BRETTANOMYCES SELECTIVE AGAR

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Selective medium for the isolation and enumeration of *Brettanomyces/Dekkera* from wine and beer.

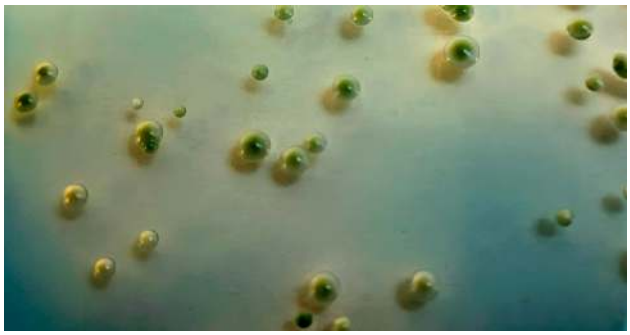
2 - COMPOSITION - TYPICAL FORMULA *

DEHYDRATED MEDIUM, AFTER RECONSTITUTION WITH 1 L OF WATER

Peptone	5.000 g
Malt extract	3.000 g
Yeast extract	3.000 g
Dextrose	10.000 g
Bromocresol green	0.022 g
Thiamine	0.02 g
Cycloheximide	0.010 g
Chloramphenicol	0.100 g
Coumaric acid	0.100 g
Yeast Nitrogen Base	3.000 g
Agar	20.000 g

READY TO USE PLATES

Brettanomyces Selective Agar	44.200 g
Ethanol 95%	16.000 mL
Purified water	1000 mL



Brettanomyces Selective Agar: *Dekkera bruxellensis*

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The name *Brettanomyces* dates back to 1904, when this yeast was identified in the laboratory of the Carlsberg brewery by N.H. Claussen during studies on British Ale beers.[1]

Brettanomyces is a non-spore forming yeast genus of *Saccharomycetaceae* family which includes five different species (*B. custersianus, B. naardenensis, B. nanus, B. anomalus* and *B. bruxellensis*). The genus name *Dekkera* is used interchangeably with *Brettanomyces*, as it describes the teleomorph or sporiform shape of the species *anomalus* and *bruxellensis*.

Dekkera bruxellensis is worldwide considered one of the main causes of wine spoilage.[2] Infected wines develop distinctive, unpleasant aromas due to volatile phenols (4-ethyl-phenol and 4-ethyl-guaiacol), produced by this species. However, this species is also known for its positive contribution to the acetic acid flavour to Belgian Lambic beers and to the fermented and sweetened tea Kombucha.[2]

The formulation of Brettanomyces Selective Agar medium is based on the work of Rodrigues *et al.*[3] and on subsequent modifications of original medium, aimed at obtaining growth with shorter incubation times.[4,5]

The medium contains glucose as carbon and energy source, cycloheximide to prevent *Saccharomyces* growth, chloramphenicol to prevent bacterial growth, p-coumaric acid as the precursor for production of 4-ethyl-phenol. Peptone, malt extract and yeast extract provide carbon, nitrogen and vitamins for microbial growth. Yeast Nitrogen Base and thiamine are growth factors for yeasts. Ethanol improves recovery of *Brettanomyces* while bromocresol green is an acid production indicator.

4 - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 44.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation. DO NOT AUTOCLAVE. Cool to 45-50°C, add 16 mL of 95% ethanol, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	green-bluish, limpid
Final pH at 20-25 °C	5.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Brettanomyces Selective Agar	Dehydrated medium	4012322 4012324	500 g (11,3 L) 5 kg (113 L)
Brettanomyces Selective Agar	Ready-to-use plates	491232	3 x 10 plates, ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops, swabs, pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, membrane filters, ancillary culture media and reagents.

8 - SPECIMENS

Wine, beer. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice.

9 - TEST PROCEDURE

Inoculate an aliquot of the sample on the medium surface or filter the sample through a sterile membrane (pore size 0.45 µm).
Place the membrane filter on medium surface, ensuring that no air is trapped underneath and incubate at 25-30°C for 5-7 days.

10 - READING AND INTERPRETATION

After incubation, observe the microbial growth and record the specific morphological and chromatic characteristics of the colonies.
Brettanomyces/Dekkera spp. grow with small, bright, very convex, nearly hemispherical colonies, with straight or lobed edge with a creamy consistency; the colony colour is between beige and greenish-yellow, with a yellow halo since the acid production turns the

indicator from greenish-blue to yellow. The detection of 4-ethylphenol by its phenolic smell, aids in the differentiation of *Dekkera/Brettanomyces* spp. from other yeast species.

11 - User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ T / ATM	Expected results
D. bruxelliensis ATCC 36234	30°C / 5-7 days/ A	growth with a typical smell
S. cerevisae ATCC 9763	30°C / 7 days/ A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - Performances characteristics

Prior to release for sale a representative sample of all lots of dehydrated and ready-to-use Brettanomyces Selective Agar is tested for productivity and selectivity by comparing the results with Sabouraud Dextrose Agar (SDA).
Productivity is tested by a quantitative test with the target strains *D. bruxelliensis* ATCC 36234, and *D. anomala* ATCC 10562; the membrane filters on the medium are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 30°C for 5-7 days. The colonies are enumerated on Test Batch (TB) and SDA and the productivity ratio ($Pr=CFU_{TB}/CF_{SDa}$) is calculated. If Pr is ≥ 1 and if the colonies' morphology, colour and smell are typical, the results are considered acceptable and conform to the specifications.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: Salmonella Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Saccharomyces cerevisae* ATCC 9763. The growth of non-target strains is totally inhibited.

13 - Precautions and warnings

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated Brettanomyces Selective Agar is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - Storage conditions and shelf life

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Claussen NH. On a method for the application of Hansen's pure yeast system in the manufacturing of well-conditioned English stock beers. Journal of the Institute of Brewing,1904.
2. Schifferdecker AJ, Dashko S, Ishchuk OP, Piškur J. The wine and beer yeast Dekkera bruxellensis. Yeast 2014; 31(9): 323–332.
3. Rodrigues N, Gonçalves G, Pereira-da-Silva S, Malfeito-Ferreira M, Loureiro V. Development and use of a new medium to detect yeasts of the genera Dekkera/Brettanomyces. J Appl Microbiol 2001; 90: 588–599.
4. Couto JA, Barbosa A, Hogg T. A simple cultural method for the presumptive detection of the yeasts Brettanomyces/Dekkera in wines. Lett Appl Microbiol 2005; 41(6):505-10.
5. Benito S, Palomero F, Morata A, Calderon F, Palmero D, Suárez-Lepe JA. Identifying yeasts belonging to the Brettanomyces/Dekkera genera through the use of selective-differential media African Journal of Microbiology Research Vol. 6(34), pp. 6348-6357, 6 September, 2012.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First issue	2024/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.