Also known as
OSA

Specification
Solid medium for the culture of aciduric organisms especially those associated with the spoilage of citrus products and their derivatives.

Formula * in g/L
Casein peptone 10.00
Yeast extract 3.00
Orange serum 5.00
Dextrose 4.00
Dipotassium phosphate 3.00
Agar 17.00

Final pH 5.5 ±0.2 at 25 °C
* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 42 g of powder in 1 l of distilled water and boil to dissolve the agar. Distribute in suitable containers and autoclave for 15 minutes at 121ºC. Avoid unnecessary overheating so as to minimise the darkening (caramelisation) and loss of gelification of the medium.

Description
Orange Serum Agar was developed in the 1950's by Hays and coworkers for the detection, enumeration and isolation of spoilage microoganisms in fruit juices and products derived from citrus. Products with a low pH have microbial growth restricted to that of aciduric microorganisms. In a later study it was shown that Orange Serum Agar pH 5.4 was the most suitable medium for the isolation of lactic acid bacteria, especially (*Lactobacillus* and *Leuconostoc*) and yeasts that produce (buttermilk off-odour) in citrus fruits.
Orange Serum Agar is not a differential Agar but a culture medium in which the orange extract provides a favourable acidic environment in which aciduric microorganisms can be recovered including those damaged by food processing. Tryptone provides the main source of carbon and nitrogen, providing optimal growth conditions. Yeast Extract supplies Group B complex vitamins that stimulate growth and the phosphate provides an osmotic buffer for cell survival. Dextrose is a supplementary source of carbon and the agar is a solidifying agent.

Technique
The International Fruchtsaft-Union (IFU) recommends the use of Sérum d'orange Agar in several standardized methods, using the plate count method:
1. Prepare 10-fold serial dilutions of the sample using a suitable diluent such as buffered Peptone Water.
2. Distribute aliquots of 1 ml of the diluted sample in sterile Petri dishes.
3. Add 20 ml of molten sterile medium cooled to 45 °C, gently swirl the dish to mix the sample and medium properly.
4. Allow it to solidify and incubate à 30 ±1 °C for 48 hours before enumeration. If there is no growth extend the incubation to 5 days, reading daily before giving a negative result.
Generally the colonies of yeasts and moulds are distinguished by their morphology but those of aciduric bacteria need to be Gram stained and examined microscopically to be appropriately categorised.

Quality control
Incubation temperature: 30 ± 1 ºC MicroAE **Incubation time:** 48 h-5 days

Inoculum: Practical range 100 ±20 CFU. min. 50 CFU (productivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Lactobacillus fermentum ATCC® 9338	Productivity > 0.50	CO2
Saccharomyces cerevisiae ATCC® 9763	Productivity > 0.50	-
Aspergillus brasiliensis ATCC® 16404	Productivity > 0.50	-
Lactobacillus plantarum ATCC® 8014	Productivity > 0.50	-
Leuconostoc mesenteroides ATCC® 9135, WDCM 00108	Productivity > 0.50	-

References

· HAYS, G.L. (1951) The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Fla. State Hortic. Soc. 54:135-137.
· HAYS, G.L. & D.W. REISTER (1952) The control of 'off-odour' spoilage in frozen concentrate orange juice. Food Technol. 6:386-389.
· HATCHER, W.S., M.E. PARISH, J.L. WEIHE, D.F. SPLITTSTOESSER & B.B. WOODWARD (2001) Fruit Beverages, en Compendium of Methods for the Microbiological Examination of Foods. 4th ed., F.P. Downes & K. Ito, editors. APHA Inc., Washington D.C., USA.
· IFU Method No. 2 (1996) Total Count of Potential Spoilaging Microorganisms of Fruits and Related Products. International Federation of Fruit Juice Producers. Microbiological Methods (2004). Schweizerischer Obstverband. Postfach CH-6302 Zug.
· IFU Method No. 6 (1996) Mesophilic & Thermoduric-Thermophilic Bacteria: Spores Count. D-II Mesophilic Anaerobic Sporeforming Bacteria: Spores Count. International Federation of Fruit Juice Producers. Microbiological Methods (2004). Schweizerischer Obstverband. Postfach CH-6302 Zug.
· IFU Method No. 7 (1998) 'Sterility' Testing of 'Aseptic Filled Products', 'Commercial Sterile Products' and 'Preserved Products'. International Federation of Fruit Juice Producers. Microbiological Methods (2004). Schweizerischer Obstverband. Postfach CH-6302 Zug.
· IFU Method No. 10 (1998) Microbiological Examination of Potential Spoilaging Microorganisms of Low Acid and High pH Vegetable Products. International Federation of Fruit Juice Producers. Microbiological Methods (2004). Schweizerischer Obstverband. Postfach CH-6302 Zug.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MURDOCK, D.I., J.F. FOLINAZZO & V.S. TROY (1952) Evaluation of plating media for citrus concentrates. Food Technol. 6:181-185.
· MURDOCK, D.I. & C.H. BROKAW. (1958). Sanitary control in processing citrus concentrates. I. Some specific sources of microbial contamination from fruit bins to extractors. Food Technol. 12: 573-576.
· STEVENS, J.W. (1954) Preparation of dehydrated agar media containing orange juice serum. Food Technol. 8:88-91.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).