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Bacteriological Investigation of Carboniferous Rock Salt from Pugwash, Nova Scotia

By **Heinz Dombrowski, Carlo G. I. Friedlaender, Robert Kühn** and **Douglas H. Loring**[1])

With 4 figures in the text

ABSTRACT

Evidence has been found for the presence of living bacteria in rock salt from Pugwash, Nova Scotia.

Geological and mineralogical observations indicate that the salt is primary in origin, of late Mississippian age, and that the bacteria found in the salt are of the same age.

A morphological comparison was made between the appearance of the bacteria in thin section of the rock salt and the bacteria after isolation.

ZUSAMMENFASSUNG

In Steinsalz von Pugwash, Nova Scotia, sind lebende Bakterien nachgewiesen worden.

Mineralogische und geologische Beobachtungen weisen auf primäre Bildung des Salzes und auf spät-Mississippian Alter; die im Steinsalz festgestellten Bakterien sind als gleichen Alters anzusehen.

Die Erscheinungsformen der Bakterien im Dünnschliff und nach Isolierung werden einem Vergleich unterzogen.

The inspection of slides of rock salt from Pugwash, Nova Scotia, reveals at high power the presence of particles which, because of their shape and size, must without any doubt be considered bacteria (figs. 1 and 3). As it has been possible to isolate and regenerate living bacteria from salt of other palaeozoic salt deposits (H. DOMBROWSKI, 1963a, b, c), it appeared of interest to study the question of the survival of these bacterial inclusions in rock salt by means of culture experiments.

A sizeable sample was therefore obtained from Pugwash Mine. We acknowledge help received from N. GRAY, Dalhousie University, D. H. STONEHOUSE and Mr. J. R. MACQUARRIE, The Canadian Rock Salt Company Limited, Pugwash.

Pugwash (approx. 45° 50′ N, 63° 40′ W) lies in Cumberland County, Nova Scotia.

The general geological setting has been described by M. F. BANCROFT (1957) and R. EVANS (1965).

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A sample of about 11 kg was taken at Pugwash Mine, on the 630 foot level, at cross-cut 3J, 140 feet from pillar D 3 drift. The sample comes from Lower Windsor, A or B Zone, late Mississippian (age $\pm$ 310 m.y.). Due to the plastic behaviour of the salt and the complicated structure a more precise stratigraphical position cannot be given.

Mineralogical examination

The main questions were to ascertain whether the salt under investigation was to be considered primary, and whether contamination could account for the biogenic inclusions.

Megascopically, the sample is white to grey or yellowish. No layering is recognisable in the hand specimen. The aspect is preponderantly dull to semi-lustrous. The material is not sparry and coarse grained as in other parts of the mine. The grain size is fine, about 2 mm in diametre. There are a number of dark grey inclusions of dense appearance in the shape of specks, a few millimetres in size, or streaks.

The microscope reveals that these dark patches consist of fine laths of anhydrite (average 0.02 $\times$ 0.04 mm) in feathery aggregates and clusters with some additional clay material. There are very numerous tiny liquid inclusions, largely in the shape of negative crystals. Frequently, these inclusions are aligned in stringers which may be observed to cross different grains while lying in a preferential plane.

Liquid inclusions are fairly common in oceanic rock salt. The crossing of different grain boundaries indicates recrystallisation, since the inclusions are linked with a former surface of the brine. A preferential alignment of the inclusions would point to a former channel of dehydration being intersected by grain boundaries of a later recrystallisation.

Gypsum was also observed but in much smaller quantity than anhydrite. Magnesite occurs in very minute (0.14 mm) well shaped rhombohedra as a minor accessory only. The distinction from calcite or dolomite is based on X-ray diffraction.

A few crystals of a strongly pleochroic mineral were detected (n_α olive brown, n_γ blueish green; extinction angle $c/n_\alpha 14°$, sign $-$). These properties seem to indicate an acmitic pyroxene. Acmite is not too frequently found as inclusion in salt (see however CHARLES MILTON, 1957). The development here shows it to be authigenic. There are partly idiomorphic crystals and a few aggregates which could not well be accounted for as detrital. In addition there are numerous not identifiable tiny specks in a more or less parallel alignment.

The partial chemical analysis shows 94.15% NaCl, 0.03% KCl, 0.003% Br. The remainder, approximately 5%, corresponds to anhydrite, the clayey substance, dolomite and the other accessories as well as moisture content (liquid inclusions).

X-ray fluorescent analysis of the bulk sample indicates that traces of Fe and Sr are present. Further analysis was made of the salts extractable by hot water and the remaining residue. The analysis indicates that the recrystallised salt did not

contain any detectable traces, whilst the residue contains Fe and Sr. Most of the Fe is probably present as a finely divided coating, as is indicated by Fe film developed on the filter paper during decantation of the soluble salts deposited in the evaporation basin. The traces of Sr are probably associated with anhydrite or gypsum. GOLDSCHMIDT (1954, p. 248) indicates that the entrance of Sr into minerals of marine salt appears to be regulated by the Ca content of these minerals. In the crystallisation from aqueous solutions Sr exclusively replaces the calcium ions, and not the potassium.

The KCl is probably in part absorbed on clayey substance, in part in solution in the liquid inclusions.

Referred to 100 NaCl, the content of Br is 0.0032%. According to the content of Br, the salt is of early formation although the content of Br does not correspond to the very first halite, crystallising out of the ocean when saturation concentration of NaCl is reached.

BOEKE (1908) states that a content of 0.004–0.005% Br is to be expected for the first crystallised halite. The importance of the content of Br has been more fully discussed by KÜHN (1953). Recent data by O. BRAITSCH and A. G. HERMANN (1962 and 1963) require 0.007% Br for primary halite. However, the crystal water set free at the early diagenetic recrystallisation of the metastable primary gypsum to stable anhydrite may have caused, in the vicinity of anhydrite, the content of Br to be lowered to some extent by an early diagenetic recrystallisation, through solution, of adjoining halite. In such a process the content of Br would be somewhat lowered, as some of the Br would stay in the brine which is squeezed out. Such a possibility seems probable in this case where the halite is underlain by and in contact with anhydrite. Massive anhydrite occurs approximately 200 feet to the west of the location of our sample on the 630 foot level and the halite is also overlain by large masses of anhydrite. This shows that the concentration became retrograde after the deposition of halite, as anhydrite corresponds to the next lower concentration stage in the evaporation of sea water.

The absence of layering in the halite could indicate that there was some fluctuation in the evaporation basin. On the other hand, diagenetic recrystallisation in connection with the transition gypsum-anhydrite may have obliterated the layering. Both these possibilities may apply here.

It is probable that there has been a rather marked turbulence during the deposition of the relatively thin layers of rock salt between anhydrite. In fact, in accordance with the Ochsenius bar theory, we must assume a strong reverse current at the bottom of the basin to account for the loss of more strongly concentrated brine which otherwise would have formed potash salts.

In conclusion it may be stated that the features observed on the sample are those of a primary rock salt. The low content of Br as well as the absence of layering are explained by the special depositional conditions.

Contamination of our sample from outside sources is considered unlikely because of the depth of this sample below the surface and its position within the deposit. In any case the grain boundaries will act in the way of a filter in a fine grained irregular material such as our salt specimen.

If, on the basis of geological and mineralogical evidence, the salt is considered primary and the action of contamination can be ruled out, it may be safely inferred that the inclusions of biogenic particles are of the same age as the salt.

Bacteriological examination

Five smaller pieces (of from 360 g to 455 g) were prepared from the inner part of the large (almost 10.5 kg) salt specimen. These smaller pieces were investigated bacteriologically. Extraordinary precautions had to be taken to avoid unwanted secondary infection. The procedure of isolation of bacteria was the same as laid down in a previous paper (DOMBROWSKI, 1963a, p. 453).

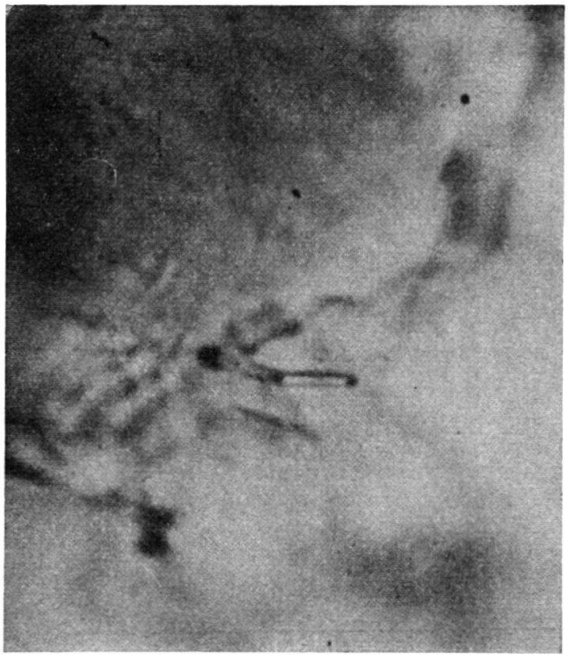

Fig. 1 (native in thin section). 'Pugwash 2'. Bacteria in halite. Short chain, with 160° link Approx. 1600:1.

Illustration 1 shows two bacillar bacteria joined to form a short chain. On one of them, a spore is clearly recognisable in terminal position. The angle between the two parts of the chain is 160°.

Figure 3 shows a single cluster of a larger number of single bacteria. On some of them, the terminal development of the spore may distinctly be observed. It must be stressed that the bacteria as well as the spores are completely embedded in the salt. They are not located in capillary fissures or in sutures within the salt crystals, nor are they observed in the hollow inclusions that are partly filled with brine.

Three of the five pieces investigated yielded living bacteria while two pieces proved sterile. Each of the three pieces with bacterial growth contained two different species which were classified according to usual bacteriological procedure. Two of the six species were found to be identical. Only five different species have therefore been isolated out of the Pugwash rock salt.

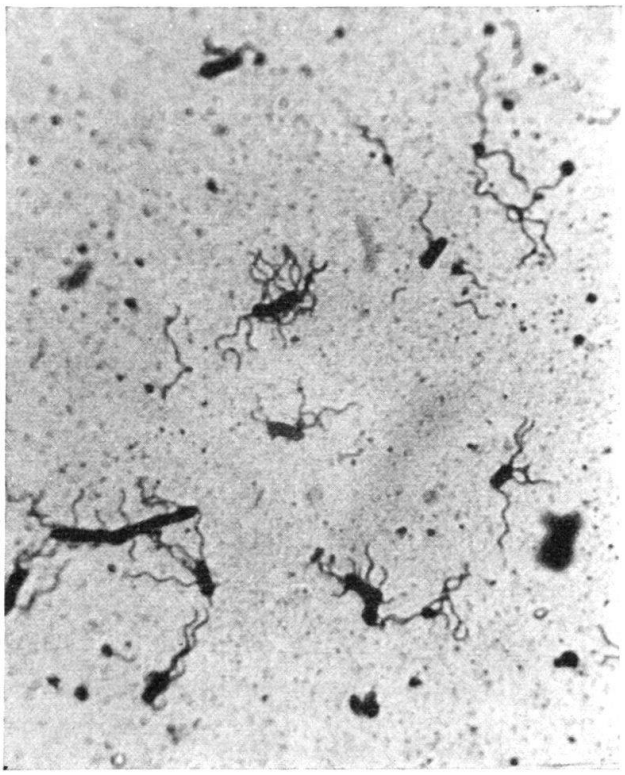

Fig. 2 (culture). ' Pugwash 2' staining after ZETTNOW. Peritrichal flagellae, showing tendency of formation of short chains with 160° link. Approx. 1600:1.

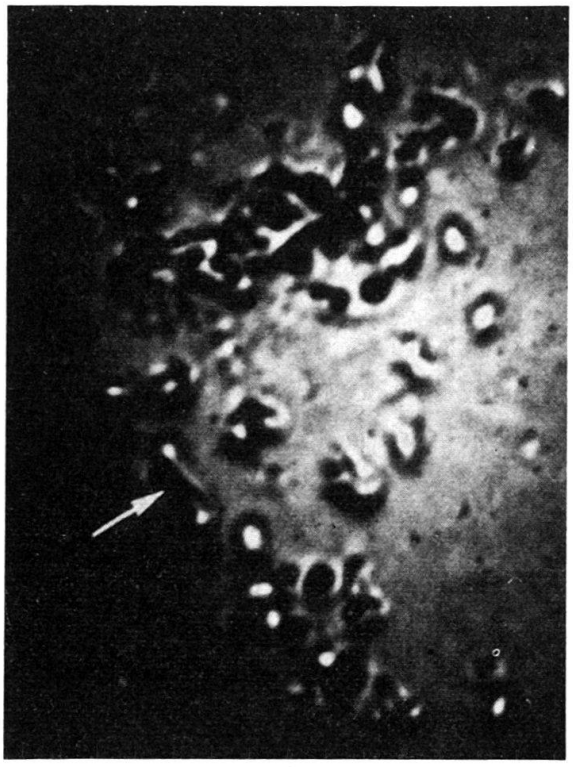

Fig. 3 (native in thin section). Accumulation of bacteria. Partly showing terminal development of one spore (' drum-stick shape' = Trommelschlegelform). Approx. 1200:1.

Morphology

The observations on the morphology of the Pugwash bacteria are put down in the Table.

The bacteria from Pugwash are, with one ecxeption which is gram-labile, gram-positive. All of them are spore-forming; some have flagellae, some are without.

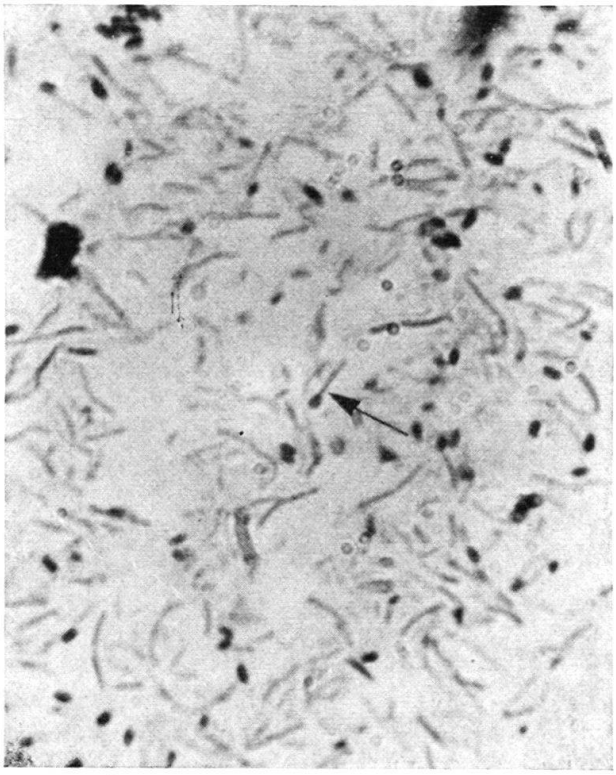

Fig. 4 (culture). ' Pugwash 4 '. Staining of the spores after HABS. Bacteria partly showing terminal swelling by one spore (' drum-stick shape ' = Trommelschlegelform) Approx. 1200:1.

Fig. 2 shows Pugwash Strain 2; silver impregnation after Zettnow reveals peritrichal flagellae. The figure shows tendency of short chains with interlinking angle of 160°. The similarity with the bacteria as observed in thin section in the rock salt, fig. 1, is hardly accidental. The same relation pertains to figs. 4 and 3. Fig. 3 shows in thin section bacteria and terminal spores, with drum-stick shape. Fig. 4 shows the same shape in living bacteria isolated from Pugwash rock salt and coloured after HABS. (See arrows figs. 3 and 4.) The bacteria in fig. 4 correspond to Pugwash Strain 4.

Physiology

All results on the physiological and biochemical properties of the Pugwash strains are put down in the Table. There is a relative lack in ferments. Strains 3 and 6 do not possess any saccharolytic ferments or alcohol. Strains 1 and 2 have only three such rather indistinct ferments while Strain 4 has only five faintly indi-

cated sugar breaking ferments. The results were obtained on cultures of 10 days and were found to be the same after 4 weeks. None of the germs is of delayed fermentation type.

A determination of the species could be carried out only with data on recent bacterial vegetation. It is rather uncertain that the representatives of the recent flora are endowed with the same chemical properties as the palaeozoic organisms. Therefore, no attempt at determination of the species based on the ascertained biochemical data has been made as this would necessarily have resulted in erroneous indications.

The lack of fermentation activity of the Pugwash bacteria is in conformity with the behaviour of bacteria which have been isolated from other salt deposits (DOMBROWSKI, 1963a; KUZNETSOV et al., 1963). From Lower Cambrian to Permian, there is a continuous increase of the fermentation activity of living isolated bacteria. However, a strain has been isolated from Permian salt which – similar to Pugwash Strains 3, 5 and 6 – does not show any fermentation activity in the variegated series.

One cannot tell whether these organisms are to be considered initial or end members of a development series.

There is no doubt about the geological age of the bacteria found in the Pugwash salt. On the other hand, these bacteria should certainly not be considered typical representatives of the bacterial flora of that time but rather specialists that succeeded in meeting the exacting requirements of survival.

The Morphological and Physiological Characters of Bacteria from Pugwash, Nova Scotia, Canada

No. of «Pugwash strain»	1	2	3	4	6
Morphology					
Spore forming	+	+	+	+	+
Motile with flagella	+	+	–	–	+
Gram	+	+	+	±	+
Physiology					
Starch hydrolysis	+	–	+	+	–
Nitrate reduction	–	–	–	–	–
Indole production	–	–	–	–	–
Pigment production	–	–	–	–	–
Gelatin liquefaction	–	–	+	–	–
H_2S production	+	+	+	(+)	+
Salt tolerance	+	+	+	+	+
Methyl red test	+	–	+	+	–
Voges-Proskauer test	–	–	–	–	–
Hemolysis	–	–	–	–	–

Acid from:					
Glucose	±	−	−	±	−
Laevulose	+	+	−	−	−
Sucrose	−	−	−	−	−
Maltose	−	±	−	±	−
Lactose	−	−	−	±	−
Raffinose-hydrate	−	−	−	−	−
Rhamnose-hydrate	−	−	−	±	−
l-Arabinose	−	−	−	±	−
Salicin	−	−	−	−	−
Inulin	−	−	−	−	−
Xylose	−	−	−	−	−
Trehalose	−	−	−	−	−
Dulcitol	−	−	−	−	−
Inositol	−	−	−	−	−
Mannitol	±	±	−	−	−

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