither of the two strains showed any deviation from other properties. The results we obtained when repeating our examinations were the same as yielded by the previous ones. Owing to its variable relation to hydrolysis of starch we suppose that the strain 259 is a variety of the strain 260 (2), which we have called *Pseudomonas adhaerens*.

FAMILY *BACILLACEAE* — GENUS *BACILLUS*

A) DISCRIPTIONS OF STRAINS

258 — *Bacillus Uniflagellaris,* n. sp.

Morphological properties: Long rods measuring 0,43 to 0,99 micron by 0,71 to 7,11 microns, with rounded ends. Occurring singly or in pairs, but most frequently in chains. At a temperature of 44° C appear longer, rod-shaped forms, curved as vibrios. Gram-positive. Well stained with gentian violet and methlyene blue, but not so well with fuchsin. Failineg to retain acid fuchsin. Spore-forming. Motile by means of a single polar flagellum.

Growth on agar slant: Abundant growth, crenulate, flat, diffuse, rugose surface, opaque, brittle consistency, white in colour.

Gelatin stab: Total liquefaction of gelatin after a fifteen days' growth.

Growth in broth: Slight, persistent clouding, abundant, flocculent sediment.

Growth in anorganic broth: Absent.

Growth on potato (with sea water added): Profuse growth, white in colour.

Growth on potato (with distilled water added): Abundant growth, white colony.

Agar colony: (48 hours) Iregular, curved margins, opaque fine granular surface.

Gelatin colony: The form of the colony not noticeable owing to quick liquefaction of gelatin.

Relation of temperature: Opt. 25 to 27° C, max. 44 to 46 C, min. about 12° C.

H-ion concentration: Opt. 7,28 to 6,11 max. 11,52 growth still present at 5,13.

Chromogenesis in media: Gelatin, agar, and potato-white.

Production of indole: None.

Production of Hydrogen sulfide: None.

Hydrolysis of starch: Hydrolyzes starch whith a slight formation of sugar.

Relation to Oxygen: Facultative anaerobic.

Relation to NO_3*:* No production from NH_4; reduction of NO_3 to NO_2.

Relation to NH_3*:* Production from peptone.

Relation to NO_2*:* No destruction of NO_2.

Relation to carbohydrates: Able to attack glucose, sucrose, xylose, mannite, dextrose, maltose, and sorbitol, with the formation of acid, but not gas. Not attacking lactose, dulcitol, inulin, starch, galactose, and arabinose (no acid formation).

Habitat: Sea water.

B) DISCUSSION

The strain 258, according to its characters, belongs to *Bacillaceae* family, i. e. to the genus *Bacillus,* but it is motile by means of a single flagellum. In his Manual of Determinative Bacteriology (1948). Bergey gives the characteristics of this family and adds: »Rod-shaped cells capable of producing spores, either with peritrichous flagella or nonmotile; monotrichous flagellation has been reported but is doubtful.«

According to the characters of the genus *Bacillus,* i.e. rod-shaped forms, sometimes in chains, sporangia usually do not differ from vegetative cells. At times they show rough colonies. As they usually oxydize carbohydrates more or less completely with the formation of acid, but without the accumulation of characteristic products, these strain would correspond to the genus *Bacillus.* The occurrence of spores in this strain does not allow the hypothesis that it belongs to the genus *Pseudomonas,* which is most frequently represented by forms with a single polar flagellum. The Bergey Manual, moreover, dealing with a series of species from the genus Bacillus, contains no reference to the number and position of their flagella, but only the statement that they are motile. By considering all these facts we assume that the strain 258 belongs to the genus *Bacillus.*

The strain 258 is most closely related marine strain to *Bacillus thalassokoites* (Zo Bell-Upham, 1944). Beside a large number of common properties owned by these two strains, there are also considerable differences. *Bacillus thalassokoites* is capsule-forming, but not so the strain 258. The former is peritrichous and the latter has a single polar flagellum. Their ability to develop pigment is different (on potato medium only), as the former produces a bright yellow pigment on this medium, and the latter develops none (on the other employed media either), and does not turn potato dark. *B. thalassokoites* produces a heavy membraneous pellicle and a scanty flocculent sediment in ordinary broth, whereas the strain 258 exhibits no pellicle but has an abundant flocculent sediment. Their growths on agar slant differ likewise, *B. thalassokoites* producing a moderate growth of butyrous consistency, and the strain 258 showing an abundant,

flat, rugose growth of fragile consistency. They differ in sizes too, *B. thalassokoites* measuring 0,4 to 0,5 micron in width and 1,6 to 2,8 microns in length, and the strain 258 measuring 0,43 to 0,99 micron in width and 0,71 to 7,11 microns in length. These diferences baing significant enough, we do not consider the strain 258 as a variety of *B. thalassokoites* but as a separate species which we have called *Bacillus uniflagellaris.*

SUMMARY

Examinations of some bacterial strains present in the Adriatic have been carried out. Descriptions and classifications are given for thirteen bacterial strains found in the Kaštela Bay.

Five out these thirteen strains belong to the family *Micrococcaceae* i. e. four from the genus *Micrococcus,* and one from the genus *Sarcina.*

Seven out of these thirteen strains belong to the family *Pseudomonadaceae,* all of them representing the genus *Pseudomonas.* While four out of these seven strains represent separate species, three of them are considered varieties. It is assumed that one of these strains is a variety of the described species called *Pseudomonas adhaerens.* But the question remains open whether two of the three strains are varieties of the third one, characterized as a separate species, Pseudomonas variabilis, or are, perhaps, all the three only varieties of a hitherto not described species.

One of the examined strains is considered representative of the family *Bacillaceae,* that is from the *Bacillus.*

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O NEKIM NOVIM MORSKIM BAKTERIJAMA

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Kratak sadržaj

Izvršena su ispitivanja nekih bakterijskih sojeva Jadrana. Izneseni su opisi i klasifikacija za trinaest bakterijskih sojeva, koji su nađeni u Kaštelanskom zaljevu.

Od ovih sojeva pet vrsta pripada porodici *Micrococcaceae,* i to: četiri iz roda *Micrococcus,* a jedna iz roda *Sarcina.*

Sedam sojeva pripada porodici *Pseudomonadaceae.* Oni su svi predstavnici roda *Pseudomonas.* Četiri predstavljaju posebne vrste pod imenom *Pseudomonas adhaerens.* Međutim, za tri soja ostavilo se otvoreno pitanje, da li dva od njih predstavljaju varijetet trećega, koji smo obilježili kao posebnu vrstu *Pseudomonas variabilis,* ili su možda sva tri varijeteti neke vrste, koja do sada nije opisana.

Za jedan dio ispitanih sojeva smatra se da je pripadnik porodice *Bacillaceae,* i to iz roda *Bacillus.*

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