COMMUNICATION TO THE EDITOR

Differential Media for the Isolation of Bacteria and Fungi from Plated Barley Kernels

DEAR SIR:

The agar plating method is frequently used to isolate and identify seed-borne microorganisms. While a standard method for the determination of fungi in barley kernels is proposed by the American Society of Brewing Chemists, no accepted method has been adopted by the Society for the detection of bacteria in barley. The Society states that "the satisfactory determination of the bacterial count of barley and malt is complicated by the many types of bacteria present as well as the interference of certain very common molds, for example *Alternaria* . . . An accurate determination would require the development of specific nutritional conditions which would inhibit the development of molds and permit the growth of bacteria . . ." (2). In lieu of an accepted method, the Society refers the reader to the method for the total bacterial count in flour used in the milling industry (1). The latter method, however, is more cumbersome and cannot be related to data derived from whole-kernel platings.

The advent of selective antibiotics has made it possible to effectively suppress the growth of one class of organisms to the advantage of another. We have developed a series of differential culture media that utilize this principle of selective inhibition. While the method was originally devised to assess the relative bacterial, yeast, and fungal populations of plated barley kernels, it has also been successfully used to evaluate a number of other crop seeds.

The series consists of a basal medium such as potato-dextrose agar, malt agar, or other common culture media; the same medium plus

50 p.p.m. Chloromycetin (chloramphenicol) to suppress bacterial growth; and the basal medium plus 100 p.p.m. Acti-dione (cycloheximide) which selectively suppresses the growth of yeasts and filamentous fungi[1]. We routinely use potato-dextrose agar as the basal medium, although most common culture media work equally well. The sample to be plated normally consists of 100 randomly selected seeds which are surface-disinfected to remove foreign, superficial contamination. We use ethyl alcohol and liquid commercial bleach (1:2 by volume) as the surface disinfectant. The seeds are placed in the disinfectant for 1 minute, transferred aseptically to Petri plates containing the nutrient medium, and incubated for 3 to 5 days at room temperature (about 25°C.). After incubation the number of seeds supporting microflora are recorded. By plating a sample on these three media, an estimate of the number of bacteria, yeasts, and fungi can be obtained; at the same time the problems of microfloral antagonism and the masking of small or slow-growing colonies by the more aggressive species are reduced.

Several samples of barley and durum wheat from diverse locations were plated on this series of media. The samples were chosen on the basis of varying microfloral content. The results are shown in Table I. While a limited amount of fungal growth on the Acti-dione

TABLE I

PERCENTAGE OF SURFACE-DISINFECTED KERNELS YIELDING MICROFLORA FROM SAMPLES PLATED ON DIFFERENTIAL MEDIA[a]

SAMPLE AND ORIGIN	BASAL MEDIUM		ACTI-DIONE MEDIUM		CHLOROMYCETIN MEDIUM	
	Fungi/Bact.		Fungi/Bact.		Fungi/Bact.	
Barley						
Liberty (North Dakota, 1961)	16	90	2	98	18	0
Hannchen (California, 1961)	16	96	0	90	16	2
Chevalier (Australia, 1961)	20	28	2	56	52	0
Trophy (North Dakota, 1962)	98	44	20	74	98	2
Durum						
Wells (South Dakota, 1962)	94	38	36	68	98	2

[a] One hundred kernels per sample plated on each type of medium after surface disinfection. Plates incubated 4 days at 25°C.

medium and bacterial growth on the Chloromycetin medium occurs, this growth is always sparse (Fig. 1). The reaction of the basal medium is not altered by the two additives; e.g., in the series shown in Table I, the pH of the three media was 5.4. We have found that the common bacterial invaders of barley kernels grow well at this pH level, but are suppressed on acidified agar.

[1] Acti-dione furnished by the Upjohn Co., Kalamazoo, Michigan. Chloromycetin furnished by Parke, Davis & Co., Detroit, Michigan.

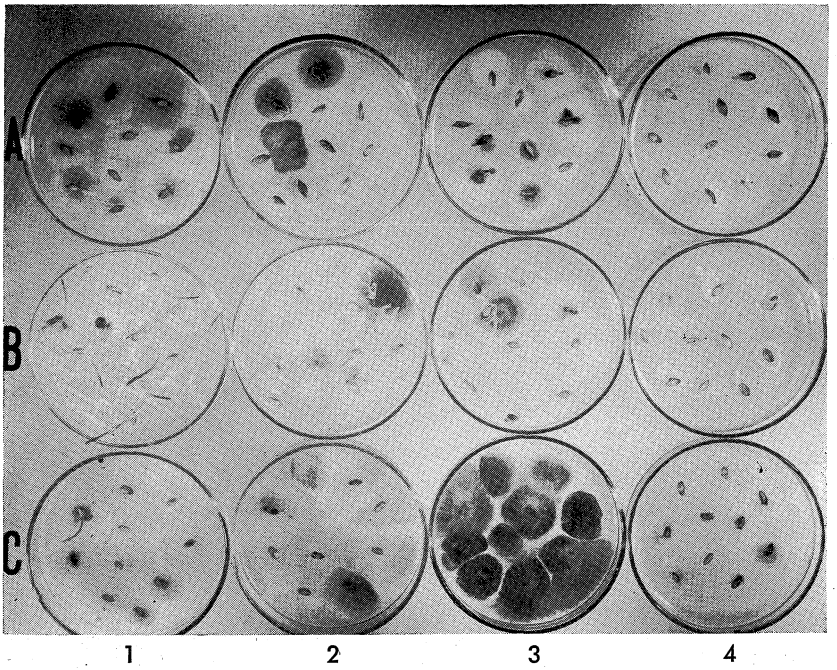

Fig. 1. Barley and durum kernels plated on differential media after surface disinfection. A, Trophy barley; B, Chevalier barley; C, Wells durum. 1, nutrient agar; 2, potato-dextrose agar; 3, potato-dextrose agar + 50 p.p.m. Chloromycetin; 4, potato-dextrose agar + 100 p.p.m. Acti-dione.

Both of the additives described in this communication are available in powdered form, are easily added to culture media, and are not noticeably affected by heat-sterilization (3). Our studies on seed microflora are still in progress and a subsequent, more detailed account will be submitted for publication at a later date.

EVAN H. PEPPER
RICHARD L. KIESLING
Department of Plant Pathology
North Dakota State University
Fargo, North Dakota

April 18, 1962

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. AMERICAN SOCIETY OF BREWING CHEMISTS. Methods of analysis (6th ed.). The Society; Madison, Wisconsin (1958).
3. STECHER, P. G. (ed.), *et al.* The Merck index of chemicals and drugs. (7th ed.). Merck & Co., Inc.: Rahway, N.J. (1960).