Studies on the Fucoidan-Utilizing Microorganisms III

—Screening of Fucoidan-Utilizing Fungi and Its Cultural Characteristics—

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Four types of fucoidan-utilizing fungi were screened from samples collected in the coastal sea area of Japan and its cultural characteristics and utilization of fucoidan were examined.

All of screened fungi grew well in presence of higher concentration of NaCl. Strain HAG-056 and HSW-102 grew well at neutral and alkaline side of pH, while strain HSS-101 and HSS-102 at acidic side. The optimum temperature was 25°C.

From the test of utilization of gagome-fucoidan by these fungi, it was estimated that HSS-101 had a superior utilizing ability against gagome-fucoidan.

Fucoidan is a sulfated polysaccharide, which is specific in brown algae. Although there had been some reports on enzymes that could hydrolize fucoidan, almost of them are mainly on glycohydolase in midgut, midgut gland or pancreas of fish or cellfish.[1)~4)] Furthermore, there has been a few reports of the types of marine microorganisms with fucoidan-utilizing ability.

Since in 1959 Yaphe and Morgan reported on marine bacteria[5)] that could utilize sulfated polysaccharides, such as fucoidan, many researchers have been attempted to isolate marine bacteria with fucoidan-utilizing ability and some bacteria were isolated. We also reported on fucoidan-utilizing bacteria isolated from the sea sand collected in the coastal sea areas of Hiroshima bay and Aki-nada in the latest paper.[6),7)]

However, we find no reports of fungi and fungi-like microorganisms that could utilize fucoidan and its analogous.

The fucoidan-degrading enzymes have an important meaning in biochemical elucidation of the structural problem in fucoidan or in new utilization of enzymatical products of fucoidan in medical and pharmacological regions. In this paper, we reported evidence of fucoidan-utilizing fungi and its cultural characteristics.

Materials and Methods

Chemicals

Toluidine blue (8GX) was purchased from Merck. Polypeptone was from Digo Nutritional Chemistry Corp. Yeast extract was from Oriental Yeast Ins. Camp. Purified agar was from Difco Lab. Other chemicals were usual commercial products.

Preparation of Fucoidan

Gagome fucoidan was prepared by method of Fujikawa.[8)]

Gagome fucoidan was extracted from gagome combu, *Kjellmaniella crassifolia*, by hot water and collected by CPC precipitation. The collected fucoidan was purified by method of

Barium precipitation. The purified fucoidan was used as substrate.

Sample collection

Sea sand, seaweeds and sea water were collected at random at the coastal sea area of Japan (24 points, from Kyusyu to Hokkaido).

Sea sands and seaweeds thrown on the shore were collected within one meter from waterside.

They were stored at 4°C until for use.

Screening and Cultivation of Fucoidan-utilizing fungi

Fucoidan-utilizing fungi were screened by the enrichment culture technique at 25°C from sea area samples.

The fucoidan-containing medium (SFC medium) was used for the screening of fucoidan-utilizing fungi. The basal medium was composed of NH_4NO_3, 2.0g; ferric citrate, 2.0g; yeast extract, 2.0g; gagome fucoidan, 5.0g; in 1000ml of artificial sea water ($MgSO_4 \cdot 7H_2O$, 2.0g; $MgCl_2 \cdot 6H_2O$, 2.0g; $CaCl_2 \cdot 2H_2O$, 0.05g; Na_2PO_4, 0.5g; in 1000ml of distilled water). The pH was adjusted to 8.0. Pure cultures were obtained by repeated plate culture on SFC medium solidified with 1.8% agar. By this procedure, four strains were screened.

The pure cultures were stored at 4°C until for use.

Judgment of Fucoidan-utilizing ability of Fungi

Judgment of Fucoidan-utilizing ability of isolated fungi was performed according to the modified method in a previous paper.[7),9)]

A small quantity of mycelium was translated on the SFC-T agar plate medium which contained toluidine blue in SFC medium. These medium were incubated at 25°C for 3 days. Strains with clear de-coloring was regarded as fucoidan-utilizing fungi.

Analytical methods

Growth of fungi was monitored by measuring of dry weight of their mycelium formed on SFC medium.

Screened fungi were cultivated on 30ml of SFC liquid medium at 25°C. Their mycelium was harvested by filtration (TOYO .ROSHI, membrane filter TM-2, mesh 0.45). They were dried at 80°C for 1 h and weighted.

Comparison of utilization of gagome-fucoidan by screened fungi was determined by measuring of dry weight of the mycelium.

Results and Discussion

Screening and Morphology

Although many fucoidan-utilizing microorganisms were separated from many samples which were collected at the coastal sea area

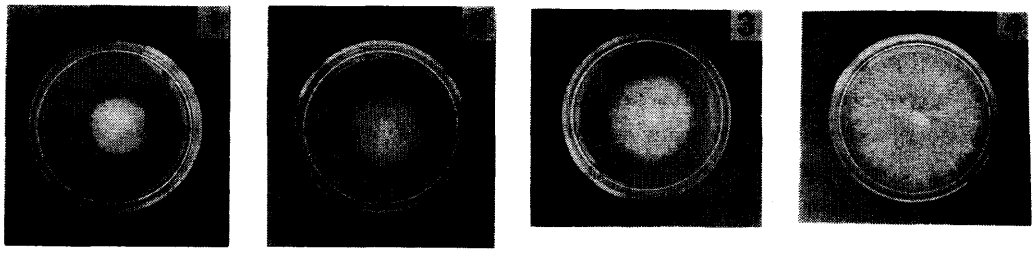


Photo. 1-4 Four days old colonies of Fucoidan-Utilizing Fungi Isolated from Sea Sand, Sea water and Seaweeds.

1: Isolate HAG-056, 2: Isolate HSS-101, 3: Isolate HSW-102, 4: Isolate HSS-102.

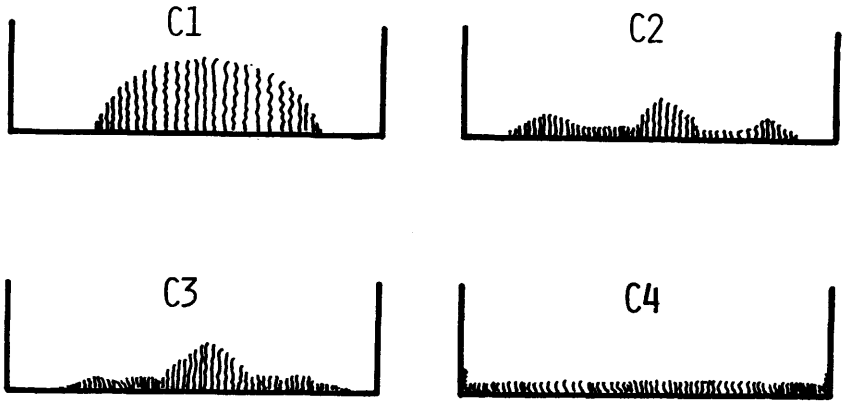


Fig. 1 Schematie Diagram of 4 days Type of Fucoidan-Utilizing Fungi Formed on SFC medium.

C1; Isolate HAG-056. C2; Isolate HSS-101, C3; Isolate HSW-102, C4; Isolate HSS-102.

of Japan, a few fucoidan-utilizing fungi were screened Growth of the fungi on the SFC-T agar plate medium was observed after 2 days incubation and the decolored zone was formed around the mycelium of these colonies. It was suggested that these fungi could utilize fucoidan.

By their colony shapes, they were divided into four types. They were named as strain HAG-056, HSS-101, HSW-102 and HSS-102(note: HAG; marine algae, HSW; sea water, HSS; sea sand).

All of them formed peculiar mycelium, as shown in Photo. 1-4 and Fig. 1. Strain HSS-102 grew very fast on SFC agar plate medium.

These shapes of colonies were as follows: strain HAG-056; long and soft aerial mycelium was formed only in the center of medium and the mass color of colony was white or pale gray. The brim of colony was smooth. Strain HSS-101; moderate mycelium was formed over the whole of medium and the mass color of colony was white and pale yellow. The brim of colony was rough. Strain HSW-102; moderate mycelium was formed over the whole of medium and the mass color of colony was white or pale brown. The brim of colony was rough. Strain HSS-102; very short and radical aerial mycelium was formed over the whole of medium and the

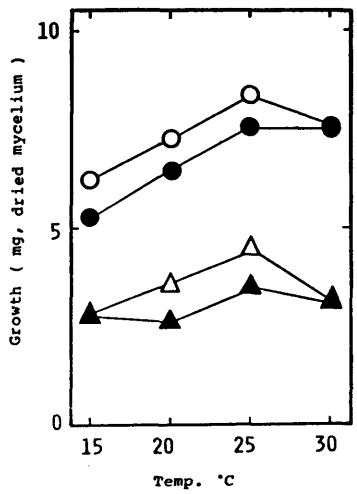
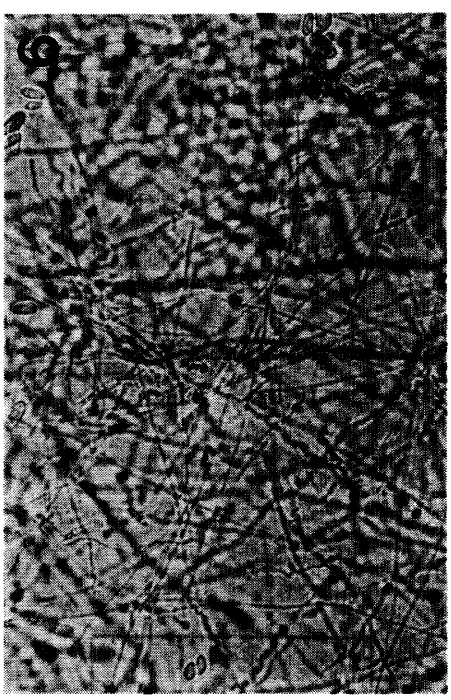
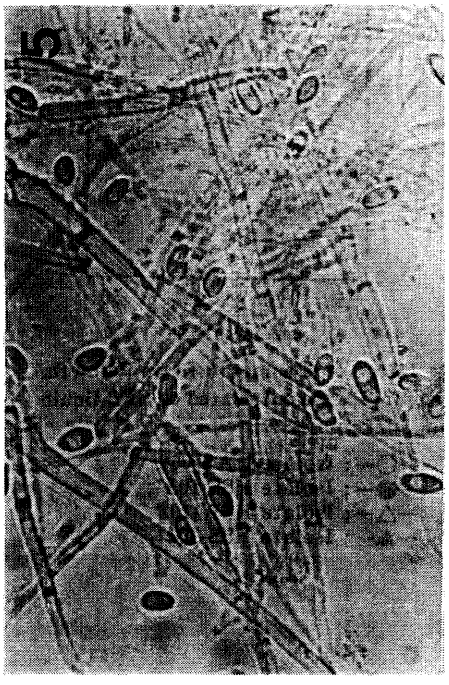


Fig. 2 Effect of Temperature on Growth. Isolated fungi were cultivated on SFC liquid medium.

—○—; Isolate HAG-056
—●—; Isolate HSS-101
—△—; Isolate HSW-102
—▲—; Isolate HSS-102





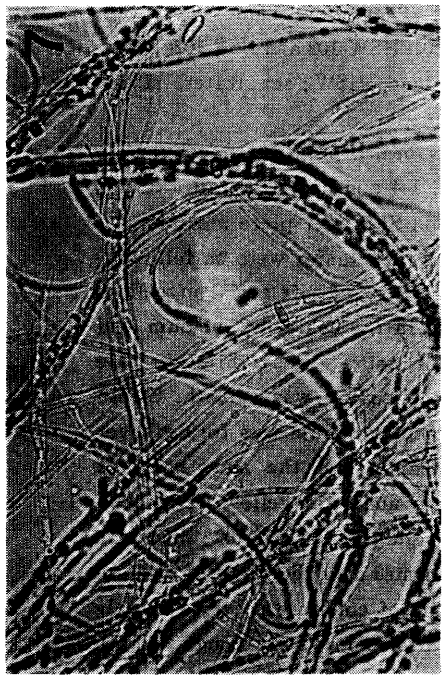


Photo. 5-8 Hyphae of Fucoidan-Utilizing Fungi.
5; Isolate HAG-056 (×400), 6; Isolate HSS-101 (×200),
7; Isolate HSW-102 (×400), 8; Isolate HSS-102 (×400).

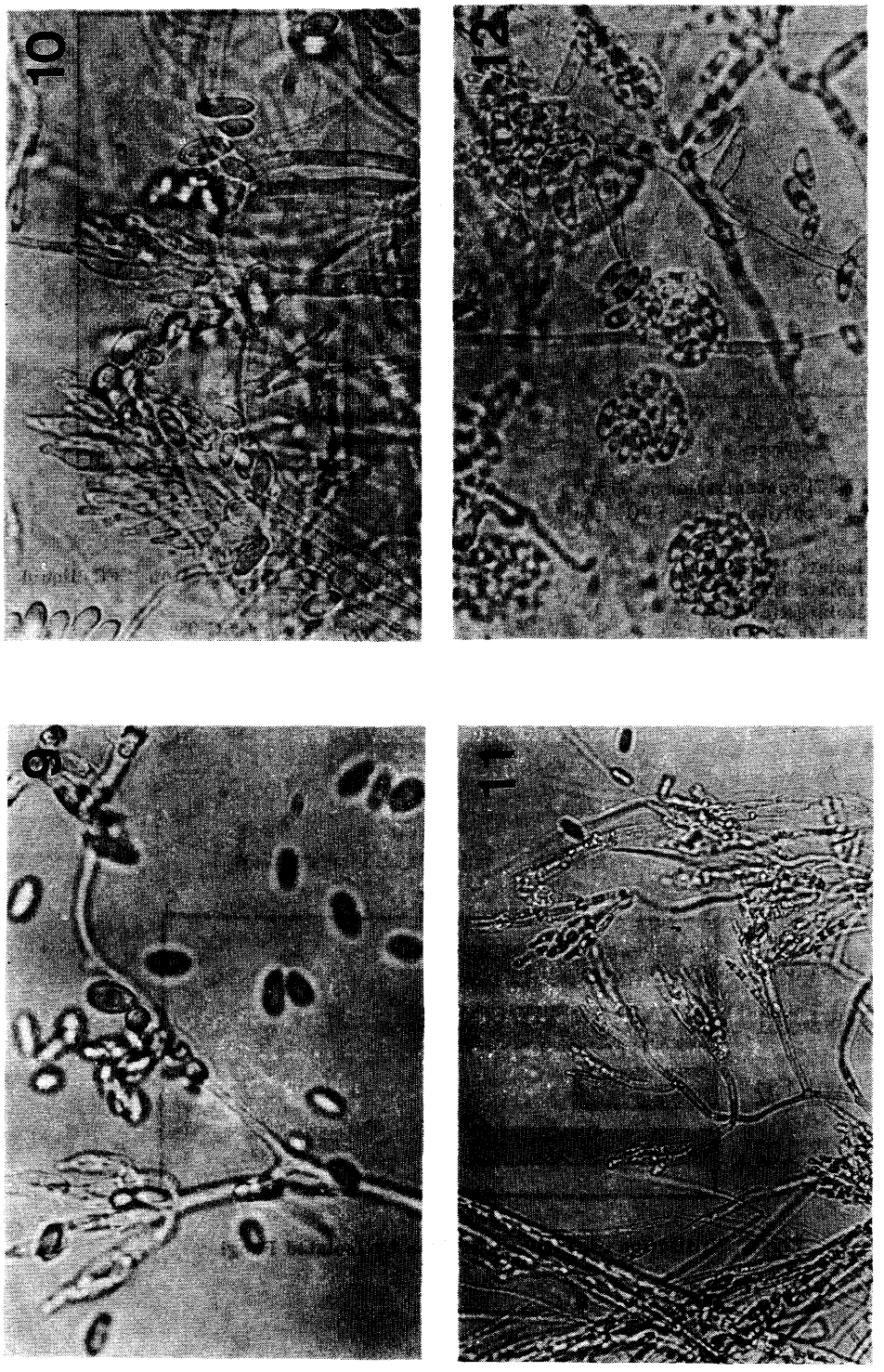


Photo. 9-12 Conidiophore and Conidia of Fucoidan-Utilizing Fungi.
9; Isolate HAG-056 (×400), 10; Isolate HSS-101 (×400),
11; Isolate HSW-102 (×200), 12; I olate HSS-102 (×400).

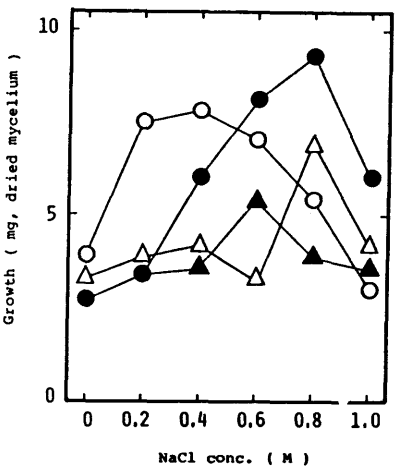


Fig. 3 Effect of NaCl concentration on Growth.
Isolated fungi were cultivated on SFC liquid medium.

—○—; Isolate HAG-056
—●—; Isolate HSS-101
—△—; Isolate HSW-102
—▲—; Isolate HSS-102

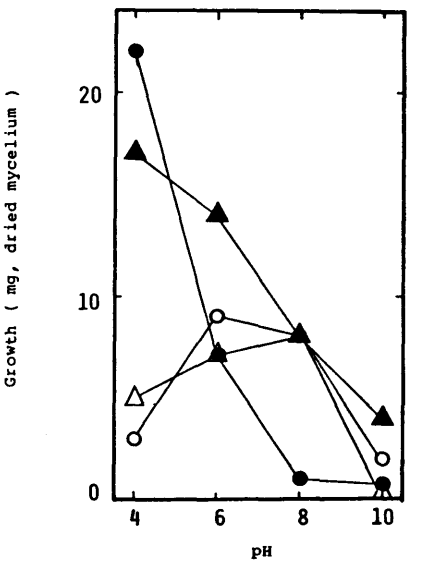


Fig. 4 Effect of pH on Growth.
Isolated fungi were cultivated on SFC liquid medium.

—○—; Isolate HAG-056
—●—; Isolate HSS-101
—△—; Isolate HSW-102
—▲—; Isolate HSS-102

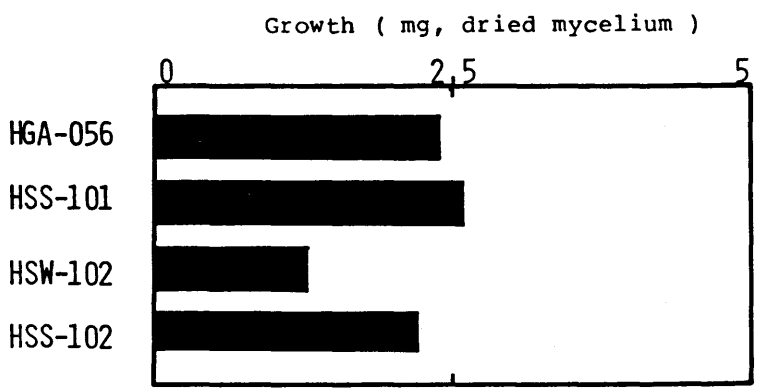


Fig. 5 Utilization of Gagome-Fucoidan by Isolated Fungi.

mass color of colony was pale yellow. The brim of colony was very rough.

Microscopic observation of haphae, conidiophore and conidia of fungi was shown in Photo. 5-12.

Strain HAG-056 and strain HSS-101 formed fine or moderate and irregular haphae, which had conidiophore but no septa and symmetrial monoverticillata. Strain HSW-102 formed moderate and irregular haphae, which had metulae but no septa and was symmetrial monoverticillata. The haphae formed a rhzomorph. Strain HSS-102 formed ahort and irregular haphae, which had many minute particles and small vacuoles. The conidia were formed sherialy not sterigmata but top of conidiophore.

No sexual reproduction was observed. These fungi were not identified. Cultural Characteristics.

Effect of Temperature on Growth

After 7day's incubation, effect of temperature on growth of screened fungi was shown in Fig. 2.

The optimum temperature was thus 25°C.

Effect of NaCl concentration on Growth

As shown in Fig. 3, all fungi grew at 0-1.0M concentration of NaCl. The optimum concentration was as follows: strain HSS-101, strain HSW-102 and strain HSS-102; 0.6-1.0M, strain HAG-0.56; 0.2-0.6M, even at 1 0M NaCl, strain HSS-101 and strain HSW-102 grew very well.

Effect of pH on Growth

All of the fungi grew at the pH range of 4.0 to 10.0 (Fig.4). At pH 10.0, these fungi grew slightly at first but stoped after 3 days. The optimum pH was follows: strain HAG-056; 6.0, strain HSW-102; 8.0, strain HSS-101 and strain HSS-102; they grew well at acidic side of pH.

From these results, it was suggested that all of screened fungi are halophilic and strain HSW-102 is alhalophilic within them. The fact that these fungi have this characteristics on growth seemed to be proper, ecause they screened mainly from samples collected at coastal sea area.

Comparison of Utilization of Screened Fungi against Gagome-Fucoidan

At the moie suitable condition for these fungi, utilization of Gagome-fucoidan by them was investigated. As shown in Fig. 5, content of their mycelium formed on SFC medium was 2.4, 2.6, 1.3 and 2.2mg for strain HAG-0.56, strain HSS-101, strain HSW-102 and strain HSS-102, respectively.

From this results, it was estimated that strain HSS-101 in tested isolates had a superior utilizing ability against gagome-fucoidan.

As reported by J. Kohlmeyer & E. Kohlmeyer, typical marine-fungus, such as *corollospora maritima*, *Arenariomyces trifurcatus* and *Carboshaerella leptosphaeioides*, were separated from a sandy beach.[10] In this time, these fungi were not screened. This seemed to be due to use unsuitable medium for them.

It was very lucky that all of 4 strains isolated in this time could grow well on such medium.

However, there is necessity for measuring more closely growth condition of different kind fucoidan, mozuku-fucoidan or futomozuku-fucoidan, as a carbon source, acquisition of various types of fucoidan-degrading enzymes is expected (no data).

In any case, screening of these fungi is very significant in the point of utilization of these fungus, its productive enzymes and enzymatic products of various fucoidan in medical or industrial region.

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要　　約

フコイダン資化性微生物に関する研究（第3報）

—フコイダン資化性糸状菌の分離とその生育特性—

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日本沿岸域より採集したサンプルよりフコイダン資化性糸状菌を分離し，その生育特性とフコイダン資化性について調べ，次のような結果を得た。

1）海砂，海藻，海水等のサンプルより，フコイダンを唯一の炭素源とするＳＦＣ培地により，４株のフコイダン資化性糸状菌を分離した。

2）４株の分離菌をＨＡＧ-056，ＨＳＳ-101，ＨＳＷ-102，ＨＳＳ-102株と名付け，その生育特性を調べた。分離菌は，全て食塩の存在下でよく生育し，最適温度は25°Ｃであった。ＨＡＧ-056とＨＳＷ-102は，中性，微アルカリ性で，ＨＳＳ-101とＨＳＳ-102は，酸性領域でよく生育した。

3）ガゴメフコイダンに対する資化性の比較より，ＨＳＳ-101が最もよく生育することから，優れた資化能力を有するものと推定された。

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