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STUDIES ON THE CULTURE OF BACTERIUM ORYZAE (UYEDA ET ISHIYAMA) NAKATA. (PRELIMINARY REPORT).

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With respect to culture of the organism responsible for the bacterial leaf blight disease of the rice plant, namely, Bacterium Oryzae (Uyeda et Ishiyama) Nakata, Ishiyama (1922) gives it some attention in his paper, but there is a lack of detail. Under these circumstances, the author felt that there was need for more knowledge concerning the best medium for the culture of this bacteria, and this paper represents the results of research on several media.

In the course of this experiment, the author received advice and aid from many members of the Pathology Laboratory of this station, to whom he is greatly indebted.

1. Types of Culture Media and Bacterial Growth.

The bacteria used in the current experiment was isolated in 1949 by the Pathology Department of the Institute of Agronomy Research, Ministry of Agrioulture and Forestry, the pathogenicity of the strain being proven at the author's experimental station. The experiment was conducted during the period, April 1951 - March 1952. The bacteria was grown for three to five days at a temperature of 25° to 30°C in a 2% potato sucrose agar medium, and then transferred with a 4mm diameter platinum wire loop to a streak slant culture and a deep stab culture. The cultures were incubated for four to five days at temperatures in the range of 25°-30°C. About three to five tubes each of the slant and stab cultures were made per experiment, the experiment being repeated two to three times. The basis of the medium was 15 to 25 grams of agar to 1 liter of water, 10cc per test tube, sterilization in a high pressure autoclave, and the pH determined by

colorimetry. The various types of culture media used and their compositions are as follows:

a. Potato sucrose agar medium: Potato 200gr. sucrose 20gr.

b. Same as above without sucrose.

c. Sucrose medium: Sucrose 10gr. KH_2PO_4 1 gr. $MgSO_4 \cdot 7H_2O$ 0.5 gr.

d. Dextrose medium: Dextrose 20 gr.

e. Bouillon: Meat(bonito) extract 10gr. peptone 10gr. MaCl 5 gr.

f. Dextrose bouillon: Same as above plus dextrose 10gr.

g. Czapek medium: $MgSO_4 \cdot 7H_2O$ 0.5gr. KCl 0.5gr. K_2HPO_4 1 gr. $NaNO_3$ 2 gr. $FeSO_4$ 0.01 gr. sucrose 30 gr.

h. Richard's medium: KNO_3 10gr. KH_2PO_4 5 gr. $MgSO_4 \cdot 7H_2O$ 2.5 gr. sucrose 50 gr.

i. Conn's medium: KH_2PO_4 5 gr. $MgSO_4 \cdot 7H_2O$ 5 gr. $Ca(PO_4)_2$ 0.5 gr. ammonium titrate 10 gr.

j. Rice straw broth medium No.1: Rice straw (old, dried) 50 gr. sucrose 10 gr.

k. Rice straw broth medium No.2: Rice straw (new stems) 250 gr. sucrose 10 gr.

l. Rice straw broth medium No.3: Rice straw (new leaves) 250 gr. sucrose 10 gr.

The pH values of these different types of media after sterilization and the degree of bacteria growth in each of the media are shown in Table 1.

Table 1

(1) 各種培養基	(a) 馬鈴薯	(b) 同 (無糖)	(c) 蔗糖	(d) 葡萄糖	(e) ブイヨン	(f) 葡萄糖ブイヨン	(g) ツアペック	(h) リチャーダ	(i) コーン	(j) 稻藁 1	(k) 同 2	(l) 同 3
pH	5.6	5.6 ~5.8	°5.8 ~6.0	5.0	5.0~5.2	4.6~4.8	°6.2 ~6.4	4.6~4.8	°6.2 ~5.4	5.2 ~5.4	5.4 ~5.6	5.2 ~5.4
(2) 斜面培養	##	##	+	+	##	###	+	+	±	+	+	+
(3) 穿刺培養	##	##	+	+	+	###	+	+	±	##	##	##

(1) Type of medium. (2) Slant culture. (3) stab culture.

(a) Potato sucrose agar
(b) Potato agar
(c) Sucrose
(d) Dextrose
(e) Bouillon
(f) Dextrose bouillon
(g) Czapek
(h) Richard's
(i) Conn's
(j) Rice straw broth No. 1
(k) Rice straw broth No. 2
(l) Rice straw broth No. 3

Remarks: Note that figures with the symbol ° means that the medium has been normalized with an N-solution of NaOH.

The present experiments showed that bacterial growth was best with the 2% potato sucrose agar medium and the 1% dextrose bouillon agar medium, and that with the rice straw broth, growth was observable with the stab culture but poor with the slant culture, also that with the potato agar medium without sucrose, bacterial growth was considerably poorer compared to the medium containing sucrose. In the case of the bouillon media, there was similar growth in the media with and without sucrose for the first two or three days, but thereafter in the medium without sucrose, the surface of the medium where bacterial growth was absent turned milky white, which seemed to inhibit the growth. In the cases of the sucrose, the dextrose media, or the synthetic cultures, i.e., the Czapek, Richard's, and Conn's media, there was minimal growth of the bacteria.

Since the experiments showed that the bacteria grew the best in a dextrose bouillon agar medium, we ran further cultures with one or the other of the elements missing. All of the cultures were intermittently sterilized thrice in a Kooh sterilizer. The results are shown in Table 2.

Table 2

	無ペプトン (3)	無肉エキス (4)	無 NaCl (5)	葡萄糖ブイヨン (6)
pH	4.6~4.8	4.6~5.0	4.6	4.6~4.8
斜 面(1)	##	##	###	###
穿 刺(2)	##	##	###	###

(1) Slant culture.
(2) Stab culture.
(3) No peptone.
(4) No meat extract.
(5) No NaCl.
(6) Dextrose bouillon.

It is seen that the absence of peptone or meat extract causes very poor bacterial growth, while there is little difference in the growth in the absence of salt.

Since the meat extract used in the experiment was made from the flesh of the bonito, bouillon agar and dextrose bouillon agar mediums with extracts prepared from beef and bonito were compared, with the result that there was little difference in the pH value or the degree of growth of the bacteria in these media.

2. Relation Between Type and Quantity of Sugar and Bacterial Growth.

Since in the case of the potato and the bouillon media, it was seen that the addition of sugar had a beneficial effect on bacterial growth, sucrose, dextrose, and maltose were combined with potato broth or bouillon to test for possible differences in the effect of different sugars. In the case of the potato broth agar medium, 20gr of sugar of each type was added to 1 liter of distilled water together with 200gr of potato; in the case of the bouillon agar, there was 10gr each of meat extract, peptone, sugar, and 5gr of salt. To each of the media was added 20gr of agar, and following sterilization, the pH value and the saccharinity by means of a refractormeter were measured, after which the media were inoculated. The pH value, the saccharinity, and the condition of bacterial growth of the various media are shown in Table 3.

Table 3

	ブドウ糖(1)		蔗糖(2)		麦芽糖(3)		無(4)	
	馬鈴薯(8)	ブイヨン(9)	馬鈴薯(8)	ブイヨン(9)	馬鈴薯(8)	ブイヨン(9)	馬鈴薯(8)	ブイヨン(9)
pH	4.8~5.0	4.6~4.8	4.8~5.0	4.6~4.8	4.8~5.0	4.6~4.8	5.2~5.4	5.0~5.2
糖度(%)(5)	3.6~3.8	5.0~5.2	3.4~3.6	4.4~4.6	3.4~3.6	4.2~4.4	1.6~1.8	3.4~3.6
斜面(6)	##	##	##	##	##	##	+	++
穿刺(7)	##	##	##	##	##	##	+	++

(1) Dextrose.
(2) Sucrose.
(3) Maltose.
(4) No sugar.
(5) Saccharinity.
(6) Slant culture.
(7) Stab culture.
(8) Potato.
(9) Bouillon.

It can be seen from the above results that there is little difference with respect to the type of sugar involved, in other words, bacterial growth is favorable, while on the other hand, the absence of sugar implies poor growth.

Since it was shown that the type of sugar made no difference, the quantity of the sugar, in this case, sucrose, was

varied in six steps: 0.5, 1.0, 2.0, 5.0, and 10.0%, and no sugar, with respect to potato broth and bouillon media. The pH value, saccharinity, and the condition of growth of the various media prepared in this case are shown in Table 4.

Table 4.

		0.5%	1.0%	2.0%	5.0%	10.0%	無(1) 糖
馬鈴薯 (2)	pH (4)	5.2~5.4	5.0~5.2	5.0~5.2	4.8~5.0	4.8~5.0	5.2~5.4
	糖 度 (%)	1.6~1.8	2.2~2.4	3.4~3.6	6.0~6.2	10.2~10.4	0.8~1.0
	斜 (5) 面	##	##	##	##	##	+
	穿 (6) 刺	##	##	##	##	##	+
アイヤン (3)	pH (4)	4.6~4.8	4.6~4.8	4.6	4.4~4.6	4.4	4.6~4.8
	糖 度 (%)	3.6~3.8	4.6~4.8	6.0~6.2	9.8~10.0	13.8~14.0	3.2~3.4
	斜 (5) 面	##	##	##	##	##	##
	穿 (6) 刺	##	##	##	##	##	##

(1) No sugar. (3) Bouillon. (5) Slant culture.
(2) Potato. (4) Saccharinity. (6) Stab culture.

It is seen that for a range of 0.5 to 10.0% concentration of sugar, the saccharinity determined with a refractometer was 1.6 to 10.4% for the potato broth and 3.6 to 14.0% for the bouillon, and that within this range, bacterial growth was good with very little variation between the different cultures.

3. Relation Between pH and Bacterial Growth.

NaOh and HCl, normal solutions, were added in appropriate amounts to the potato broth, the latter then intermittently sterilized thrice in a Koch sterilizer, the pH values measured, and then inoculated. The pH value ranges involved in this experiment were 1.8-2.0, 3.0, 4.0, 5.0 (no addition of reagent), 7.2-7.4, 8.8, 9.0-9.2, 10.6, 10.8-11.0, and it was found that the limits for growth were 4.0 and 8.8, with no bacterial growth possible for pH values less than 3.0 or higher than 9.2.

4. Discussion and Summary.

According to the experiments as described here on the growth of Bacterium Oryzae (Uyeda et Ishiyama) Nakata on various types of culture media, it was found that growth of this bacterium was best on bouillon glucose agar and potato sucrose agar, with the growth being relatively poor in the cases of the other media tested. It was also shown that there was little difference in the effect on growth between the sugars sucrose, dextrose, and maltose, that is to say, growth was equally good, and that the degree of growth was fairly uniform for the range of sugar concentration 0.5 to 10.0%. The saccharinity, measured with a

refractometer, of the bouillon was higher than that of the potato broth, that is to say, the saccharinity of the bouillon without sugar corresponded to the saccharinity of the potato broth containing 2% sugar, and considering the fact that bacterial growth was favorable in the case of the potato sucrose culture medium, the saccharinity measured with a refractometer is not a sound indicator of the growth limit condition. In the case of the sugar bouillon, it was seen that the meat extract, the peptone, as well as the sugar were all essential to bacterial growth, and also that there was little difference between the use of bonito or beef meat extracts. With respect to the effect of the pH value, it was found that growth occurs for pH values in the range 4.0-8.8 only, and details regarding this point will be reported on another occasion. Comparing the slant culture specimens with those of the stab culture, it was observed that in the early stages, growth seemed to be more favorable with the stab culture specimens, and this could be due to a denser bacteria population transplanted by the stab method of inoculation, also perhaps due to some secretion of the multiplying bacteria which makes the nutrients more easily absorbable, but regarding this point, the experiment should be repeated.

It should be noted that with the exception of the pH experiment, all of the other experiments were with a solid culture medium in which agar was added. With respect to liquid as well as various other types of culture media, the life of this bacterium on various types of media, the toxicity of the culture filtrate, and other details, further research is required. (Received 2 August 1952.)

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