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Comparison of Rose-Bengal Chloramphenicol Agar and Dichloran Glycerol Agar (DG18) for Enumeration and Isolation of Moulds from Raisins

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Abstract

Two different selective media as Rose-Bengal Chloramphenicol (RBC) Agar and Dichloran Glycerol (DG18) Agar were used for isolation and enumeration of molds from raisin, which is the main economic crop in Turkey and somewhere else. A total of 129 raisin samples were collected randomly during 1998-2000, of 94 were taken from several field vineyards and 35 from two different raisin packaging houses. Although after microbiological examinations no significant differences were found in relation with their fungal count ($p>0.05$), there was a marked variation ($p<0.05$) in terms of fungal diversity between the two media. Thus 53 species belonging six genera were obtained with DG 18 agar, whereas 74 species from 12 genera with RBC agar. There were 39 species common in the two media. The results warrant the need to use two selective media with different moisture or water activity in order to isolate and/or enumerate a more representative mycoflora including toxigenic and/or pathogenic from raisins.

Key words: Selective media, dried fruit, xerotolerant fungi.

INTRODUCTION

Fungi and their concomitant mycotoxin in food are mainly developed according with the moisture contents. Thus moisture levels can induce a microbial ecological succesion in food [3]. Toxin production capability by fungi can be limited or impeded by low water activity (a_w). Pardo et al. showed that ochratoxigenic isolates are adapted to low water activity conditions and may easily colonize stored grain [10]. Fungi can grow at relatively lower moisture conditions than bacteria. Although their growth range is between 0.97 and 0.99 a_w, some of them can grow easily within 0.80 to 0.85 range of a_w (*1*) however, a_w for fungal growth can be different from those required for production of mycotoxins [3,5,8]. Thus, in the enumeration of fungi in foods the substrates used traditionally in media have high water activity, ranging between 0.997 and 0.999 [6]. Although such media are satisfactory for enumerating and isolating moulds from fresh foods such as fruit, vegetables, dairy products, meat etc., they are inadequate for sampling the fungal flora of dried or semidried foods such as dried fruits e.g. raisin, spices, condiments, confectionary, stored cereals, cereal products and nuts [6].

Media of reduced a_w have been used for many years for the isolation and enumeration of moulds. These media have traditionally been based on NaCl although some workers have preferred to use sucrose based media [6].

Pitt and Hocking [15] found that a common isolation medium for xerophilic fungi could be based on glycerol but not on NaCl as a_w–limiting solute. Glycerol, an ideal solute in terms of utilization and low cost, can be a possible alternative. Then, Hocking and Pitt [6] developed a low water activity medium (0.95 a_w) containing 18% (w/w) glycerol and 2 μg of dichloran per ml for enumerating the fungal flora of dried foodstuffs. This medium is called DG18 agar and is recommended for the enumeration and isolation of xerophilic moulds from dried and semidried foods, whereas, RBC agar is a selective medium for the enumeration of yeast and moulds from a wide variety of foodstuffs [2]. Both media are widely used in mycological research.

This study was performed to compare fungal counts and species diversity based on enumeration and isolation from two different culture media, “Rose-Bengal Chloramphenicol Agar” (Oxoid, CM 549) and “Dichloran-Glycerol (DG18) Agar Base” (Oxoid, CM 729) in raisins.

MATERIALS and METHODS

Raisin samples

Totally 129 raisin samples were collected randomly during 1998-2000. Ninety four samples were taken from different fields vineyards and 35 samples from two different raisin packing houses in Turkey.

Preparation of the raisin samples for enumeration and isolation

Raisin samples, collected from different parts of the heap, were partitioned into subsamples and placed into sterile polyethylene bags to carry them to the laboratory. They were kept in a deep freeze at –20° C until preparation. Then one kg of raisin was diluted at 1:3 sample:water, w/v) ratio and homogenized in a blender at high speed. Fifty g was taken from the homogenate and 450-ml-distilled water was added and

homogenized in shaker at 20 °C for 30 minutes. Therefore, the homogenate was 10^{-1}, from which a series of dilutions at 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared.

The media used for isolation and identification of moulds

Every dilution was plated into each media, RBC agar and DG18 agar as 2 replicates according to the "Pour Plate Method" [9]. After incubation at 25 °C for 5-7 days, colonies in each plate were counted with the naked eye and multiplied by the dilution factor and 3 to determine cfu g^{-1}.

For maintaining isolates, the colonies were cultivated on Malt Extract Agar (MEA, Oxoid CM 59) slants and kept at + 4 °C.

Identification of the isolates

For the identification of the cultures, each isolate was inoculated on both Czapek-Dox Agar (CZ, Oxoid CM97) and MEA and also the characteristics of penicilli type determined on CYA [11,14]. Fungal colonies were identified according to standard mycological procedures [4,12,16-19].

Statistical analysis

Each time, the experiments were done using 3 replicates. The mean was calculated, and a standard deviation and T-test were performed as a statistical analysis.

RESULTS

Comparison of mould counts from both DG18 and RBC agar cultures

To compare the relative performance of DG18 agar and RBC agar as fungal count from media on a total of 129 raisin samples were evaluated. The results from the statistical analysis, showed no differences between the two media ($p>0.05$) with respect to their fungal counts. The mean numbers of fungi isolated from DG18 agar was 2.8×10^5 cfu g^{-1} (range from 8.5×10^2 to 9.9×10^6 cfu g^{-1}) from RBC was 2.1×10^5 cfu g^{-1} (range from 1.0×10^3 to 8.3×10^6 cfu g^{-1}).

Comparison of mould diversity from both DG18 and RBC agar cultures

There were marked differences ($p<0.05$) from the point of view of fungal diversity between the two media. RBC showed the greater species diversity. The species isolated from RBC agar and DG18 agar are listed in Table 1. In the comparison of the two media for isolating moulds from raisins, 53 species belonging to six genera were isolated from DG18 agar, whereas 74 species belonging to 12 genera were isolated on RBC agar. Thirty-nine of the identified species were isolated on both media. Thirteen species were identified only on DG18 agar and thirty-five isolated only from RBC agar.

Table I. Mould species isolated from Rose Bengal Chlorampenicol (RBC) Agar and Dichloran Glycerol (DG18) Agar media.

Media / Mould species	RBC Agar	DG 18 Agar
Absidia ramosa (Lindt) Lendn.	+	–
Alternaria alternata (Fr) Keissler	+	+
A. pluriseptata (Karst. and Har.) Jorstad	+	–
Aspergillus aculeatus Iizuka	+	+
A. auricomus (Guéguen) Saito	+	–
A. awamori Nakaz.	+	+
A. carbonarius (Bainier) Thom	+	+
A. carneus (V. Tiegh.) Blochwitz	+	+
A. ficuum (Reich.) Hennings	+	+
A. flaschentraegeri Stolk	+	–
A. flavo-furcatis Batista and Maia	+	+
A. flavus Link ex Gray	+	+
A. foetidus (Naka.) Thom and Raper	+	+
A. foetidus var. *acidus* Naka., Simo and Wat.	–	+
A. foetidus var. *pallidus* Naka., Simo and Wat.	+	+
A. fumigatus Fresen.	–	+
A. heteromorphus Batista and Maia	+	+
A. japonicus Saito	+	+
A. niger van Tieghem	+	+
A. ochraceus Wilhelm	+	+
A. oryzae (Ahlburg) Cohn.	–	+
A. parasiticus Speare	+	+
A. petrakii Vörös	–	+
A. phoenicis Thom	+	+
A. pulverulentus (Mc Alpine) Thom	+	–
A. puniceus Kwon-Chung & Fennell	–	+
A. sclerotiorum Huber	+	–
A. spinulosus Warcup	+	+
A. sydowi (Bain. Sartry) Thom & Church	–	+
A. terreus Thom	+	–

A. terricola Marchal	–	+
A. tubingensis (Schöber) Mosseray	+	+
A. unguis (Weill & L. Gaudin) Thom & Raper	+	–
A. ustus (Bainier) Thom & Church	+	–
Cladosporium cladosporioides (Fres.) de Vries	+	+
C. herbarum (Pers.) Link ex S.F. Gray	+	+
C. oxysporum Berk. & M.A. Curtis	+	–
C. uredinicola Speg.	+	+
Fusarium oxysporum E.F. Sm. & Swingle	+	–
Glomerularia sp.	+	–
*Mucor circinelloides f. circinelloide*s van Tiegh.	+	–
Penicillium albicans Bainier	+	–
P. atramentosum Thom	–	+
P. brevicompactum Dierckx	+	+
P. canescens Sopp	+	–
P. carneo-lutescens Smith	+	–
P. caseicolum Staub.	+	+
P. chrysogenum Thom	+	+
P. citrinum Thom	–	+
P. corylophilum Dierckx	–	+
P. corymbiferum Westling	+	+
P. cyaneo-fulvum Biourge	+	+
P. cyclopium Westling	+	+
P. decumbens Thom	+	+
P. digitatum (Pers:Fr) Sacc.	–	+
P. duclauxii Delacroix	+	–
P. echinulatum Raper & Thom ex. Fassat.	+	–
P. expansum Link	+	–
P. funiculosum Thom	+	–
P. godlewskii W. Zalessky	–	+
P. granulatum Bain.	+	+
P. herquei Bain. Sartory	–	+
P. lanoso-viride Thom	+	–
P. lanosum Westling	+	+
P. martensii Biourge	+	–
P. meleagrinum Biourge	+	–
P. miczynskii W. Zalessky	+	+
P. nigricans Bain.	+	–
P. notatum Westling	+	–
P. olivino-viride Biourge	+	+
P. oxalicum Currie and Thom	+	+
P. puberulum Bainier	+	+
P. raistrickii G.Sm.	+	–
P. roseo-purpureum Dierckx	+	–
P. rubrum Stoll	+	+
P. simplicissimum (Qudemans) Thom	+	+
P. solitum Westling	+	–
P. steckii Zleski	+	+
P. stoloniferum Thom	+	+
P. tardum Thom	+	–
P. thomii Maire	–	+
P. urticae Bain.	+	–
P. variabile Sopp	+	–
P. viridicatum Thom	+	–
Rhizopus oryzae Went & Prinsen Geerlings	+	+
Spicaria sp.	+	–
Stachybotrys chartarum Corda	+	–
Trichoderma hamatum (Bonord.) Bainier	+	–
Species number	**74**	**53**

DISCUSSION

The higher diversity shown by RBC demonstrated the microbial ecological succession is induced by the selective media. The selective effect of the media on the fungal diversity is also shown that there was a 1.4 fold differences between the two media regarding the fungal diversity, although the difference in water activity was narrow between the two media (0.95-0.98). Cuero et al. also demonstrated a microbial ecological succession induced by different water activities in corn and rice [3].

Other authors have shown higher fungal counts on DG18 Agar than on modified RBC (Dichloran Rose Bengal Chloramphenicol, DRBC) however they didn't report any fungal diversity and/or microbiological ecological succession, as we demonstrated [7]. Also, our result didn't show any difference on the fungal total counts between the two selective media ($p>0,05$).

Wu et al. [20] compared the two sampling media, DG18 agar and MEA, for environmentally viable fungi collected in a hospital environment [20]. They found that the airborne fungal concentrations are higher from the DG18 agar plates than from MEA plates. In addition, the number of different genera present was greater on the DG18 agar plates than on the MEA plates. They reported that DG18 agar plates appear to be more effective in collecting more fungal colonies in terms of both quantity and the types of genera.

The initial low water activity (0.50-0.61) of the raisins seems to have also established an initial microbial ecological succession. Thus stimulating more spore formation than mycelia. Therefore the faster germination fungal spores such as *Aspergillus foetidus* var. *pallidus*, *A. aculeatus* were also the faster to be recovered on the RBC from raisin samples. Perhaps the difference between xerotolerant and xerophilic is based on narrow water activity, according the type of substrate. Petrovic et al. have reported that strains which can grow between a_w 0.80- 0.90 should be considered as xerotolerant and those that can grow under a_w 0.80 as xerophilic [11].

The herein results, suggest that in raisin both xerophilic and xerotolerant fungi are found as a result of a microbial ecological succession induced by both raisin and media water activity.

The results also warrant use at least two different selective media with different water activity for more realistic results for sampling dried fruits such as raisins. It was reported that no single medium is satisfactory for the detection, isolation and enumeration of all the fungi in foods although Dichloran Glycerol 18 % agar performed well enumeration of fungi [1].

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