

The Role of Corn Seed Hemicellulose for Cereal Fresh Preservation

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Abstracts

The storage of corn seeds in the fresh state is very difficult. This reason is presumably ascribable to the unstability of hemicellulose constructing the cell membrane. The hemicellulose was composed of xylose, arabinose mainly, and smaller amounts of galacturonic acid, glucose and galactose. The mean molecular weight was about 730,000 by a gel-filtration method. The yield of the hemicellulose from corn seeds amounts to about 1.5%. It is hydrolyzed by an exo-enzyme of one strain of bacteria isolated from corn seed. The mode of the hydrolysis is that of an endo type. This bacterium is a native one, namely, utilizes preferably the hemicellulose as only carbon source for the growth. At 15°C it can grow with difficulty in stab and plate culture, but at 5°C it can not grow at all.

This bacterium is quite resistant to heat treatment, since even after heating at 120°C for 20 min., a significant number of bacteria appeared in the sterilized medium. The bacterium can grow within pH 6-10 range.

Thus, we are expecting that corn seeds will be stored safely by controlling temperature and spraying of the acidic solution.

The storage of corn seeds in the fresh state is very difficult. The harvested corn seeds with a corn cob are pulled out from the stem. It is stored free from damage in the straw wrapper on the field at the Aso mountain district, Kumamoto Prefecture, Japan. The environmental temperature at that district is 14° Celsius on an average for a year.

But the corn seeds alter soon after being brought to the base of the mountain where the temperature is relatively high.

As this reason, it is presumed to be ascribable to the result of the unstability of

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hemicellulose constructing the cell membrane of corn seeds. Hereupon we considered hemicellulose as cold 5% sodium hydroxide soluble matters according to E. Schulze's definition (1).

In Fig. 1, the upper is a schematic expression of cell wall of a fresh corn seed. The outer circle is a cell membrane which contains a few amount of hemicellulose. The inner part contains carbohydrate, protein and fat & oil.

The lower is a scheme of cell wall of a deteriorated corn seed. There is no hemicellulose. Thus, carbohydrate, protein and fat & oil are skimmed out, and then gradually change to acidic matters.

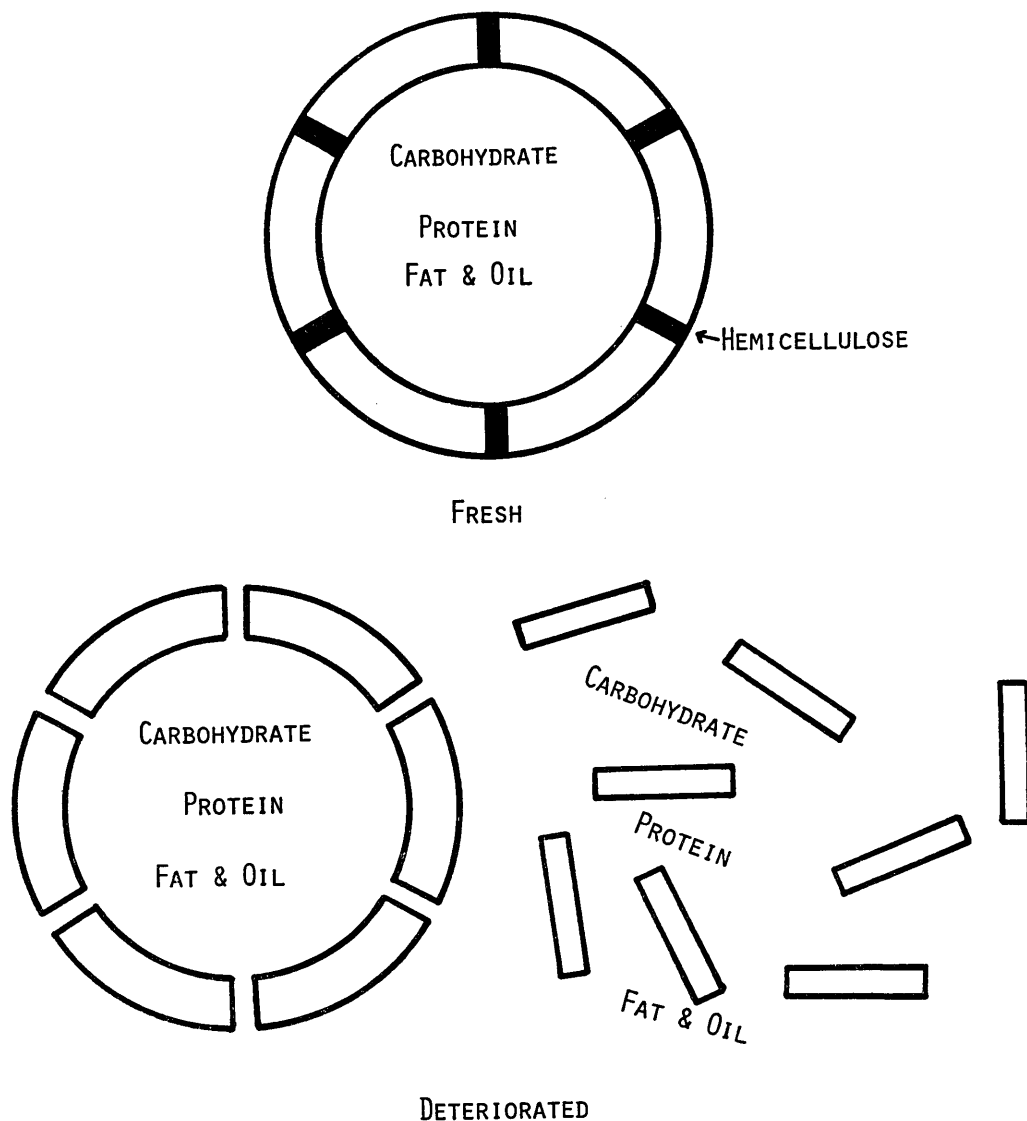


Fig. 1. Schematic expression of Fresh and Deteriorated Corn seed.

In the meanwhile, an apparent increasing of water-soluble sugars occurs during the storage of corn seed powder. The disappearance of hemicellulose in the corn seed is also demonstrated under the storage. In Fig. 2, the right one shows microscopically that starch granules in the stored corn seed powder exist in a naked state without cell-membrane-like material. On the contrary, the left one shows that starch granules in unstored corn seed are wrapped with cell-membrane-like material.

The former, that is, naked granules are the result of lack of hemicellulose which is one of constituents of cell-membrane-like material. As stated above, the increasing of water-soluble sugars in the stored corn seed powder occurs by this same reason.

When powdered corn-seeds were stored in a refrigerator for 8 months and then their water soluble matters were extracted at 20°C for 1 hr., it was showed that the total sugars in the sample stored increased very much as compared with the original one.

The corn seed (stored in an ice box after powdered and sifted) was extracted with 5 volumes of water for 6 hrs at about 50°C. The pH was adjusted to 4.72, with acetate buffer.

The extract was filtered, hydrolyzed, neutralized, and deproteinized. Paper chromatograms of the hydrolyzate showed glucose and uronic acid. They were presumed to be cold 5% NaOH soluble substances, that is, hemicellulose.

This polysaccharide is very susceptible to enzymatic attack and thus would be hydrolyzed to sugars.

The carbohydrates constituting raw corn seed were separated in four fractions as following, namely, soluble part in hot 80% methanol (I), hot water soluble parts (III) and hydrolyzed matters of III (II), and cold 5% NaOH soluble (hydrolyzed) parts (IV).

Paper chromatographies and chemical reactions as to these parts showed that free sugars were glucose, fructose and xylose, constituting sugars of the fraction II were glucose, xylose and fructose (not yet confirmed), and component sugars of the fraction IV were glucose and uronic acid (not yet confirmed).

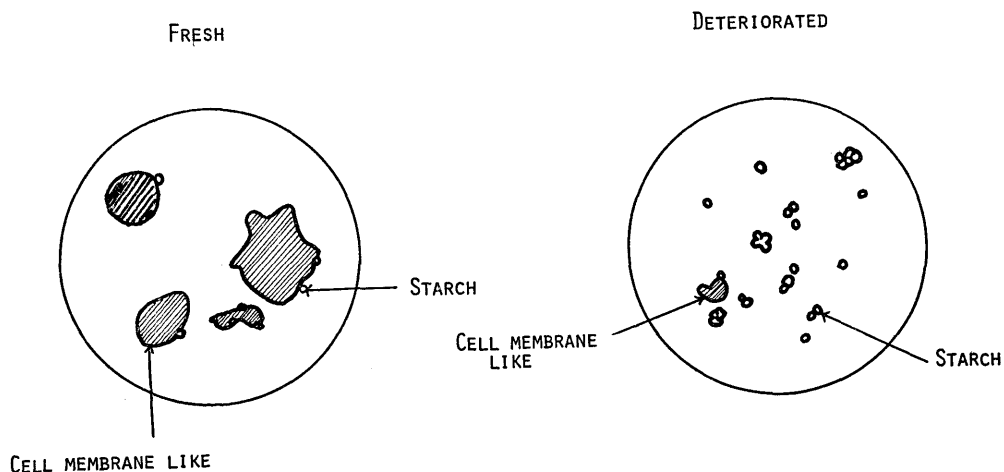


Fig. 2. Powder.

In section of a corn seed granule eaten by worms, it is observed that worms eat ways into the corn seed from the side of corn cob instead of the surface of it. This phenomenon is presumed to be the result of the dissolution of hemicellulose, too.

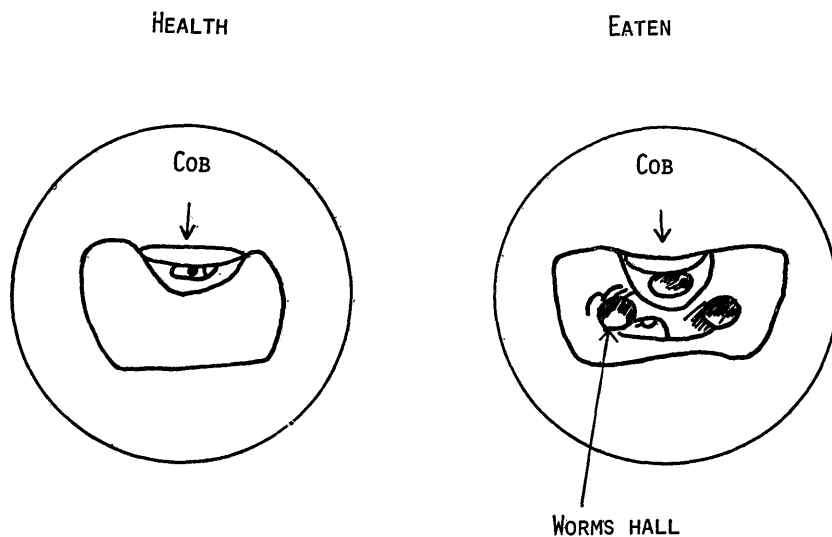


Fig. 3. Section.

The yield of the hemicellulose from corn seeds amounts to about 1.5%.

The separation of the hemicellulose from corn seeds is summarized as following. Namely, powdered corn seeds were defatted with ether by a Soxhlet's extractor.

The resulting powder was suspended in M/200 $\text{Ca}(\text{AcO})_2$ buffer (pH 5.5) and incubated at 37°C with the bacterial α -amylase (ratio 10,000 to 1) for 6 days. The pH of the medium was always maintained at 5.5, and in the duration of this amylase treatment. The filtration of the medium and crushing of the dry residue in a ball mill were repeated, until the iodine reaction showed negative.

The residue was then treated with 0.5% ammonium oxalate solution for 6 hours at 100°C . After filtration, the residue was extracted with 5% NaOH solution under nitrogen gas at room temperature. The extract was neutralized with HCl until pH 2 (methyl orange) and then mixed with methanol (ratio 1 : 2). By this treatment, a white precipitate was formed. So our adopted hemicellulose might be correspond to the hemicellulose-B (O'Dwyer, 1926) in the point of solubility in an organic solvent (2).

After being filtered, the residue was washed with 80% methanol until the washing became free from the halogen ion, then the concentration of methanol was increased to absolute.

Finally it was washed with ether. The hemicellulose thus obtained was stored in a desiccator with calcium chloride.

When starting materials are rich in starch, starch must be removed beforehand care

fully, because the existence of starch disturbs the separation of hemicellulose.

According to the up-to-date process, horse bean starch is removed by Taka-diastrase, and rice starch by salivary amylase. The process of removing starch by the former method is unsuitable for the separation of hemicellulose, because the hemicellulose is dissolved also by the Taka-diastrase solution. In the case of the latter it takes a lot of time to separate the starch itself. The best way to eliminate the above mentioned faults was to use bacterial α -amylase. This α -amylase treatment is very effective, because it has no influence on the hemicellulose.

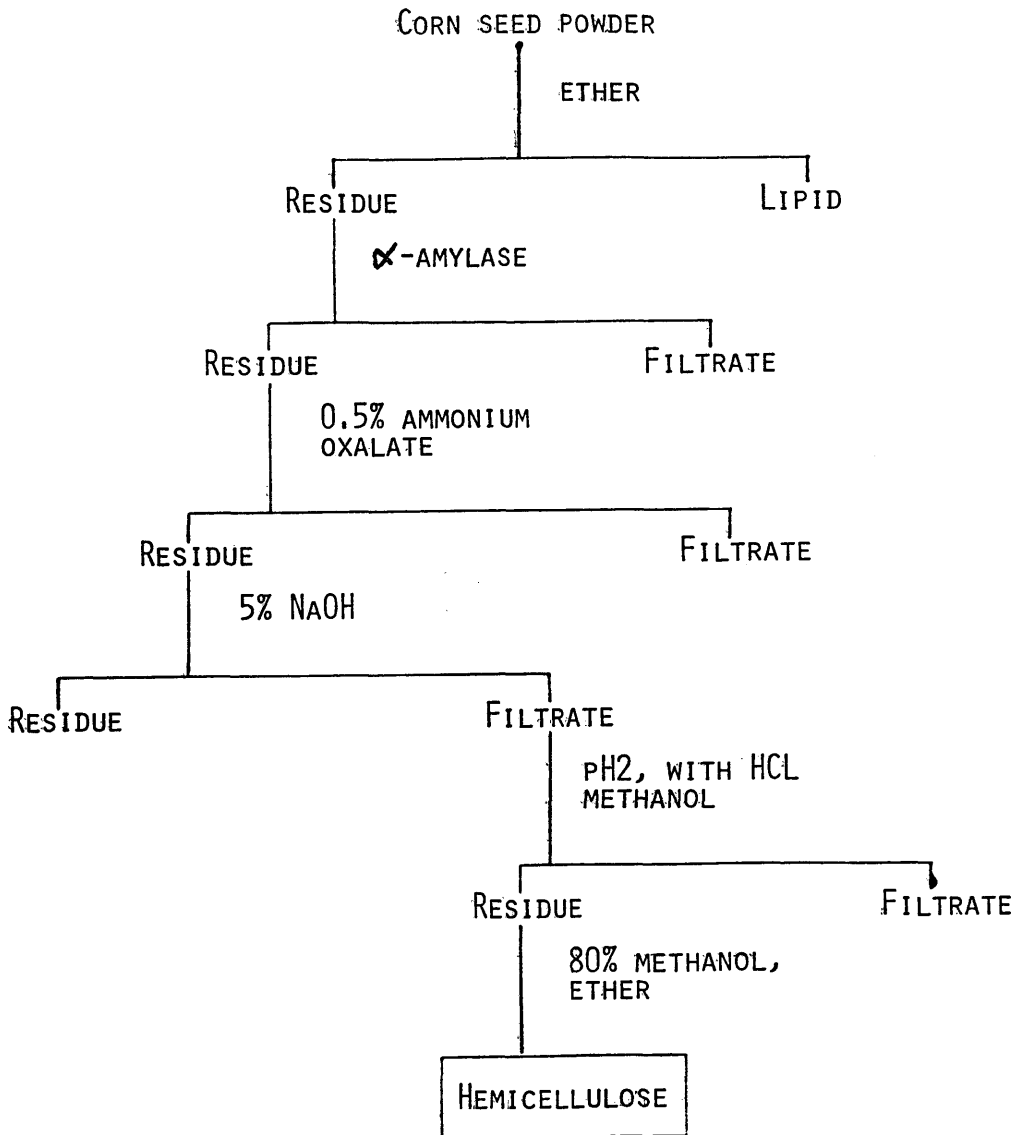


Fig. 4. Separation

The hemicellulose extracted from corn seeds is dissolved in water as a milky white and semi-transparent solution. The hemicellulose thus obtained was brownish, colloidal state in appearance and contained 0.027% ash.

Paper chromatographies and chemical reactions of the hydrolysate showed that the hemicellulose composed of xylose, arabinose, and smaller amounts of galacturonic acid, glucose and galactose. The solubility of the hemicellulose was estimated as about 50%. By adding an extract of corn seeds the solubility of hemicellulose increase very much.

The dissolution of hemicellulose is promoted by adding Taka-diestase mentioned previously. The corn seed suspension and Taka-diestase solution may contain hemicellulose decomposing enzymes. However, during this dissolution, reducing sugars did not increase apparently. Hence, the enzymes might be debranching or scissing one.

The mean molecular weight of the hemicellulose was estimated as about 730,000 by Andrew's gel filtration. In this experiment, Bluedextran 2,000(M.W. 2,000,000) and Dextran T 500 (M.W. 500,000) were used for the standard materials.

Using a screening medium containing inorganic nitrogen source and corn seed hemicellulose as sole carbon source, we isolated a hemicellulose decomposing strain belonging to wild lactic acid bacteria from a corn seed.

In the Fig. 6, the method of havesting is mentioned. Namely 2 g of crushed corn seeds were incubated in 10ml basal medium (K_2HPO_4 0.1%, $MgSO_4$ 0.02%, $NaNO_3$ 0.2%, and corn seed hemicellulose 0.5%) for 2 days at 37°C. The culture broth was adjusted to pH 8.0 with phosphate buffer.

In this culture, the pH of medium and the number of the bacterium decreased in allowing to stand. So the control of pH was carried out day by day, then the stable growth of the bacterium was attained.

The uniformly crowded medium was selected and adopted.

In the plated culture basal medium was used and an aerobic culture for the selection was applied.

Elapsed 2 days, an appeared colony was round circle. In the liquid culture, basal medium was also used. The growth of the strain reached to the stational phase in about 23 hours. And its generation time was about 90 minutes. A viable cell number appeared on a plate culture, using a diluted medium, was counted to estimate a growth curve in the basal medium. The generation time was examined by means of an order. In this case a synchronous growth was adopted at the first of the stational phase.

The composition of the suitable medium for the culture of tentatively enumerated No. 101 bacterium which produces the hydrolysing enzyme (hemicellulase) toward corn seed hemicellulose had been searched beforehand. Twenty eight kinds of the medium were scrutinized. As the carbon source xylose, arabinose, glucose, galactose, soluble starch and corn seed hemicellulose were examined. The nitrogen source were ammonium primary phosphate, ammonium sulphate, sodium nitrate and peptone. Sodium nitrate was used as referring nitrogen source for cellulose decomposing bacterium. This salt was very useful in a screening of hemicellulose decomposing bacterium to prevent a contamination.

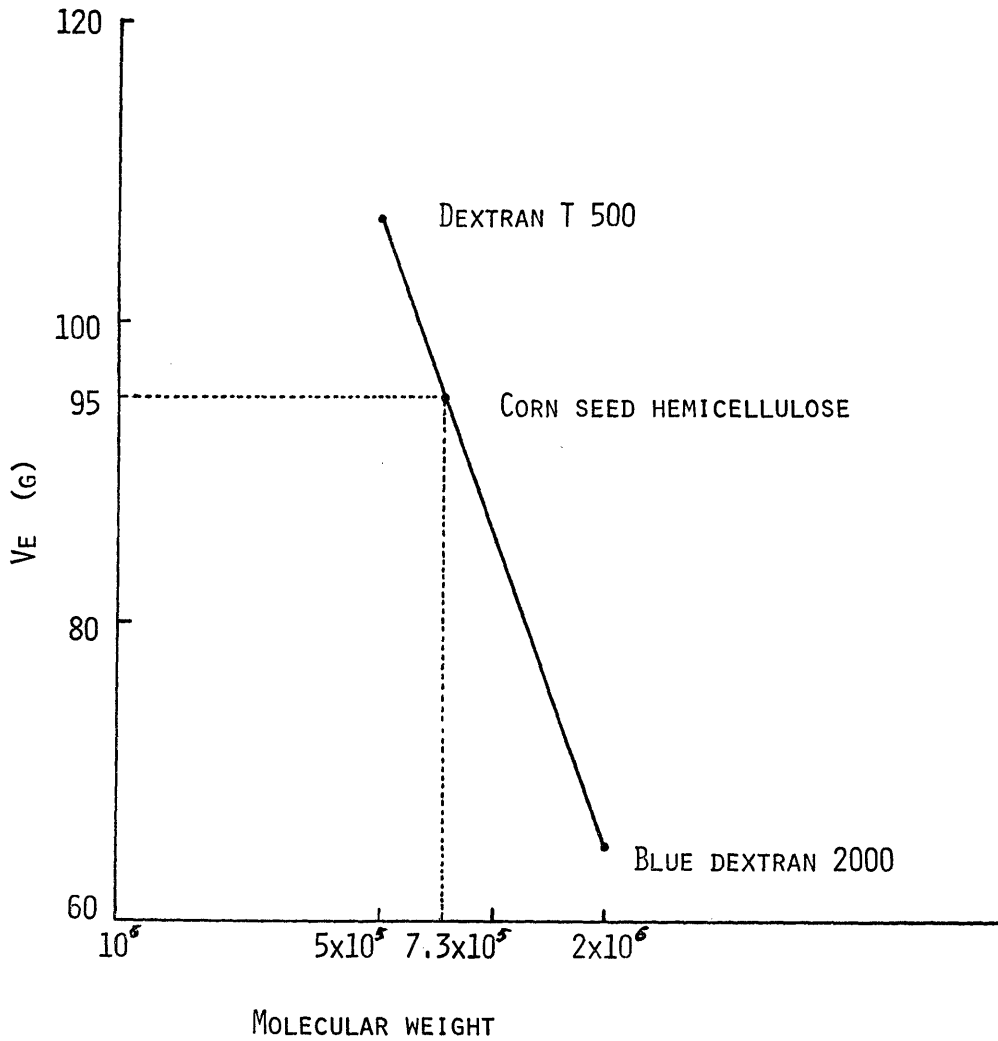


Fig. 5. Molecular weight.



Fig. 6. Screening.

Protein was determined by the Lowry et.al. method (3). The hemicellulase activity was determined by the decrease of a viscosity in the reaction system containing 10ml of 0.2% phosphate buffer solution (pH 5.7) of corn seed hemicellulose and 1ml of enzyme solution.

The reaction was carried out at 37°C for 30 minutes with a Ostwald's viscosimeter

The method of calculation is as follows. The activity on viscosity is calculated by means of the equation :

$$a = \frac{t_1 - t_2}{t_1 - t_0} \times 100$$

in which

a = activity in percent ;

time required to falling ;

t₀ = buffer alone

t₁ = buffer + sample at starting point

t₂ = / + / after digestion

The activity as to the increase of reducing power was analysed by Somogyi-Nelson's method as a xylose quantity (4).

The results showed that corn seed hemicellulose and corn seed powder which was free from fat, starch and pectin were suitable carbon source, and sodium nitrate was beneficial as the nitrogen source.

Table 1. Medium.

N	C	Xylose	Arabinose	Glucose	Galactose	Soluble starch	Hemicellulose
		(A) 1.2	1.8	0.5	1.6	1.4	3.7
NH ₄ H ₂ PO ₄	(B) 10.3	1.8	9.7	4.2	4.2	14.8	
	0.9	0.9	0.2	0.8	1.4	2.9	
(NH ₄) ₂ SO ₄	3.4	8.9	3.1	2.9	3.0	17.1	
	—	—	0.3	0.9	0.6	5.2	
NaNO ₃	4.7	1.6	3.2	4.0	4.5	59.8	
	1.1	0.9	1.4	2.4	2.2	4.3	
Pepton	5.9	1.5	5.9	1.1	6.6	25.6	

(A) : Protein (B) : Activity

During the cultivation of this bacterium, it was found that the bacterium was quite resistant to heat sterilization, since even after being heated at 120°C for 20 minutes, a significant number of bacteria appeared in the sterilized medium. The bacterium presumably contains spores resistant to the heat treatment. In order to recognize spores in bacterium, it was cultured in malt juice for 16 hrs and 48 hrs, then each of cultured medium was mixed. An aliquot of it was stained successively with 5% aqueous chromic acid, phenol fuchsinic acid, and methylen blue solution. At these treatments, the pink coloured spore was observed microscopically in the blue colored cell (Möller). The bacterium could grow within pH 6-10 range.

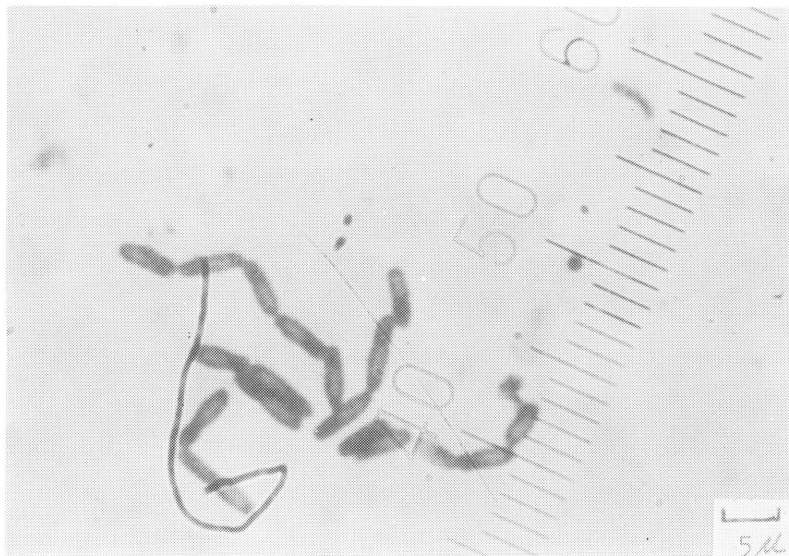


Fig. 7. Spores. ($\times 1,500$)

The final identification of this hemicellulose hydrolyzing bacterium has not been accomplished yet. But until now quite a few of its properties was surveyed as follows: the form was rod with rounded ends, length was $2-10\mu$, motility none, gram stainability was positive, surface growth in 5% yeast extract was positive, acid production was weak, and catalase reaction was positive.

Table 2. Comparison

	No. 101 bacterium	Microbacterium (Bergey')
Form	Small rods with rounded ends	Small rods with rounded ends
Length	$2-10\mu$	$0.5-30\mu$
Motility	None	None
Stainability	Gram+	Gram+ Granulations demonstrable with Methylene blue stain.
Growth	Surface growth with 5% yeast extract	Good surface growth on media supplemented with milk or yeast extract.
Acid production	Weak	Weak
Lactic acid production	+	L-lactic acid
Catalase	+	+
Optimum temperature	$14-37\text{ }^{\circ}\text{C}$	32°C

A crude preparation of the hemicellulase was purified by gel filtration.

A culture filtrate obtained from 7 day's incubation was subjected to the fractionation by ammonium sulfate. The precipitate settled by 60% saturation was further fractionated by successive applying of Sephadex G-25, G-75 and G-100. The eluates were lyophilized separately.

Although the enzyme preparation was still impure, the mode of the enzymic action was presumably an endo type.

At that way, a mixed solution composed of 200 mg of corn seed hemicellulose as a substrate, 200 mg of crude enzyme preparation and 50ml of 0.1 M pH 5.7 phosphate buffer was incubated at 37°C.

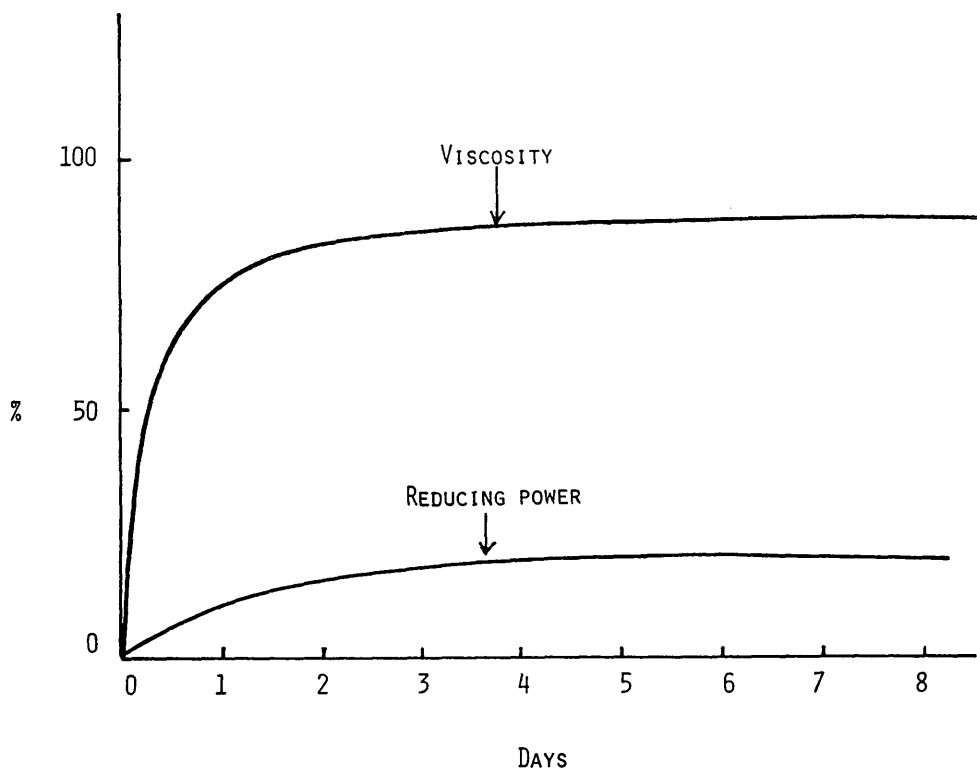


Fig. 8. Hydrolyze.

In the terms of decrease of the viscosity and the increase of the reducing powers, the activity of the enzyme was measured. The former aims to the end-hydrolyzing action and the latter focused to the exo-hydrolyzing action.

On the whole, this bacterium is a native one, namely, utilizes preferably the hemicellulose as only carbon source for the growth. At 15°C it can grow with difficult in a stab and plate culture, but at 5°C it can not grow at all. Furthermore, it is very weak against slightly acidic medium.

Thus, we are expecting that corn seeds will be stored safely by controlling temperature and spraying of the acidic solution.

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