Studies on the Smut of Johnson Grass. I. Preliminary Studies on Sporidial Cultures.

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For some time the senior author has been interested in variation in fungi, and in the detection of variants. Although other smuts have been rather thoroughly investigated for variation, we are not aware of such studies on the smut of Johnson grass. The local abundance of material of both the parasite and its host, and the relative ease of culture of the host and the sporidial stage of the parasite made this fungus an ideal subject for investigation. This paper is a report of preliminary investigations on growth in tube culture of the sporidia of the smut of Johnson grass.

Six collections of smutted infloresences of Johnson grass were made during October from the same general locality, the north edge of the Durant High School football practice field bordering Willow Street, just west of 7th Street. The collections and the sporidial cultures derived from them were designated by numbers, prefexed by the initial "J". Chamydospore characteristics of all six accessions were quite similar. The average diameter was about eight microns, the color, an olive brown. The collection J-4 differed from the others in that the stem bearing the smutted infloresence

was about as tall as adjacent healthy stems. All other collections were from stems much shorter than the healthy stems. As yet it has not been possible to complete the identification of the smut beyond the genus, *Sphacelotheca*.

Chlamydospores were plated out on Difco potato dextrose agar by spreading dilute suspensions of the spores over the agar. Sporidia were transferred from the resulting growth to Difco nutrient agar slants for stock cultures. Inoculations were usually made by washing sporidia from the slants and transfering small droplets of the resulting suspension. Liquid cultures were always in five milliliter amounts in tubes averaging 13 millimeters internal diameter.

Sporidia of J-1 and J-2 were first cultured in nitrate broth, glucose-peptone broth, and tryptophane broth made according to the formulas in the bacteriology laboratory manual of Peltier, Georgi, and Lindgren (1). The tests for acetyl-methyl-carbinol, indole, and nitrate reduction were made according to the tests outlined in that manual. J-2, 3, and 4, were then inoculated into Koser's citrate broth (1) and Koser's broth modified by the substitution of an equal weight of sodium acetate for the sodium citrate, for one series, and of sodium succinate for the citrate for another series. Inoculations of J-2 and J-3 were also made in urea solution, casein solution, and on Difco nutrient gelatin. The formulas for the urea and casein solutions are in Allen's manual of soil bacteriology (2). Casein was substituted for sodium caseinate called for in the manual.

After a week of incubation each tube was examined for growth by the microscopic examination of the sediment. The results were of sufficient interest to set up an expanded series of cultures, all based on Koser's citrate broth, but with various carbon sources substituted for the citrate. The carbon sources were added in amounts to give weights of carbon equal to that present in the original citrate broth. Two sub-series were set up, one with the sodium ammonium phosphate as in the basic Koser's solution, one with potassium nitrate as the nitrogen source and in the amount to give a weight of nitrogen equal to that in the basic solution. Eleven different carbon sources, plus controls with no carbon, were used for both nitrogen sources. These were: sodium citrate, sodium acetate, sodium succinate, glucose, fructose, lactose, maltose, sucrose, mannitol, glycerol, and pyruvic acid. In addition, nitrate, tryptophane, and glucose-peptone broths were prepared, and also another modification of Koser's citrate broth with potassium nitrite and glucose substituted for the original nitrogen and carbon sources. All six accessions of the smut were used as inocula for these media. After five days of incubation all cultures were examined macroscopically for growth, and appropriate chemical tests were made.

In liquid culture all growth was in the bottom of the tube, with the possible exception of J-5. This was in contrast to the habit of two accessions of Bermuda grass smut which were grown on a few media, and which grew on the surface.

The results of the earlier tests may be summarized as follows: J-2, 3, and 4, grew in glucose-peptone, tryptophane, nitrate, and casein broths and on nutrient gelatin, but not in urea solution. The tests for acetyl-methyl-carbinol and indole were all negative. The gelatin was liquified in all cases. The tests for nitrate reduction were all positive, but these results are of doubtful validity. In later tests, only J-5, and not J-1, 2, 3, 4, and 6, showed nitrate reduction. Negative tests for acetyl-methyl-carbinal and indole were again secured, this time for all six accessions.

The growth in tubes of J-5 was not as in other tubes. All cultures of J-5 in which growth occurred were cloudy. Microscopic examination of stained smears from cultures of J-5 failed to show contaminating organisms; the results of streaks on nutrient agar are not yet available.

In the expanded series of growth tests, based on Koser's citrate broth, the apparent utilization of the several carbon sources in most cases did not appear to be critically dependent on the nitrogen source. Cultures J-2, 3, 4, and 6, grew in all carbon sources in the sodium ammonium phosphate subseries, with the exception of J-2 in mannitol, in which it did not grow, J-2, 3, 4, and 6 grew in all carbon sources in the potassium nitrate sub-series, but not as well as in the other sub-series. J-2, 3, 4, and 6 grew in the potassium nitrite-glucose medium. J-1 did not grow in the nitrite- glucose medium, nor in the pyruvic acid, mannitol, and acetate media of the sodium ammonium phosphate sub-series, and did not grow in any of the potassium nitrate sub-series, of modified Koser's broth.

The term "apparent utilization" of the carbon source is used because J-2, 3, 4, and 6 showed some growth in the controls with no carbon in both the sodium ammonium phosphate and potassium nitrate sub-series. The source of error, if any, which could explain this is not apparent at present.

Results of the growth of J-5 are not reported because of the possibility of the presence of contaminating organisms.

On the basis of the evidence secured so far, it would seem that J-1 is considerably different from J-2, 3, 4, and 6. The latter four are very similar if not identical. Thus it has been shown that there is variation in the smut of Johnson grass and this variation can be detected by cultural studies of the sporidia. Other questions of great interest have been raised and will require further study.

REFERENCES

1. PELTIER, GEORGE L., CARL E. GEORGI, AND LAWRENCE F. LINDGREN. 1946. Laboratory manual for general bacteriology. New York: John Wiley and Sons.

2. ALLEN, O. N. 1949. Experiments in soil bacteriology. Minneapolis: Burgess Publ. Co. p. 47.