OSMOPHILOUS AGAR

A solid medium for the research of osmophilous yeasts in foods according to the CeNAN rules.

Code KM4145

Typical formula	**(g/l)**
Fructose	60.0
Yeast Extract	5.0
Agar	15.0

pH 7.0 ±0.2

Directions
Dissolve 80g of powder in 1 litre of distilled water, heating up slightly to boiling. Distribute into containers and sterilise in autoclave at 121°C for 15 minutes.

Description
This solid culture medium has been formulated according to the Food and Nutrition National Centre (CeNAN) suggested rules for the examination and total counting of osmophilous yeasts in honey, fruit juices and derivatives. According to this normative, osmophilous yeasts are those that grow well in media with high osmotic pressure. Yeasts that tolerate higher sugar concentrations have been associated to several species of the type *Zygosaccharomyces* and they are the agents that alter dry fruits, fruit concentrates and syrups. The normative specifies that the total count of osmophilous yeasts is the number of yeasts that grow from 1g of food on Osmophilous Agar.

Method
The recommended technique is the massive seeding of the sample dilutions. To do this, put aliquotes of 1mL in Petri plates and pour 15 ml of melted and cooled to 47°C medium over them, mixing homogeneously. Once solidified, incubate for a 3 days period at 20-22°C. Medium acts selectively due to the high sugar concentration. Moreover the species of *Zygosaccharomyces* use to appear also *Saccharomyces rouxii* and *S. mellis*, which may be easily detected by their fermentative characteristics:

	Glucose	Fructose	Manose	Mannitol
S.rouxii	+	+	+	±
S.mellis	+	+	+	-

References
Centro Nacional de Alimentacion y Nutrición (1976). Normas recomendadas para el Examen Microbiológico de Alimentos. Madrid. Suplementos 12 y 16.
CeNAN (1982). Técnicas para el Análisis Microbiológico de Alimentos y Bebidas. Madrid.

OGYE (Oxytetracycline, Glucose, Yeast Extract) AGAR BASE

A basal medium for the enumeration of yeasts and moulds

Code: KM1078

Typical formula	**(g/l)**
Yeast Extract	5.0
Glucose	20.0
Agar	12.0
Biotin	0.1 mg

pH 7.0 +/- 0.2

Directions
Suspend 37g of OGYE Agar Base in 1000ml of cold distilled water and heat to boiling. Autoclave at 115°C for 15 minutes and cool to approximately 50°C. Reconstitute under aseptic conditions two vials of Oxytetracycline Supplement with sterile distilled water, and add to the base medium.

Description
It has been demonstrated by Mossel et al. (1962) that acidic media are not completely suitable for counting yeasts and moulds in foods for two reasons:

1) Yeast cells stressed by heat, do not tolerate the acid conditions necessary to inhibit bacterial contaminants.
2) Yeast and mould growth is often limited by the presence of acid-tolerant bacterial flora.

The addition of oxytetracycline to a neutral pH medium has proved to be particularly suitable since it provides higher yeast and mould counts than previously used acid pH media. These observations were confirmed by Buttiaux and Catsaras, and by Sainclivier and Roblot, in a study carried out on 4000 clinical and food specimens. Mossel et al. (1970), estimated the accuracy of yeast and mould counts using OGYE Agar. Furthermore, they observed a complete inhibition of *Bacillaceae.* Under certain experimental conditions and when testing certain foods, the use of oxytetracycline alone was not sufficient to obtain reliable yeast and mould counts (Put, 1974). In particular, Mossel et al. (1979) observed that, with very proteinaceous foods heavily contaminated with Gram-negative rods, it is necessary to use both oxytetracycline and gentamicin in order to obtain complete inhibition of contaminants. Moreover, when a higher incubation temperature is required the use of oxytetracycline in proteinaceous food tests has been seen to be less suitable because it is 50% inactivated when incubated for 5 days at 37°C (Mossel, 1970; Put, 1974). To avoid this inconvenience and also when it is necessary to limit the overgrowth of moulds *(Neurospora* and *Rhizopus* spp.), and the production of aerial mycelia, the use of Rose Bengal Agar is advised. ISO 7954, for the enumeration of yeasts and moulds at 25°C recommends the use of Chloramphenicol Glucose Yeast Extract Agar or alternatively the use of OGYE Agar Base supplemented with 100mg/ltr of oxytetracycline HCl.

Method
Prepare a series of suitable dilutions of the sample. Transfer 1ml aliquots to an empty 9cm petri dish. Add approximately 5ml of medium prepared as described above. Mix gently turning the plates. Incubate for 5 days at 22 +/- 2°C checking for the formation of aerial mycelia after 2 days. Count the colonies in plates containing

50-10 colonies after 5 days or in any countable plates when aerial mycelia threaten to obscure further readings after 2 days.

User quality assurance (25°C - 3 days)

Productivity control
C.albicans ATCC 10231: good growth
A.niger ATCC 16404: good growth
P.cyclopium ATCC 16025: good growth
S.cerevisiae ATCC 9763: good growth

Selectivity control
E.coli ATCC 25922: inhibited
B,subtilis ATCC 6633: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared flasks (medium base): 3 months at 2-8°C
User prepared plates: 7 days at 2-8°C

References
Buttiaux, R. et Catsaras, M. (1965).. Annls, Inst. Pasteur, Lille, 16,167.
ICMSF (1978). Microrganisms in Foods, Their Significance and Methods of Enumeration. University of Toronto Press, 157-159.
Jarvis, B. (1973). J. App. Bact. 36, 723-727.
Mossel, D.A.A., Visser, M. and Mengerink, W.H.J. (1962) Lab. Pract. 11, 109.
Mossel, D.A.A., Kleynen-Semmeling A.M.C., Vincentie H.M. (1970). J. App. Bact. 33, 454
Mossel, D.A.A et al. (1979). J. App. Bact. 39, 15
Put H.M. (1974) Arch. Lebensmittel Hyg. 25, 73.
Sainclivier M. and Roblot A.M. (1966) Annls, Inst. Pasteur, Lille, 17, 181.

Orange Serum Agar

A medium designed to investigate organisms involved in the spoilage of citrus products including fruit juices etc.

Code: KM1077

Typical formula	**g/ltr**
Tryptone	10.0
Yeast Extract	3.0
Orange Extract	5.0
Glucose	4.0
Di-potassium phos.	3.0
Agar	7.0

pH: 5.5 ± 0.2

Description
A medium developed for the investigation of organisms involved in the spoilage of citrus products including fruit juices and citrus concentrates. The low pH of these products restricts the growth of organisms to those capable of tolerating an acid environment such as yeasts and moulds and bacteria belonging to the genera Bacillus, Lactobacillus, Leuconostoc, Streptococcus and Clostridium. By having a low pH and incorporating orange extract, Orange Serum Agar is the ideal isolation medium.

Technique in use
Weigh 42 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes. Bring to the boil swirling frequently, cool to 47ºC. and dispense into containers. Sterilise by autoclaving at 115ºC. for 15 minutes.

Appearance: Amber gel.

Q.C. organisms L. acidophilus
Pen. roquefortii

Prepared Medium: Store plates up to 7 days at 4ºC in the dark. Containers up to 3 months at 15-20ºC in the dark.

Inoculation: Pour plate technique.

Incubation: 3 days at 30ºC. for bacteria, 5 days at 30ºC for yeasts and moulds.

Interpretation: Count bacterial colonies and yeast/moulds separately. Calculate the colony forming units (C.F.U.'s) per ml of the sample allowing for dilution factors.

References
Hays G.L. and Reister D.W. (1952) The control of "off-odour" spoilage in frozen concentrated orange juice. Food Tech 6 p386. Murdock D.I.

Hays G.L. (1951) The isolation, cultivation and identification of organisms, which have caused spoilage in frozen concentrated orange juice. Proc. Florida State Hort. Soc.