New Iodinin (1,6-phenazine-diol-5,10-di-N-oxide)-producing Bacteria

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A great many reports on microorganisms producing various phenazine pigments, have been presented. In many cases, those pigments are produced by *Pseudomonas* spp. in form of soluble pigments, such as pyocyanin, phenazine-1-carboxylic acid and oxychlororaphine by *Ps. aeruginosa*[1,2,3,4,5], phenazine-1-carboxylic acid by *Ps. fluorescens*[6], phenazine-1-carboxylic acid and 2-phenazinol-1-carboxylic acid by *Ps. aureofaciens*[7,8,9,10], oxychlororaphine by *Ps. chlororaphis*[11], 2, 9-phenazine-diol-carboxylic acid, 1, 8-phenazine-diol-10-oxide, 8-amino-1-phenazinol, phenazine-1,6-dicarboxylic acid and 1,8-phenazine-diol by gram-negative Venezuelan bacterium[12], and a little amount of iodinin by *Ps. iodina* IFO 3558. On the other hand, many reports have been presented that gram-positive bacteria and actinomycetes produced phenazine pigments, especially many strains of the genus *Microbispora* (*Waksmania*) and the genus *Streptosporangium* would produce iodinin. NONOMURA and OHARA studied on the taxonomy of the genus *Microbispora* and the genus *Streptosporangium* in details, and they reported that most of the strains produced a large amount of water-insoluble, benzine-soluble violet crystal in various kinds of agar media[13]. GERBER and LECHEVALIER reported that *Microbispora* (*Waksmania*) *aerata* produced 1,6-phenazine-diol, 1,6-phenazine-diol-5-N-oxide as well as iodinin[14], *Microbispora amethystogenes* produced iodinin[14], and *Microbispora parva* produced it in a small amount[14]. *Streptosporangium amethystogenes var. non-reducens* produced a large amount of iodinin on oat meal agar[15]. In the genus *Streptomyces*, antibiotics, griseolutein A and B that *Streptomyces griseoluteus* produces are phenazine derivatives[16], and *Streptomyces thioluteus* excretes yellow pigment, 1,6-phenazine-diol in the medium[17], and also is reported to produce 1,6-phenazine-diol and 1,6-phenazine-diol-5-N-oxide in addition to iodinin[18], and to produce 1-phenazinol, 6-methoxy-1-phenazinol, 1,6-dimethoxy-phenazine[19]. Malloch strain of *Nocardiaceae* produced iodinin, 1,6-phenazine-diol, and 1,6-phenazine-diol-5-N-oxide in various kinds of culture media[20]. With gram-positive bacteria, *Brevibacterium crystalloiodinum*, which SASAKI et al. isolated from red spoilt miso[21] was observed to produce iodinin and 1,6-phenazine-diol[22]. *Ps.* (*Chromobacterium*) *iodina*, which SNEATH considered to be gram-positive[23] and so GERBER termed *Brevibacterium iodinum*, produced 1,6-phenazine-diol and 1,6-phenazine-diol-5-N-oxide in addition to iodinin[24], but *Ps. iodina* that was maintained in the collections of the Institute for Fermentation, Osaka, was regarded as gram-negative, as described formerly[25]. SUZUKI et al. reported that *Arthrobacter paraffineus* produced

iodinin and 1,6-phenazine-diol only when hydrocarbons were utilized as a sole source of carbon[26].

As described above, gram negative bacteria could not produced phenazine-N-oxide with a few exception, but gram positive bacteria and actinomycetes produced phenazine-N-oxide pigments, such as iodinin and 1,6-phenazine-diol-5-N-oxide, and the author also has isolated gram positive bacteria, *Brevibacterium* spp., that produced a large amount of iodinin over its colony and in culture media, in the sea to the south of South Africa in the Indian Ocean and in the sea to the east of Japan in the Pacific Ocean. Phylogenic importance may be found in this aspect in future. The author is carrying out on morphological and physiological studies with these isolates, and this report presents the determinations of iodinin producing bacteria.

DESCRIPTIONS

Brevibacterium maris (Harrison, 1929) Breed, 1953.

Strain : Ma-11.

Strain Ma-11 was isolated from a surface sea water sample, that collected by Dr. H. Meguro at the station in the Indian Ocean during the fifth Japanese Antarctic Research Expedition 1960-1961. A few small orange-colored colonies were detected from 8 of 22 samples, and as a result of determination of these orange-colored colony-forming microorganisms, most of them were regarded as *Br. fulvum*, but strain Ma-11 was distinguished in respect to production of deep violet crystal with bronze luster over the surface of orange-colored colony, and various physiological properties, such as nitrate reduction, gelatin liquefaction, weak starch hydrolysis, acid formation from glucose, positive methyl red test, and litmus milk alkaline. As this strain was sphere or coccoid in shape, it was regarded as *Micrococcus* sp. in the former report[27]. And it was distinguished from *Micrococcus colpogenes* in respect to sugar utilization, pigment production, and litmus milk. Though strain Ma-11 had different properties from *Micrococcus aquivivus* in respect to motility, nitrate reduction, gelatin liquefaction, and hydrogen sulfide formation, it was considered as *Micrococcus aquivivus*-like species, because of similar morphological and cultural properties except iodinin production[28]. But, it was concluded that strain Ma-11 should be identified with *Br. maris*, as *Br. maris* was reported to be coccoid when being cultured at 37° C, in addition to similar physiological and cultural properties[29]. Iodinin production of strain Ma-11 had been observed until the third transplantation. At present it cannot produce iodinin, but there is no change in other microbiological characteristics.

Coccoid, 0.5 by 0.5 to 0.6 micron. Non-motile. Gram-positive.

Agar slant : Moderate, filiform, orange-colored growth. Sea water broth : Moderate turbidity. Fresh water broth: Moderate turbidity.

Catalase positive. Oxidase positive. Nitrates reduced to nitrites. Ammonia produced from pepton. Ammonium salts utilized as a sole source of nitrogen. Nitrogen-deficient medium : No visible growth. Gelatin not liquefied. Starch may be hydrolyzed. Acid but no gas from glucose. Slight acid from maltose, mannitol, xylose and salicin. No acid or gas from lactose and sucrose. Hydrogen sulfide not produced. Indole not pro-

duced. Citrate may be utilized as a sole source of carbon. p-Hydroxybenzoate utilized. Milk : Alkaline. Methyl red positive. Acetylmethylcarbinol not produced. Fluorescent soluble pigment not produced. Salt concentration favorable for growth : O to 12 per cent sodium chloride Aerobic. Temperature favorable for growth : 10 to 37° C. pH favorable for growth : 6.0 to 9.6. Source : Sea water.

Brevibacterium stationis var. iodininofaciens nov. var.

Strains : Po-35 and Po-36.

Both strains Po-35 and Po-36 were isolated from sea water sample, collected at a depth of 99 m at the station 7 (145°00′E, 39°03′N, Sept. 12, 9 : 00, 1962) in the Sea to the East of Japan in the Pacific Ocean and their properties were identical with those of *Brevibacterium stationis* (ZoBell and Upham, 1944) Breed, 1953, except such properties as typical rods, inactive glucose utilization, optimum temperature, 30° C, no change in milk and color of colony, white, but turning yellowish brown to pale reddish brown and subsequently covered by deep violet iodinin crystal with bronze lustre in 3 days. *Br. stationis* shows such properties as ovoid cells, acid formation from glucose, optimum temperature 20 to 25° C, and milk alkaline. Achromogenic mutant strains derived from sectors in colony of the original strain or isolated by higher temperature screening are similar to the original strain in respect to many properties, except no or little production of iodinin. Therefore, the original strains, Po-35 and Po-36 should be regarded as a new variety of *Br. stationis*, regardless of iodinin production.

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Rods, 0.6 to 0.7 by 1.0 to 1.5 microns. Non-motile. Gram-positive.

Agar slant : Moderate, filiform, white to yellowish brown, pale reddish brown covered with iodinin crystal. Sea water broth : Moderate turbidity. Fresh water broth : Moderate turbidity.

Nitrates reduced to nitrites. Urease negative. Ammonia produced from pepton. Ammonium salts not utilized as a sole source of nitrogen. Gelatin stab : stratiform liquefaction. Starch not hydrolyzed. No acid from glucose, lactose sucrose, maltose, mannitol, xylose, salicin, and glycerol. Hydrogen sulfide not produced. Indole not produced. Milk : No change. Methyl red negative. Acetylmethylcarbinol not produced. Aerobic. Temperature range for growth : 5 to 35° C, optimum 30° C. pH range for growth : 6.0 to 10.0, optimum, 6.0 to 8.0. Source : Sea water, in the Sea to the East of Japan in the Pacific Ocean.

SUMMARY

New iodinin (1,6-phenazine-diol-5,10-di-N-oxide)-producing bacteria were isolated from sea water. One was isolated from surface sea water samples, collected in the Indian Ocean, during the fifth Japanese Antarctic Research Expedition 1960-1961, and identified with *Brevibacterium maris* (Harrison, 1929) Breed, 1953. The others were isolated from the sea water, collected at a depth of 99 m at the station in the Sea to the East of Japan in the Pacific Ocean and regarded as a new variety of *Br. stationis* (ZoBell and Upham, 1944) Breed, 1953, *Br. stationis var. iodininofaciens.*

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Fig. 1. Cells of iodinin producing bacteria, *Brevibacteriums stationis var. iodininofaciens* Po-36, grown on sea water agar slant for 24 hr. at 30°C.

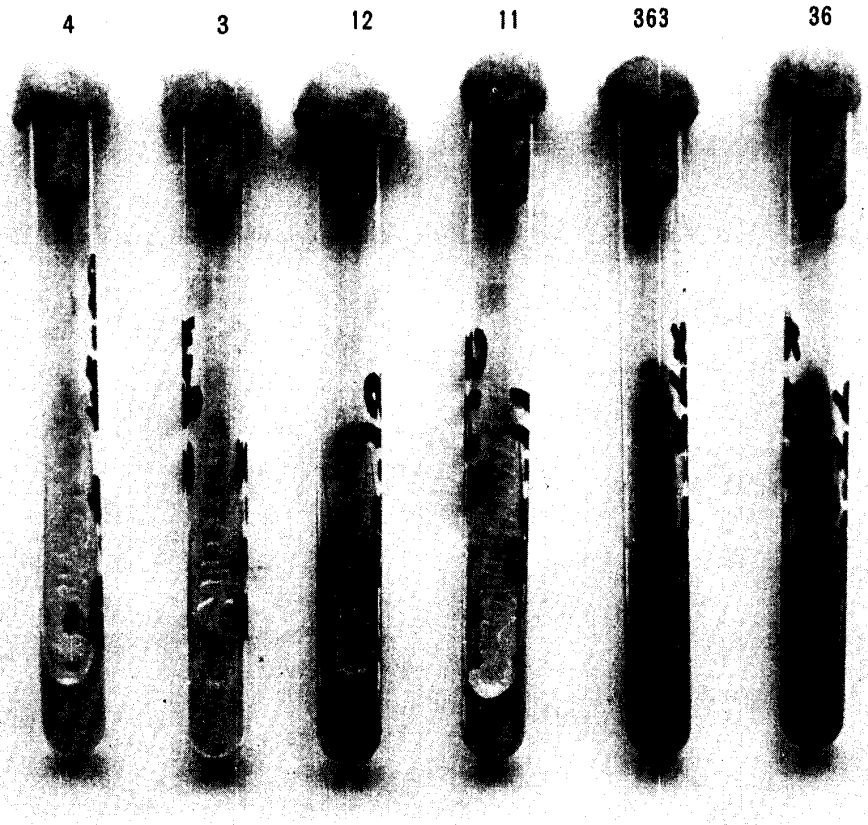

Fig. 3. Agar slants of iodinin producing bacteria, *Brewibacterium stationis var. iodininofaciens* and its achromogenic mutant strains. Right to left. Po-36 : The original strain. Po-363 : A strain for iodinin production, gained from the original strain by plating. Po-363-11 and Po-363-12 : Strtains, derived from achromogenic sectors tha appeared on the nutrient agar at 30°C, and partially losing the ability of iodinin production. Po-363-3 : A strain, derived from achromogenic sector that appeared on the nutrient agar at 30° C, and completely losing the ability of iodinin. Po-363-4 : A strain, derived from achromogenic sector screened at higher temperature 35°—40° C, on the sea water agar, and completely losing the ability of iodinin production.

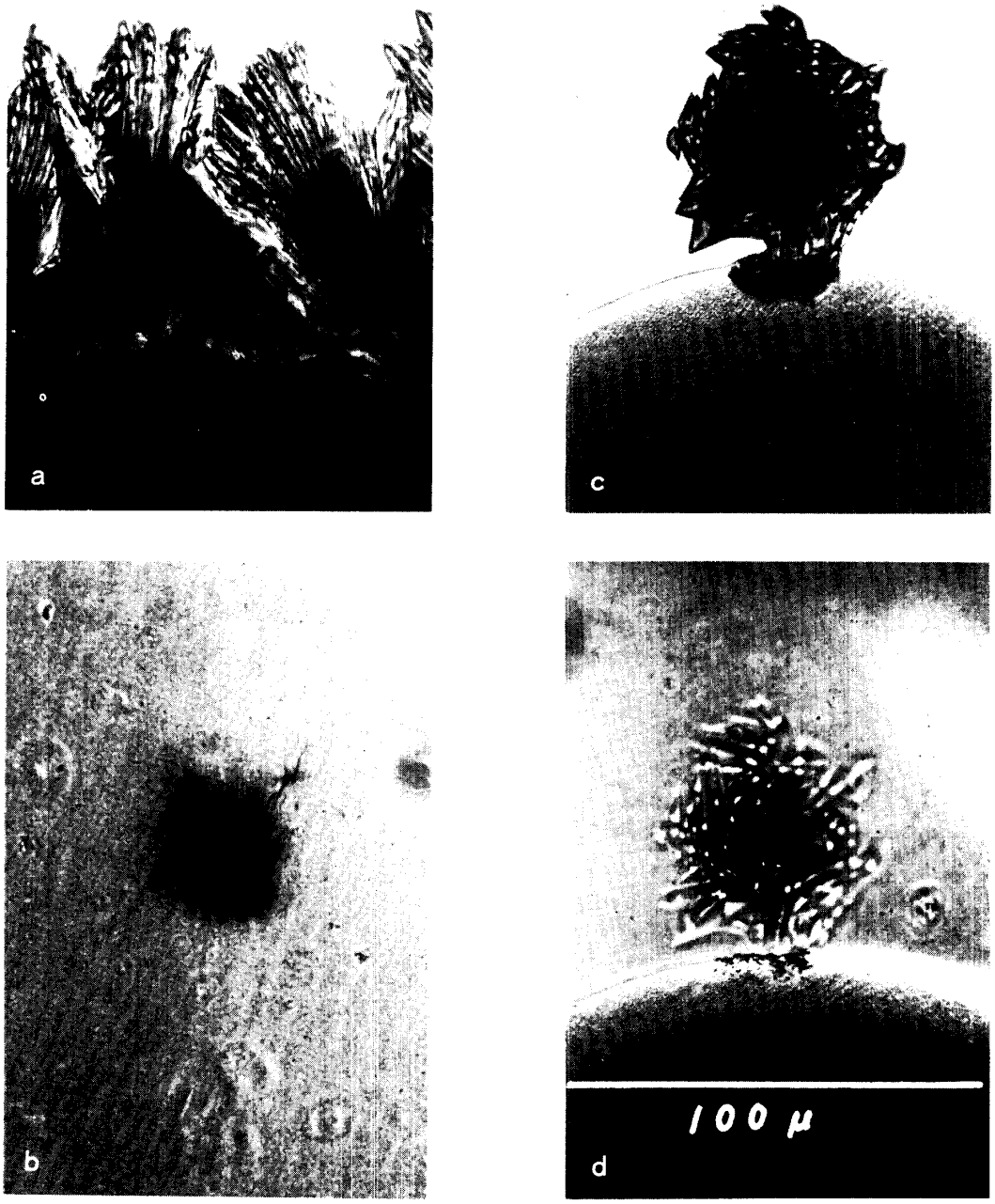

Fig. 2. Iodinin crystals produced by *Brevibacterium stationis var. iodininofaciens* on and in the agar medium.

a. Sword-like iodinin crystals on the edge of colony on the agar medium.
b. Iodinin crystals in the agar medium, on which strain Po-36 was cultivated.
c. Iodinin crystals on the edge of colony on the agar medium.
d. An impression of iodinin crystals, left after iodinin in Fig. 2 c. was washed off with chloroform.