A METHOD FOR THE PREFERENTIAL ISOLATION OF YEASTS

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Received on September 29, 1965

Abtsract

A plate method for the preferential isolation of yeasts from various ecosystems is described. The method is based on the use of tellurite at a concentration of 15 mg/L and streptomycin at 100 μg in proteose peptone yeast extract dextrose agar.

Introduction

While investigating the microbial ecology of sewage and sludges (Dias & Bhat, 1964) considerable difficulty was experienced in the isolation of yeasts though these organisms were found to be present in appreciable numbers in the ecosystems. The usual media recommended for their isolation were generally ineffective in the sense that a large number of bacteria developed on the plates rendering the isolation and the enumeration of yeasts difficult if not impossible. A search was therefore made for a suitable inhibitor(s) which could selectively suppress the bacteria from growing. Potassium tellurite, together with certain antibiotics which by themselves do not give satisfactory results, was found to be effective for the purpose. Utility of tellurite as a bacteriostatic agent in the isolation of *Corynebacterium diphtheriae* has been recognised for several years (Anderson *et al.*, 1931).

Materials and Methods

Several media, listed below, into which tellurite was incorporated, were tested for their suitability for the preferential isolation of yeasts: Proteose peptone yeast extract dextrose agar (PPYED) made with proteose peptone (Difco) 0.5 *g*; yeast extract (Difco) 0.1 *g*; dextrose 2.0*g*; agar 2.5*g*; and streptomycin 100 μg: Sabouraud agar (SA) containing rose Bengal 0.033 *g* and aureomycin 35 μg: Potato dextrose agar (PDA) with streptomycin 100 μg: Hertz and Levine agar (1942): Littman's oxgall medium (1947) and Di Menna's medium (1960). The *pH* of all media, after autoclaving, was adjusted to 5. Glucose was autoclaved separately as a 20 per cent solution for incorporation into the basal media.

The usefulness or otherwise of certain antibiotics (Cooke *et al.*, 1954) was also ascertained in the course of investigation. The stock solutions were prepared by dissolving antiobiotics in sterile distilled water. The antibiotics and potassium tellurite were incorporated into the sterile media cooled at 50°C and immediately poured into petri dishes.

The plates were dried at 37°C for 24 hours prior to inoculation. A variety of inocula such as soil, fæces, cowdung, sewage, activated sludges, tomato, and grapes (0.1 ml.), suitably diluted where necessary in sterile distilled water, were used. The plates were incubated at room temperature (15 to 30° C) for 5 days. Stained smears of all colonies that developed in a representative sector of a plate were examined.

Results and Discussion

The effect of potassium tellurite on the isolation of yeasts is shown in table I It is clear therefrom that most of the bacteria got inhibited and that the plates contained almost exclusively yeast colonies. Though the techniques allowed only rough estimates, it was abandantly clear from the results that

TABLE I

Effect of tellurite in media on the selectivity of yeasts from activated sludge and sewage

Media	Dilution used	Activated Sludge		Sewage	
		No. of colonies/plate	Yeasts per cent	No. of colonies/plate	Yeasts percent
PDA	10	Innumerable	.....*	Innumerable	.....
PDA + tellurite	10	101	63	18	65
PPYED	10	Innumerable	.....	Innumerable	.....
PPYED + tellurite	10	84	100	43	100
Hertz & Levine	10	Innumerable	.....	Innumerable	.....
Hertz & Levine + tellurite.	10	96	65	17	67
SA	10	Innumerable	.....	Innumerable	.....
SA + tellurite	10	65	100	46	100
Littman Oxgall	10	122	38	Innumerable	.....
Littman Oxgall + tellurite.	10	46	100	18	100
Di Menna	10	212	21	Innumerable	.....
Di Menna + tellurite	10	44	100	18	100

* Due to overcrowding growth of bacteria per cent could not be calculated.

it is an useful tool for the isolation and enumeration of yeasts. Sabouraud's agar no doubt yielded maximum number of yeasts with raw sewage as inoculum but it proved to be less suitable with activated sludge as inoculum. Proteose peptone yeast extract dextrose agar, on the contrary, yielded maximum recoveries of yeasts from both the sewage and activated sludge. This finding, together with the fact that this medium can be prepared with ease, led to its selection as the medium of choice for subsequent studies.

The effect of varying concentrations of tellurite on the growth of bacteria and its relative influence on the yeast populations is shown in table II. The extent to which streptomycin exerts its beneficial effect in limiting the bacterial growth is presented in table III. The antibiotic, it would seem, restricted in particular, the growth of Gram negative bacteria developing on the plates but have little effect on the Gram positive types.

TABLE II

Effect of various concentrations of tellurite on yeast counts
(Inoculum : Sewage)

PPYED medium	Yeast colonies per cent
Without tellurite	..... *
With tellurite 5 mg/L	55
„ 7 mg/L	63
„ 10 mg/L	73
„ 12.5 mg/L	90
„ 15 mg/L	192
„ 17.5 mg/L	100

* Due to overcrowding growth of bacteria per cent could not be calculated.

TABLE III

Effect of streptomycin on the selectivity of tellurite media

PPYED Medium	Yeast colonies per cent
Without streptomycin and tellurite	..... *
With tellurite : without streptomycin	75
With tellurite and streptomycin	100

* Due to overcrowding growth of bacteria per cent could not be calculated.

The applicability of the method was then ascertained by employing other ecosystems as inocula (table IV). It was evidenced that the technique was successful irrespective of the nature or origin of the inocula Of particular interest was the success achieved with the faecal samples tested. Lobo (1950) had shown the unsuitability of the usual media for isolating yeasts from fæces and this led him and his associates (1953) to devise an enrichment method. The utility of tellurite media for the direct isolation of yeasts is worthy of note in this context.

TABLE IV

Suitability of tellurite medium for isolation of yeasts from various ecosystems

Ecosystem	Dilution used	PPYED medium	No. of colonies/plate	yeasts per cent
Soil	10^1	Without tellurite	Innumerable	— *
		With tellurate	9	100
Faeces	10^1	Without tellurite	Innumerable	—
		With tellurite	21	100
Cowdung	10^1	Without tellurite	Innumerable	—
		With tellurite	25	100
Toddy	10^4	Without tellurite	42	100
		With tellurite	42	100
Tomato juice	10^1	Without tellurite	35	100
		With tellurite	35	100
Grape juice	10^1	Without tellurite	56	100
		With tellurite	56	100

* Due to overcrowding growth of bacteria per cent could not be calculated.

Although it was clear from the results of plating grapes and tomatoes, that tellurite had no effect on yeasts as such, the possible adverse effect tellurite may have on laboratory yeasts cultures was ascertained by taking a mixture of 6 species of yeasts from stock cultures. It was observed (table V) that at 15 *mg*/L level (which is adequate for the inhibition of most bacteria) tellurite does not inhibit the growth of any species, though morphologically the colonies of yeasts appearing on tellurite media were of smaller size than those that developed on telluriteless media.

TABLE V

Effect of various concentrations of tellurite on a mixed population of yeasts

PPYED Medium	No. of colonies/plate
Without tellurite	270
With 7.5 mg/L tellurite	270
With 10 mg/L tellurite	270
With 15 mg/L tellurite	265
With 20 mg/L tellurite	136

* *Candida utilis* (3) NCYC 3010
Hansenula saturnus NCYC 22
Rhodotorula glutinis NCYC 59
Saccharomyces cerevtsiae var. ellipsoideus NCYC 94
Torulopsis pulcherrima NCYC 1664
Zygo-saccharomyces priorianus NCYC 176

It would seem from these results that tellurite can serve as an useful inhibitory agent for the suppression of bacteria from such systems wherefrom isolation or counting of yeasts proves to be a difficult proposition. It may be mentioned, at the same time, that the use of tellurite does not inhibit the growth of moulds and that for the isolation of yeasts from ecosystems wherein fungi dominate the use of antifungal agents may be imperative. Experience has shown however that in most instances tellurite media are successful for isolation as well as enumeration of yeasts as the moulds appear on the plates at a much later stage.

The yeasts cultures made from sewage, activated sludge and fæces by the exploitation of this medium are being currently studied in this Laboratory.

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