NASA TECHNICAL TRANSLATION NASA TT F- 14439

MICROCOCCUS PIERANTONII. NEW SPECIES OF PHOTOGENIC BACTERIA OF THE LUMINOUS ORGAN OF RONDELETIA MINOR NAEF.

G. Zirpolo

Translation of "Micrococcus pierantonii. Nuova specie di batterio fotogeno dell'organo luminoso di Rondeletia minor NAEF", Bollettino della Societa di Naturalisti, Vol. 32?, 1919, pp. 75-87.

(NASA-TT-F-14439) MICROCOCCUS PIERANTONII. NEW SPECIES OF PHOTOGENIC BACTERIA OF THE LUMINOUS ORGAN OF RONDELETIA MINOR NAEF G. Zirpolo (NASA) Jun. 1972 20 p CSCL 06M

N73-11072

G3/04 Unclas 46399

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 JUNE 1972

SOURCE: SOCIETA DI NATURALISTI
30-31; 1917-18

TITLE: MICROCOCCUS PIERANTONII.

New species of photogenic bacteria of the luminous organ of Rondeletia Minor NAEF.

Professor Dr. Giuseppe Zirpolo, member of the Society.

(Session of August 18, 1918)

Summary

Introduction

These investigations are a sequel to my two preceeding notes in which I dealt with the photogenic bacteria that develop on the skins of Sepia Officinalis L. and with those that occur in the luminous organs of Sepiola Intermedia NAEF[1].

In the present work I deal with a coccus that is alive and that constitutes the photogenic body of the luminous organ of Rondeletia Minor NAEF.

This coccus is a species new to science, not possessing the characteristics of the Micrococcus phosphoreus found by Cohn[2], in 1878, on the flesh of Salmon, following the data taken by Pflueger, nor those of the Micrococcus Pfluegeri studied by Ludwig[3], in 1887, and found by Molisch[4] on ox flesh in 1902. /76

This species I dedicated to Professor Umberto Pierantoni also because of the distinguished contribution made by him to the study of bacterial symbiosis, especially in the phenomena of bioluminescence[5].

Subject of the Study, and Technique

The material under examination is not easily obtainable, because the Rondeletia Minor NAEF lives at a depth of about 200 meters, and off Posillipo Cape (Ammontatora). It is therefore necessary to make catches specially adapted to finding it. And for this I must warmly thank the President of the Zoological Station who for the last two years was able to supply me several times with the material mentioned.

The animal would live in the basins of the little aquarium in my room for a few hours after it had been caught. It was rather slow in its movement, preferring to remain at the bottom of the basin. If one excited it, it would come up to the surface, and then creep again into a nook at the bottom. /77

If the mantle is opened, in the venntral region, one perceives an organ with lenticular shape, of yellowish color, sunk deep into the ink sac[6].

In darkness it appears luminous, like a small lamp with a light green light. On excitation with the point of a needle, the surrounding water can be observed to light up slightly, through the escape of the bacteria from the organ.

To collect the bacteria I would isolate the organ, staining it with sterile water several times, to make the surface free of the bacterial flora that might be in the external environment, so far as it was possible for me to do this.

I would then pulp the organ in a small sterile glass and make inoculations of it in a broth of cuttlefish and agar. Twenty-four hours later there would appear in the agar small colonies of a very intense green, and the broth also would show a quite vivid luminosity, which remained for a period of three or four days always the same, and then diminish gradually over the following days.

Continuous inoculations gave colonies constantly constituted by a luminous coccus which I thought I would study just because it was perfectly new to science. In the succeeding cultures and also in the old ones, there would be a coccobacillary form. This confirms the observations of Pierantoni, who in the transverse sections made of the luminous organ saw some coccobacilli in the peripheral region of the organ and some cocci in the central region.

Both these forms were luminous, and were produced one by the other — the coccobacillus by the coccus. These observations were repeated in numerous examples experienced, nor on the other hand could pollutions be assumed there, when both the forms would appear always luminous.

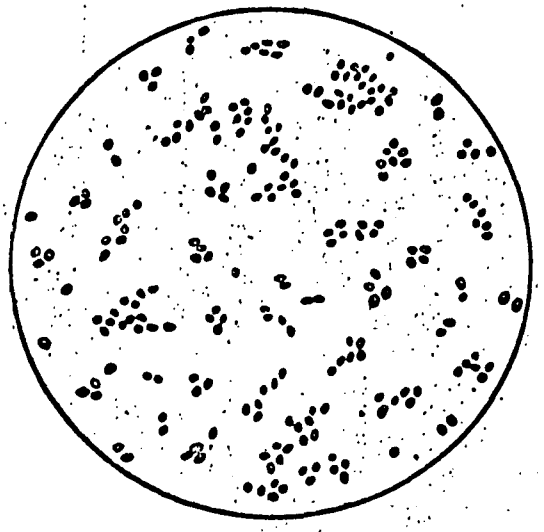
/78

The variations, besides, in the forms depends to a great extent on the culture ground. A greater or lesser concentration of salts provokes not only a luminous variation, but modifies also the form of it. In the various cultures made, I did several times prepare fresh agar and was able to note how I always, through small variations either in the quantity of cuttlefish used or through having made use of octopi or of anchovies in the concoction of the broth, observed these same variations of light and of form in the bacteria under examination.

Micrococcus Pierantonii n. sp.

Morphological Characteristics

The coccus under examination, of a not perfectly spherical shape, measures in the larger diameter μ 1,2.



$\left(1 = \frac{\text{oc. comp. 4}}{\text{ob. Imm. }^1/_{12}\text{ Kor.}}\right)$

Micrococcus Pierantonii n. sp.

It is a mobile bacteria; it does not have cilia or spores. It is stained by the ordinary aniline dies; very intensely by crystal violet, fuchsine, gentian violet, rather palely by G orange, methyl green, "Orceina", Bordeaux and Romanowsky red. It is not resistant to GRAM. Carbolic fuchsine colors it intensely at the edges with a vivid red; in the central region, palely.

The coccobacillary form attains in the larger diameter 1-8; it has well-defined outlines and it stains similarly to the coccaceous form. /79

Cultural Characteristics

The culture grounds of which I made use were very nearly the same as I used in the study of the Bacillus Pierantoni Zirpolo. I would refer the reader, therefore, to my previously mentioned note, where they will all

be found described on pages 208 and 209.

Cultures in Broth

Simple Broth

The light shown by the coccus inoculated in cuttlefish broth, is, after 24 hours, of a light green, especially after lively agitation of the body which becomes slightly turbid. The film that would form characteristically in the cultures of Bacillus Sepiae and Bacillus Pierantonii does not appear immediately in this and is palely luminous. The duration of the light was about three months.

In cultures made with broth of anchovies, after twenty-four hours, I noted a deep, intense green color, which as umed an extraordinary luminosity after lively agitation of the tube. But in the succeeding days the light became whiteish and pale and it lasted a period of two weeks altogether.

Evidently the variation in the light, as regards color and as regards intensity, depends on the quality of the broth, the saline concentration, and the purity or lack of it in the medium, and the ambient temperature.

The optimum is from 21° to 26°*. Higher temperatures gave a more rapid development, but they shortened the duration of the luminescence of the bacteria.

Broth with 1% Saccharose

The light given by the coccus in this broth was an intense, light green

---

* Centigrade

in the first three days, and afterwards it continued to fade little by little, until it became visible only on lively agitation of the tube. The broth became slightly turbid and a dense, thick, whiteish film formed about twenty days after the inoculation.

Broth with 1% Laevulose /80

Inoculations made in this broth gave light only after twenty-four hours. The bacteria collected in the bottom, giving a green light. On the succeeding days I could not observe any further luminosity developing in the tube, even after continued lively agitation of the tube.

Broth with 1% Lactose

The development of luminosity in this culture ground was scarce. The light, of a light green color, lasted for a little more than a month. Visible only after nine days. In the days following it was necessary to agitate the tube strongly to obtain a barely perceptible light.

The broth became somewhat turbid.

Broth with 1% Galactose

The light lasted only five days. It was always white, pale, and visible after agitation of the tube. Broth somewhat turbid, after eighteen days from the time of inoculation.

Broth with 1% Glucose

The luminsoity lasted only one day. The broth also became discolored after seventeen days, taking on a light yellow hue.

Broth with 1% Mannose

After two days the broth gave a quite intense light green light; then this became more and more weak. This lasted about one month. There was no formation of a film, and the broth became somewhat turbid.

Broth with 1% Monosodium Phosphate

Only twenty-four hours after the inoculation, the light became vivid; and then it became pale, light, and visible after strong agitation of the tube.

The broth became turbid after seven days and the light lasted about eighteen days.

Broth with Bisodium Phosphate

The development of the light in this broth was quite rapid. In dilutions which went from 1% to 6% I was able to note that a few hours after the inoculation the tubes were all luminous; on the succeeding day the light, of a fine light green color, was becoming more vivid, especially in the tube with bisodium phosphate at 6%, and then in that at 1% and that at 3%, and then in that at 4% and 5%. /81

The light remained vivid and with good visibility for nineteen days, after which it progressively darkened.

Culture in Broth with Lecithin

I dilated, in broth, Lecithin of Aplysia which was supplied to me by my friend Aurel Craifaleanu. I was able to note how a part of the undissolved lecithin deposited itself at the bottom of the tube, where I observed a vivid white-green light that lasted for a period of about twenty-seven days.

Culture in Milk

The light appeared only in milk in which I had dissolved sodium chloride, 3%, after twenty-four hours from the time of inoculation. It retained considerable vividness for a period of about sixty days, with a fine light green. The milk coagulated slightly.

In the other tube, without sodium chloride, there was complete absence of light.

To conclude: the cultures of the micrococcus gave a green light in the first days, and whiteish in the succeeding, until the light then went out. Its duration was maximal - in the pure broth without dissolving inside the salts; minimal - for only a few days in the laevulose and glucose broth.

With bisodium phosphate, 2%, it was more vivid and lasting than with solutions of monosodium phosphade, 1%.

With mannose, galactose, saccharose, lactose, there was moderate light which lasted for a not inconsiderable period.

Cultures in Agar

(Broth of cuttlefish, agar 3%, peptone 1%)

An inoculation made from the luminous broth gave after twenty-four hours, phosphorescent colonies, of spherical shape, flat, integral at the /82 edges, of a thickness in the central zone of one mm and of a size of 1/4 of a mm in diameter. The light is light green, quite intense. After forty-eight hours, the luminosity still persists in its vividness, and the colonies have attained to a great extent the diameter of a millimeter.

On the succeeding day the light still remained vivid, but not as much so as on the preceeding one. The colonies had attained a diameter from 1 1/4 mm up to in some cases one millimeter and a half. The light then progressively faded and the size of the colonies remained stationary.

Cultures in tubes with agar preparation and flute mouths showed after twenty-four hours colonies with a bright appearance, of spherical shape, slightly flat, with light green luminosity, and of a size of 1/3 mm. Some of them attained, after the third day, a diameter of about 2 mm, preserving a quite bright light.

On culture grounds of agar disolved in broth of anchovies, there were very small little colonies, numerous, of an intense green color, such as was noted in the case of the broth of anchovies. The colonies on the third day

attained the maximum development of 1/2 a millimeter, but the light quickly faded progressively and it disappeared completely after a few days. They are not bright in appearance, but somewhat opaque, and their coloring is a light yellow.

Agar, with Saccharose 1%

On this ground the colonies attained a size of 1/4 of a mm. Shape, spherical; integral at the edges; in appearance bright, like so many little drops of dew.

The light, of a light green color, quite intense the first few days, is whiteish on the succeeding days. It lasted about twenty days.

Agar, with Laevulose 1%

This ground was completely negative for the development of the colonies. They remained like imperceptible points, without ever giving any light.

Agar with Lactose 1% /83

The colonies attained a diameter of about two milimeters with a very intense green light. Shape, spherical, biconcentric (sic); bright; dense in the center; integral at the edges; flat. The luminosity lasted about ten days.

Agar with Galactose 1%

The colonies attained a size of 1½ mm. Shape, spherical; bicon-

centric with central zone higher, and peripheral one more depressed; of waxy appearance; with intense emerald green light. The edges with short sinuosities. The smaller colonies of convex shape and with integral edges. The luminosity lasted for about thirty days.

Agar, with Glucose 1%

This ground could not be used. Dot shaped colonies developed, without even giving any light.

Agar, with Mannose 1%

The colonies attained a maximum size of 1 millimeter. Flat; appearing as a light whiteish veil; rather dense in the center; bright; deep sinuosities at the edges; concentric; with quite vivid green light, lasting about eighteen days.

Agar, with Monosodium Phosphate 1%

The colonies scarecely attained a size of 1/3 of a mm. Shape, spherical; flattened; bright; transparent; integral at edges; with light green light, which lasted about twenty days.

To conclude: The shape, size, and luminosity varies in the different culture grounds.

Colonies of about 2 mm. diameter occurred in the grounds prepared with simple agar, and with agar with lactose; the light was very intense

in the agar with lactose, with galactose, with mannose.

The ground formed by agar with laevuclose or glucose gave negative results. /84

The duration of the luminosity also varied: maximal with galactose (thirty days), minimal with mannose (eighteen days).

Cultures in Gelatine

(Broth of culttlefish, gelatine 12%, peptone 1%)

In this ground I made inoculations by brushing on and by inserting.

A) Inoculation by brushing on: After twenty-four hours spot-shaped colonies developed, spherical, not adhering much to the gelatine, with an intense green light. The colonies attained a size of 1/3 of a mm. and the light lasted for a period of about twenty days.

B) Inoculation by insertion: After one day, along the whole of the insertion made in the tube, there developed very small colonies with a whiteish color. On the surface the little colonies, spherical in shape, gave a rather intense green light.

The colonies that remained luminous are the ones on the surface which were continuing to grow notably and to melt into each other. The gelatine started to become fluid after about fifteen days. At forty-two days, the body had in part become fluid and the light had completely disappeared.

Cultures on Potatoes

This ground did not lend itself much to the quick development and

the luminosity of the bacteria. There was a very pale light, almost imperceptible, with duration of a few days.

Cultures on Cuttlefish Muscles

The luminosity assumed by the coccus on muscles of the cuttlefish was extraordinary, superior to that of the plates with agar and the tubes with broth even when strongly agitated. The little colonies developed in multiple form, after twenty-four hours. After two days they increased, always maintaining their very vivid light, of an intense light green. The small colonies, spherical, slightly raised up, on the plane of the ground, of a yellowish white color, with a bright appearance, gave light for about twenty days, then the bright faded progressively, until it disappeared towards the 30th day from inoculation. /85

Cultures on Egg Yolk

One inoculation gave, after two days, light at some point that was pale, and light green, and lasted hardly a few hours.

Another inoculation that was made, showed how the light remained very pale always. The development of colonies was always limited and scarcely lasted three or four days. It is perhaps not a good ground for the culture of this species.

Culture on Liver of Sepia Officinalis L

The development of the luminous coccus on this ground took place

after about two days. The light presented itself as a fine light green, quite vivid, at several spots in the body.

The bacterial colonies developed on the succeeding days in the remaining parts, in such a manner as to give such a vivid luminosity that one could even read the print of books. The light lasted an average twelve days.

To conclude: In gelatine there developed spherical colonies, with an intense green light, that began to make the gelatine fluid after fifteen days; on potatoes there developed small colonies with very pale light; on cuttlefish muscles there developed multiple colonies and they had light of a very vivid green color; on egg yolk the colonies developed little and the light proved to be pale; on cuttlefish liver there was a light green light, quite vivid, but of short duration.

Pathogenetic Characteristics

In order to observe the action of the Micrococcus Pierantonii on the organism of marine animals, I made various inoculations of twenty-four hour culture in broth in examples of Sepia Officinalis L., Carcinas Moenas Leach, Portunus Holsatus Fabr, Maia Verrucosa M. Edw., and Palaemon Serratus Fabr.

In the cuttle fish I made inoculations in the eye and in the ventral subcutaneous region.

I inoculated into the eye of a cuttlefish weighing two hundred grams /86 one cubic centimeter of luminous culture. The eye became luminous and it was easy to follow the movements of the animal in the basin. This light

became progressively to diminish.

The animal died ten days after the inoculation.

In another example, also weighing 200 grams, I made an inoculation in the ventral subcutaneous region of 1 cubic centimeter. The light made its appearance immediately at the inoculated spot and in the surrounding region. On the seventh day it died.

Experiments repeated on various other examples, just about of the same weight, gave virtually identical results.

Other cuttlefish left in the basin as a control died virtually contemporaneously with those that had had the inoculation of the bacteria. It is to be deduced, therefore, that the death of the cuttlefish was perhaps due to the conditions of life to which they are subjected, they being placed in a basin where the conditions of life proved perhaps impossible for them.

Examples of Palaemon Serratus Fabr. inoculated in the ventral region with 0.5 cm^3 of culture died after a minute; others, with 0.25 cm^3, after twenty minutes. Their body, however, remained vividly illuminated for several days.

Examples of Portunus Holsatus Fabr. died after about ten minutes, with 1 cm^3 of culture inoculated.

Individuals of Carcinas Moenas Leach, inoculated with 0.50 cm^3 of culture, died after 2 minutes; with 0.25 cm^3 they survived for about twelve days.

The inoculations in the Carcinas were made in the ventral region, and in all the examples there could be discerned a pale light, which progressively disappeared, in the whole of the peripheral region of the point of inoculation. Examples of Maia Verrucosa M. Edw. always gave little light and died after fourteen days.

Conclusions

The morphological, cultural and pathogenetical characteristics of the Micrococcus Pierantonii can be summarized as follows:

A) The diameter of the coccus is μ 1.2 and the diameter of the /87 coccobacillus is 1.8; it is mobile; it stains with the ordinary aniline dies; it is not resistant to the GRAM test; it does not have cilia or spores; it coagulates milk uncertainly. The optimum is from 21° to 26°C.

B) Colonies of spherical form, flat, integral at the edges, which attain a diameter of about 1 mm., with quite intense green light. The intensity and the color of the light varies on the different grounds of the culture.

C) Makes the broth turbid, forming a very slight coating at the liquid-level, with a light green color in the first days and then a whiteish one in the succeeding days; it develops in milk with 3% sodium chloride, giving a light green light.

D) On potatoes and on yolk of hen's eggs, it develops very little; on muscles of cuttlefish it develops rapidly, giving quite an intense light.

E) Inoculated into examples of Sepia Officinalis L., it illuminates the inoculated regions and adjoining parts surrounding those regions; the animals lived about ten days. Examples of Palaemon Holsatus Fabr. died after a few minutes; of Carcinas Moenas Leach, after several days, about twelve; of Maia Verrucosa M. Edw., after about fourteen days, according to the quantity of the luminous culture injected.

Naples, Zoological Station, July, 1918.

Printing completed the 28th of February, 1919.

References

1) Zirpolo, G. - Ricerche su di un bacillo fosforescente che si sviluppa sulla Sepia Officinalis L. (Bacillus sepiae n. sp.) Boll. Soc. Nat. Vol. 30, p. 47, tav. 2-3, 1 fig. Napoli, 1917.

- - - I batteri fotogeni degli organi luminosi di Sepiola Intermedia NAEF (Bacillus pierantonni n. sp.) Boll. Soc. Natl. Vol. 30, p. 206, Tav. 6, Napoli, 1918.

2) Cohn, F. - Briefliche Mittheilungen an J. Penn, Abgedruckt in Vezameling van stukken betreffende het geneeskundig staatsoetzich in Needarland. Laarg. 1878, p. 126, da Schroter, Pilze in Kr. Flora von Schliejien, p. 146.

3) Ludwig, F. -- Micrococcus pflugeri. Bot. Centralbl. Bd. 18, No. 11, 1887.
- - - Die bisherichen untersuchungen uber photogene Bakterien. Centralbl. f. Bakt. Bd. 2, p. 372, e 401, 1887.

4) Molisch -- Ueber das Leuchten des Fleisches. Centralbl. f. Bakt, Bd. 9, p. 725, 1902.

5) Cfr. i. seguenti lavori:

Pierantoni U. -- La luce negl insetti luminosi e la simbiosi ereditaria. Rend. R. Acc. Sc. Fis. e. mat. Napoli, p. 15, 1914.

5) ------Sulla luminosita e gli organi luminosi di Lampyris noctiluca L. Boll. Soc. Nat. Napoli, Vol. 27, p. 85, 1914.

------Nuove osservazioni sulla luminosita degli animali. Rend. Acc. Sc. fis. c. mat. Napoli. Fasc. 1-2-3-, 1917.

------Organi luminosi, organi simbiotici e glandola nidamentale accessoria Boll. Soc. Nat. Vol. 30, p. 30, 1917.

------Gli organi luminosi e la luminescenza batterica dei Cefalopodi. Publ, Staz. Zool. Napoli, Vol. 20, p. 105, tav. 6-8, 1917

6) Cfr. l'elaborata descrizione che ne fa al riguardo il sullodato Prof. Pierantoni che si e occupato della morfologia degli organi luminosi e la luminescenza batterica dei Cefalopodi. Pubbl. Staz. Zool. Napoli, Vol. 2 p. 105, tav. 6-8, 1917. Per cio che riguarda le Rondeletia minor Naef cfr. p. 122-128 e tav. 6 fig. 5, 6, 7, 8, tav. 7, fig. 13, 18, 19, tav. 8, fig. 21.