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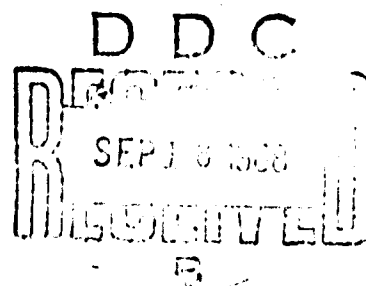
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DEPARTMENT OF THE ARMY
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A STUDY ON THE ESSENTIAL SUBSTANCE FOR THE GROWTH
OF PIRICULARIA ORYZAE

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This manuscript reports a part of an investigation on Piricularia oryzae disease spanning the years 1942 to 1945 while the author was at the western branch (Soriwon) of the Agricultural Experiment Station of the former Korean Government. At that time, the study of P. oryzae involved the cultivation of the fungus - its growth and production were excellent - when there was no basic culture medium for the successful formation of spores. Even when this fungus was grown in the synthetic culture medium of Tochinai and Nakano⁶ which is frequently used in physiological investigations of filamentous fungi, the growth of fungus filaments was small and spores were not formed. However, when the author added a rice straw broth to the culture medium, the cultivation of the fungus filaments was extremely successful and spores were also successfully formed. Furthermore, some information on the characteristic of the cause of acceleration of the culture was obtained later. A portion of the result of the investigation has already been reported in the reference by Tanaka, et al^{1,2}. This was the clue which began many important experiments. This report is a summary of the clue to these investigations.

1. The Influence on the Growth of P. oryzae by the Addition of Rice Straw Broth to a Synthetic Culture Medium

Experimental Procedure: The Tochinai solution was chosen for the synthetic culture medium (KNO₃ 2.0 gm, KH₂PO₄ 0.5 gm, K₂HPO₄ 0.5 gm, MgSO₄ 0.5 gm, CaCl₂ 0.1 gm, FeCl₃ 1% solution 0.82 cc, cane sugar 30 gm,

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distilled water 1 liter), to which was added the rice straw broth in order to observe the effect on the growth of the fungus. In order to have essentially the same consistency in the culture medium and the broth, an additional 3% of cane sugar was added to the rice straw broth. For the rice straw broth, the Ishikawa Chusei Gin Bozu was used. 50 gm of this was soaked in 1 liter of distilled water, kept at 100° C in a steam kettle, boiled for an hour, and then filtered. The liquid culture medium was distributed in 50 cc portions to each of 10 triangular 200 cc Kolbens, then after intermittent sterilization, they were inoculated with a portion of the fungus culture from a Barei Potato Agar slant. After 25 days at 27° C, the growth was examined.

Result: As can be seen in Table 1, the growth of filamentous fungi in Tochinali solution is extremely small and there was no spore formation. Contrasted to this, in the rice straw broth, it was rather good and spore formation was seen. Again in the solution where equivalent amounts of both components were mixed, the growth was extremely successful and spore formation was also remarkable. From this, it could be conjectured that the growth promoting factor of this fungus and the necessary factor for spore formation was contained in rice straw broth.

Table 1. The Influence On the Growth of P. oryzae by the Addition of Rice Straw Broth (1942)

Culture Medium Classification	pH of Culture Medium*		Spore Formation	Bacterial Dry Weight from 50cc of Solution	
	Initial	Final		Weight	Percentage
Tochinali Solution.....	6.5	6.0	-	7	1.0
Rice Straw Broth.....	5.2	7.7	+	52	7.4
Mixture of an Equivalent Amount of Tochinali Solution and Rice Straw Broth.	5.8	8.3	+	325	46.5

*Determination by Kolthoff Color Comparison Method

2. Heat Resistance of the Growth Promoting Substance for P. oryzae

In order to determine the heat resistance of the growth promoting substance for P. oryzae found in rice straw broth, the broth was intermittently heat sterilized (one hour on 100° C steam, three sterilizations at intervals of 24 hours) as well as autoclaved (16 lbs, 20 min.). Following the aforementioned procedures, the growth results obtained are presented in Table 2. The difference in growth and spore formation between either treatment cannot be distinguished, and proved that the

growth promoting substance found in rice straw broth for this fungus was heat resistant.

Table 2. Heat Resistance of Substance for Growth Promotion of P. oryzae (1942)

Culture Medium Classification	Sterilization Method	pH of Culture Medium*		Spore Formation	Bacterial Weight**
		Initial	Final		
Rice Straw Broth.....	Intermittent Sterilization	5.2	7.5	+	89
Mixture of Equivalent Amount of Tochinai Solution with Above..	ibid	5.8	7.7	+	175
Rice Straw Broth.....	High Pressure Sterilization	5.2	7.5	+	87
Mixture of Equivalent Amount of Tochinai Solution with Above..	ibid	5.8	7.6	+	163

*Determination by Kolthoff Color Comparison Method

**Bacterial Dry Weight (mg) from 35 cc of Culture Medium

3. Growth Substance for P. oryzae from the Broths of Rice Straw, Rice Chaff, Rice Bran, Polished Rice, and Broths from Their Ashes

Besides rice straw, 50 gm each of air dried chaff, bran, and polished rice were added to 1 liter of water and each extracted for 1 hour in a steam sterilizer. The broths were then filtered, 30 gm of cane sugar added, then diluted to 1 liter. A part of each was ashed and the ash (from each 50 gm of air dried material, the ash obtained was: chaff 8.577 gm, bran 4.826 gm, polished rice 2.462 gm, straw 5.161 gm) was made into a broth, and as in the case of the straw broth, cane sugar was added and the entirety made up to 1 liter. The growth of fungus in these broths alone and in broths mixed with an equal amount of Tochinai solution were compared. The results in the various broths as shown in Table 3 indicated that for growth of this fungus, bran was the best, followed by polished rice and rice straw; however, the fungus weight was less than one third that obtained from the rice bran. The chaff broth was even less effective. Comparing the growths in the cases where the Tochinai solution was mixed in, it was seen that with the rice bran, the result was entirely the same; however, there were improvements with the rice straw, chaff, and polished rice broths. Spore formation also was seen. Moreover spore formation was greatest in the case of the bran, followed by rice straw, chaff, and was much smaller with polished rice.

Next the growth of the fungus in the broth from the ash of the foregoing materials was extremely small (even with the addition of 3% cane sugar), and even on mixing with Tochinai Solution, the effect was small. Spore formation could not be detected.

Table 3. Effect on Growth of *P. oryzae* by Broths of Rice Straw, Rice Chaff, Rice Bran, Polished Rice, and Their Ashes

Culture Medium Classification	pH of Culture Medium		Spore Formation	Bacterial Dry Weight*
	Initial	Final		
Rice Straw Broth.....	5.2	7.6	++	81
Tochinai Solution Mixed with Above..	5.8	8.2	++	222
Rice Straw Ash Broth.....	5.9	6.3	—	2
Tochinai Solution Mixed with Above..	6.4	5.9	—	13
Rice Chaff Broth.....	5.4	6.9	++	33
Tochinai Solution Mixed with Above..	6.2	8.0	++	221
Rice Chaff Ash Broth.....	6.6	6.7	—	1
Tochinai Solution Mixed with Above..	6.6	5.8	—	7
Rice Bran Broth.....	5.4	6.7	#	303
Tochinai Solution Mixed with Above..	6.3	8.0	#	300
Rice Bran Ash Broth.....	6.2	6.2	—	1
Tochinai Solution Mixed with Above..	6.2	6.1	—	14
Polished Rice Broth.....	6.4	6.0	+	95
Tochinai Solution Mixed with Above..	6.6	7.0	+	212
Polished Rice Ash Broth.....	4.8	5.4	—	2
Tochinai Solution Mixed with Above..	6.2	6.4	—	7

*Bacterial Weight (mg) from 35cc of culture medium

From the foregoing experimental results it was clearly indicated that not only rice straw but also, bran, chaff and polished rice contained the growth accelerating substance for *P. oryzae*, and since acceleration was not seen in the ash broths, it was clear that the substance was an organic compound.

4. Relationship Between *P. oryzae* Growth and the Addition of Rice Straw Broth to the Synthetic Culture Medium

Since it was found that the growth accelerating substance for *P. oryzae* was present in rice straw, the relationship between the amount of broth added and the growth of the fungus was investigated. The results presented in Table 4 clearly indicated that on addition of 0.05% of the rice straw broth (a 5% straw broth solution added to make up 1% of the Tochinai solution), the fungus growth was extremely vigorous, at 0.1 - 0.2% addition there were further accelerations. Up to 3.5% there

was a gradual increase but the differences were not as striking. Furthermore, at 0.1% addition spore formation could not be seen; at 0.75 - 1.0% there were adequate formations, and on additions above this, an increase could not be seen. From the above, it was clear that an addition of 1% straw broth to the Tochinai solution contained sufficient growth accelerating substance necessary for P. oryzae growth and spore formation.

Table 4. Growth of P. oryzae on Addition of Straw Broth to Synthetic Culture Medium

Per Cent Rice Straw Broth Added to Tochinai Solution*	pH of Culture Solution		Spore Formation	Bacterial Weight**
	Initial	Final		
0.00	6.3	5.0	—	6
0.05	6.3	5.2	—	171
0.10	6.3	5.4	—	259
0.20	6.3	6.4	++	312
0.30	6.2	6.5	++	321
0.40	6.2	7.2	+	334
0.50	6.2	8.2	#	334
0.75	6.1	8.2	#	350
1.50	6.0	8.2	#	359
2.50	5.9	8.4	#	365
3.50	5.7	8.6	#	396
4.92	...	8.7	#	310
Original Rice Straw Broth Solution	5.2	8.7	#	21

*Original Broth of 50 gm straw per liter Made Up to Required Concentration

**Bacterial Dry Weight from 50 cc of Culture Solution

5. Summary

The growth of fungus filaments when P. oryzae was cultured in the synthetic medium of the Tochinai solution was extremely small, and spore formation was not observed. However, on addition of rice straw broth, growth was remarkable and spore formation was excellent. It was concluded that the growth accelerating substance for P. oryzae was present in the rice straw broth. This substance was heat stable and not deactivated by intermittent sterilization (100° C for one hour, repeated three times) nor by high pressure sterilization (16 lbs, 20 minutes). Beside rice straw, it is present in rice bran, chaff, and polished rice and since the growth accelerating effect disappears on ashing the aforementioned materials, it became clear that the accelerating substance was an organic compound. Furthermore, it was found that with rice straw the

addition of 1% of the broth to a synthetic culture medium contained the necessary and sufficient amount of accelerating factor to cause growth and spore formation.

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