

Selective Isolation of *Spirillum* Species¹

R. M. CODY

Department of Microbiology, Auburn University, Auburn, Alabama 36830

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The most widely used procedure for isolation of spirilla from natural sources is the enrichment-dilution technique described by Williams and Rittenberg (4). This technique has the disadvantage of frequently requiring several serial transfers before pure culture status is achieved. Canale-Parola et al. (1) have developed a procedure for the isolation of *Spirillum gracile* sp. n. from natural water sources. Isolation by this method is based on the selective passage of the particular species of *Spirillum* through a mem-

contaminated urine cultures. The successful recovery of leptospirae with this antimetabolite suggested that the same agent could be used for the selective isolation of spirilla. The experiments reported herein were designed to show that 5-FU has little or no inhibitory action on growth response of *S. serpens* (obtained from J. C. Ensign, University of Wisconsin, Madison), and that pure culture isolates of spirilla can be obtained from natural sources on media containing this chemical substance.

TABLE 1. Microscopic observations and nephelos values from cultures of *S. serpens* and spirilla in pond water grown in presence of different concentrations of 5-fluorouracil (5-FU)

5-FU (µg/ml)	<i>S. serpens</i>						Pond-water spirilla					
	Microscopic ^a			Nephelos			Microscopic ^a			Nephelos		
	24 hr	36 hr	72 hr	24 hr	36 hr	72 hr	24 hr	36 hr	72 hr	24 hr	36 hr	72 hr
0	2	3	3	360	820	809	1	3	3	480	483	749
25	3	3	3	358	805	807	1	3	3	518	485	713
50	3	3	3	554	812	810	1	2	2	440	703	718
75	3	3	3	361	815	808	1	3	3	382	496	>1,000
150	3	3	3	360	815	802	1	3	3	367	688	736
300	3	3	3	357	818	818	1	3	3	150	481	727

¹ Number of spirilla per microscopic field (1 = <10; 2 = >100; 3 = >1,000).

brane filter of pore size similar to the size of the organism. The utility of the method is limited to the isolation of identical or closely related species of spirilla.

Pure culture isolates of many bacterial species are often obtained by the incorporation of bacteriostatic substances in culture media for the purpose of inhibiting growth of unwanted organisms. Johnson and Rogers (13) used the pyrimidine analogue 5-fluorouracil (5-FU; supplied by Hoffman-La Roche, Inc., Nutley, N.J.) as a selective agent for securing isolates of *Leptospira* from

Stuart's liquid medium (Difco) with 10% rabbit serum (Pel-Freeze, Rogers, Ark.) and a chemically defined medium GGS described by Clark-Walker, Rittenberg, and Lascelles (2) were treated with 5-FU to give concentrations of 0, 25, 50, 75, 150, and 300 µg per ml of medium. These media were inoculated with 0.1 ml of a 24-hr GGS culture of *S. serpens* with a nephelos value of 240 (equivalent to approximately 2×10^7 cells per ml). In a similar manner, 1-ml quantities of pond water known to contain spirilla were inoculated on media containing the various concentrations of 5-FU. All cultures were incubated at 30 C and examined microscopically with a Petroff-Hausser counting chamber under dark-field illumination

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after 24, 36, and 72 hr of incubation. Microscopic examination of cultures inoculated with pond water was extended to 6 days of incubation. Growth responses were measured by use of a Coleman no. 9 Nephocolorimeter as a nephelometer immediately following microscopic examination. Nephelos readings were made only on GGS cultures. Growth on Stuart's medium was estimated from turbidity on visual inspection of cultures.

The results of microscopic observations and growth responses of spirilla in the presence of 5-FU are summarized in Table 1. In addition to spirilla, several different types of bacteria were observed microscopically in cultures made from pond water containing less than 150 μg of 5-FU per ml. These bacteria account for the high nephelos values observed with the pond water cultures. Organisms other than spirilla were not detected in any of the cultures containing 300 μg of 5-FU per ml. The nephelos readings obtained with these cultures were directly related to the growth of spirilla. The pure culture status was substantiated by microscopic examination and development of a single-colony type on peptone-

yeast extract agar. The change in nephelos readings of cultures extended to 6 days of incubation was not appreciably different from that of the 72-hr reading. Microscopic observations of cultures on Stuart's medium revealed growth characteristics of spirilla similar to those with GGS medium.

The results of this investigation show that 5-FU in a concentration of 300 μg per ml did not exhibit any inhibitory action on *S. serpens* and offer a selective technique for isolating spirilla from natural sources.

LITERATURE CITED

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