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Large Quantity Hydrogen Evolution by a Mesophilic Cyanobacterium *Lyngbya* sp. 108 Strain

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Much thought has been given to alternative energy sources as a long-term solution to the problem posed by the dwindling supply of non—renewable hydrocarbon based fuels. Among the various considerations is the evolution of hydrogen through the use of living organisms. This method has the advantages of being safer, as the main input is solar energy, and also seems well suited to countries such as Japan which lack abundant national resources. Of particular interest is the evolution of hydrogen by cyanobacteria, which have the following advantages; cyanobacteria differ from photosynthetic bacteria[1)] in that they do not require organic compounds as electron donors for photosynthesis but rather can utilize water[2-5)], in addition they can utilize atmospheric CO_2 as a sole source of carbon.

The current authors isolated cyanobacteria which evolved large quantities of hydrogen from the estuary of the Ashida River, located on the coast of the Seto Inland Sea. Initially, 314 water samples were collected. Then, 1 mℓ of a water sample was poured into a 25 mℓ test tube containing 10 mℓ of the synthetic medium. Test tubes were incubated at 30°C, under 2,000 lux from fluorescent lamps. The synthetic medium of Pringshein and Wiessner[6-7)] was employed, which was carbon free and contained vitamin B_{12} and KNO_3 as a source of nitrogen. The pH was adjusted to 8.2. Algae which grew in this medium were smeared onto a agar medium. Using this procedure, 52 strains of cyanobacteria were isolated.

To induce nitrogenase for hydrogen evolution, the isolated cyanobacteria were cultured in the above syntheric medium from which KNO_3 was restricted for a period of 1 week. This procedure was repeated four times.

To test hydrogen evolution, those cyanobacteria samples in which nitrogenase was induced were resuspended in 5 mℓ of the above synthetic medium not containing KNO_3 in a 30 mℓ Erlenmeyer flask sealed by a butyl rubber stopper. Air in the flask was replaced with argon. The flask was agitated at 30°C, under 2,000 Lux from fluorescent lamps.

Thirty one of the 52 strains of cyanobacteria were filamentous. These strains evolved the greatest quantities of hydrogen. On the other hand, unicellular strains (21 strains) did not evolve hydrogen at all, or only in small quantities even when hydrogen evolution was detected.

Fig. 1 shows the hydrogen evolving patterns for 9 strains in which substantial quantity of hydrogen were produced. Some strains showed increased hydrogen evolution in the early period of incubation which ceased soon after, while others increased greatly during the latter periods of

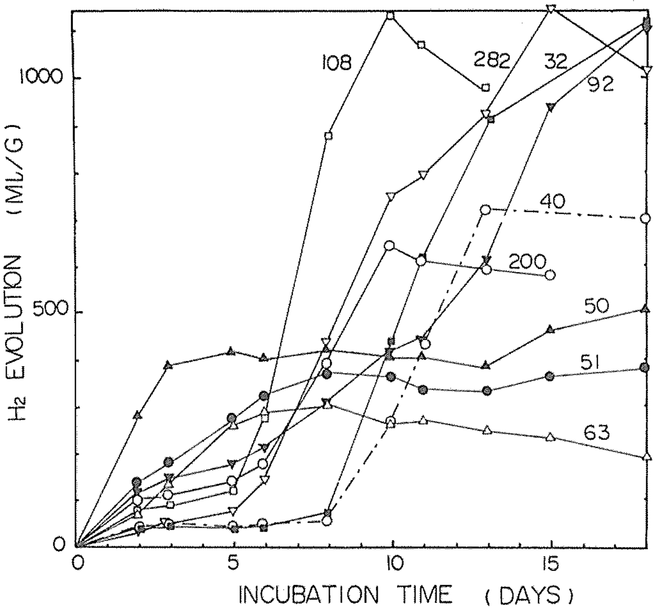

Fig. 1. Hydrogen evolution by cyanobacteria isolated from the district of Seto Inland Sea.
Numbers show the strain number of cyanobacteria.
All strains are filamentous.

incubation. From these 9 strains, we selected No. 108 as an excellent strain because the quality of hydrogen produced was greater than others, 1,120 mℓ /g of dry cells; its maximum average evolution velocity was faster, 250 mℓ /g of dry cells/day but the average velocity was 110 mℓ / g/day; and it continued evolving for an extended period.

Strain No. 108 is an unbranched, nubenthic, planktonic, cylindrical, filamentous cyanobacterium (mostly 4 μm $\times$ 60–80 μm), ensheathed without heterocyst. This strain contains the pigments, ß-carotene, phycocyanin, allophycocyanin, xantophyll, and chlorophyll a. Under the KNO_3 restricted condition, in particular, phycobilin synthesis was repressed, and sugars were accumulated. The strain was morphologically identified as *Lyngbya* sp..

The following conditions are considered optimal for the growth of the strain No. 108. 1. Temperature: 36°C. 2. Light intensisty: 2,000 Lux. 3. pH: 9.0. 4. Salinity: growth was unefected at salinities between 0 and 16‰ though inhibited at concentrations greater than 16‰. 5. Of 5 nitrogen sources utilized (nitrate, nitrite, ammonium, urea, and gaseous nitrogen) nitrate (KNO_3) supported the greatest growth. Growth was inhibited by concentrations of KNO_3 less than 0.1%. 6. Concentrations of iron had a strong influence on growth. Thus, this strain is regarded as a freshwater, mesophilic, alkaliphilic cyanobacterium.

Many papers have been published for high quantity hydrogen evolution by cyanobacteria. However, most of these attempts have incorporated flush systems with inert gas except for those utilizing marine cyanobacteria Miami BG7[8]. This *Lyngbya* sp. 108 strain can be utilized in a closed system. Also, this strain is a mesophilic, freshwater, alkaliphilic cyanobacterium capable of growth under low light intensity as compared with photosynthetic bacteria, in a medium free of carbon and organic compounds as electron donors. The velocity of hydrogen evolution is fast, moreover it continues for an extended period. Therefore, this strain could be cultured and incubated at low cost and could possibly be utilized as a local energy. However, there are some problems which remain to be resolved. Particularly, the velocity of hydrogen production is slower than that of photosynthetic bacteria. Also, the induction of nitrogenase requires a considerable length of time. Experiments are presently being conducted concerning these problems.

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中温性ラン藻 *Lygbya* sp. 108 株による水素の生産

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広島県福山市芦田川河口より，54株のラン藻を分離し，その中から水素生産能の高い No.108 株をスクリーニングした。その形状は糸状でヘテロシスト，分岐はない。No.108 株の総水素生産量は，1,120mℓ /g（藻体乾燥重量），最高時の水素生産速度は， 250mℓ /g/day（藻体乾燥重量）であった。生育条件を検討した結果，至適温度36℃，至適 pH 9.0，至適照度2,000 Lux，至適食塩濃度は16‰であった。また，窒素源として KNO_3 が有用であり，鉄の濃度による生育への影響が認められた。以上より，No.108 株は，中温性，淡水性，好塩基性藻類であり，中温性のラン藻 *Lygbya* sp. であることが分った。本 No.108 株は，密閉系で水素生産を行わせているが，現在までに報じられているラン藻による水素生産は，Flush system によるものが多く密閉系で高い値を示すものは海水性の Miami BG 7 しかない。