Chapter 11

Enumerating Distinct Yeast in the Same Food Sample

Luara Aparecida Simões, Angélica Cristina de Souza, Rosane Freitas Schwan, and Disney Ribeiro Dias

Abstract

The enumeration of yeasts in food is widely performed in the field of microbiology. When more than one yeast species coexists in a food, it is interesting to enumerate and differentiate them, for which the use of selective and differential culture media can be used, making it possible to observe the growth, texture, color, shape, and size of the colonies. Another method that can also be used is the microscopy visualization of cells. This chapter aims to show different culture media that allow characterizing different yeast colonies and different techniques for observing cells under the microscope, in samples of food and beverages (which for convenience in this chapter we will call food from now on).

Key words Differential media, CHROMagar, Selective media, Optical microscopy

1 Introduction

The enumeration and differentiation of yeasts in food is a fundamental parameter that gets an idea of the degree of the presence and role of each species present in different situations such as fermented food, food contamination, food quality, and the influence of yeast metabolism in the final composition of the food. In addition, these parameters allow detecting spoilage yeasts, which are non-desired in the food and affect the organoleptic characteristics and quality of the final product.

Traditionally, there is a great interest in yeast identification at genus and species levels, which is accomplished based on tests for phenotypic characteristics (morphology plus physiological and biochemical tests) [1]. Currently, identification using methods that apply molecular techniques is the most used because it is faster and more reliable. However, basic phenotypic information is necessary to understand how yeast survives and grows in the food, as well as to differentiate them, and this information is achieved through

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simple techniques, such as plating in selective and differential media and the observation of cells morphotypes under a microscope [2].

The selective and differential culture media are based on several factors. They can provide different sources of carbon, nitrogen, or contain concentrations of compounds that can select and differentiate yeasts according to the size, texture, color, and shape of the colonies. Another sort of medium is the chromogenic one, based on the enzymatic interaction with a chromogenic substrate, in which the different yeast species grow with a characteristic color in the colony, due to compounds with different absorbance that are released when the chromogens are degraded by enzymes specific to each yeast [3]. Other methods can be used to guarantee the efficiency of yeast differentiation, such as microscopic aspects of the colony and cell morphologies, with the verification of the presence and type of spores, presence of hyphae, production of filaments, shape, and other structures.

The use of an optical microscope allows enumerating and differencing yeast species by the shape and size of their cells, as well as by the size and number of their reproductive structures (spore formation, budding, or binary fission) [3]. Microscope counting and observation are carried out mainly by using wet mount slides and by Neubauer chamber [4]. The combination of the use of selective and differential media and microscopy visualization allows distinguishing different species of yeasts that coexist in the same food sample, and it is a widely applicable practice. In this chapter, methods for enumerating different yeasts in the same food sample will be discussed; a summary can be viewed in Fig. 1.

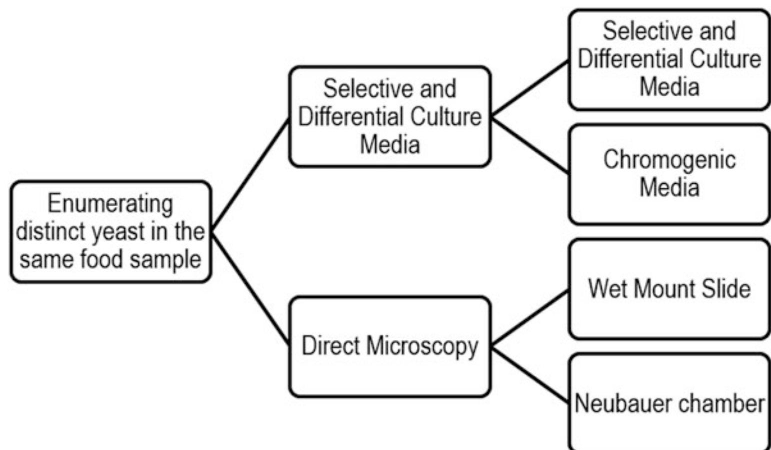

Fig. 1 Methods used for enumeration and differentiation of yeasts present in the same food sample

2 Materials

2.1 Selective and Differential Culture Media

1. Basic equipment and appropriate techniques for preparation of sample and dilution (*see* Chapter 8).
2. Equipment for plating samples (*see* Chapter 10).
3. Incubator (30 °C and 37 °C).
4. pH meter.

2.1.1 Medium (See Note 1)

Lysine agar composition (g/L, except for potassium lactate): glucose (44.5), potassium dihydrogen phosphate (1.78), magnesium sulfate (0.89), calcium chloride fused (0.178), sodium chloride (0.089), adenine (0.0018), DL-methionine (0.0009), L-histidine (0.0009), DL-tryptophan (0.0009), boric acid (0.000009), zinc sulfate (0.000036), ammonium molybdate (0.000018), manganese sulfate (0.000036), ferrous sulfate (0.00022), lysine (1), inositol (0.02), calcium pantothenate (0.002), aneurine (0.0004), pyridoxine (0.0004), p-aminobenzoic acid (0.0002), nicotinic acid (0.0004), riboflavin (0.0002), biotin (0.000002), folic acid (0.000001), potassium lactate (10 mL), agar (17.8), distilled water for 1 L of final volume.

Molybdate Agar composition (g/L): proteose-peptone (10), sucrose (40), phosphomolybdic acid (1.9), agar (15), distilled water for 1 L of the final volume.

Wallerstein Laboratory Nutrient Agar composition (g/L): bromocresol green (0.022), calcium chloride (0.125), casein enzymic hydrolysate or peptone (5), dextrose (50), ferric chloride (0.0025), magnesium sulfate (0.125), manganese sulfate (0.0025), monopotassium phosphate (0.55), potassium chloride (0.425), yeast extract (4), agar (20), distilled water for 1 L of the final volume.

CHROMagar Candida composition (g/L): peptone (10.2), chromogenic mix (5-bromo-4-chloro-3-indolyl/N-acetyl-b-D-glucosaminide and 5-bromo-6-chloro-3-indolyl phosphate p-toluidine salt) (22), agar (15), distilled water for 1 L of final volume.

2.2 Direct Microscopy

1. Optical microscope.
2. Slides or Neubauer chamber and 24 × 24 mm coverslips.
3. Filter paper.
4. Pipette.
5. Inoculating loop.
6. Immersion oil (if necessary).

Dye solution composition: methylene blue (1 g); distilled water (10 mL), sodium citrate, dihydrate (2 g).

3 Methods

3.1 Selective and Differential Culture Media

1. Add the components to sterile distilled/deionized water (*see* **Note 2**).
2. Gently heat and bring to boiling (*see* **Note 3**).
3. Mix thoroughly and adjust the pH (*see* **Note 4**).
4. Autoclavable media (Molybdate Agar and WL agar): Autoclave 15 min at 121 °C.
5. No autoclavable media (Lysine agar and CHROMagar Candida): Swirling or stirring regularly to prevent overheating.
6. Cool to 45–50 °C.
7. Pour into sterile petri dishes (*see* **Note 5**).
8. Make the respective dilutions of the food samples if necessary (*see* Chapter 8) (*see* **Note 6**).
9. Perform plating using the spreading technique (*see* Chapter 10).
10. Incubation (*see* **Note 7**).
11. Count the number of colonies and classify the different yeasts according to the reactions in the respective medium.

3.1.1 Lysine Agar

Many yeast species can grow in a liquid synthetic medium containing L-lysine as the main nitrogen source. On this medium pitching, yeasts are suppressed; the *Saccharomyces* yeasts are not supposed to grow on this media. Lysine agar is considered an excellent selective medium for estimating wild yeast contamination in commercial samples of beer, baker, wines, and other yeasts, as well as in large-scale fermentation and cell propagation [5]. Recent studies have used the Lysine medium to differentiate yeasts [6, 7, 8]. The characteristics of yeasts that grow in the Lysine agar are described in Table 1.

3.1.2 Molybdate Agar

In this medium, different species of yeast give rise to different colored colonies in the presence of molybdate, providing a count of different yeasts present in the same food sample [9]. An advantage of using this culture medium is that the growth of filamentous fungi can be completely inhibited with the addition of propionate [10] facilitating the differentiation and isolation of yeast colonies. The different yeasts according to the reactions on Molybdate agar are shown in Table 2.

3.1.3 Wallerstein Laboratory Nutrient Agar (WL)

The WL Agar differential medium contains 0.1% bromocresol green, which allows general discrimination among some yeast species based on colony color, size, and morphology [12]. The WL medium was initially developed to monitor the yeast community during industrial fermentation processes and is widely used to

Table 1
Appearance of yeasts according to growth on Lysine agar

Colony size	Species	Filaments	Other properties
0.6 mm	*Candida mycoderma*	Lobed, narrow fringe of closely filaments at the periphery	Semi-transparent border
1.5–2.0 mm	*Candida tropicalis*		Spiky, blastospore formation
2.5–3 mm	*Trichosporon cutaneum*	Long filaments	Irregular thickened center
1.5–3.0 mm	*Candida krusei*		Strongly scalloped margins
0.5 mm	*Torulopsis candida*	Very small branched filaments (fringe-like)	Colonies were often lobed
0.4 mm	*Rhodotorula mucilaginosa*	No filaments	Pink with a characteristic ovoid shape

Source: Adapted [5]

Table 2
Properties of yeast colonies on Molybdate agar

Macro colonial pigment	Species	Other properties
No extracellular opacity		
White, opaque, or colorless translucent	*Torulopsis glabrata* *Cryptococcus neoformans* *Candida krusei* *Candida mycoderma* *Candida zeylanoides* *Candida lipolytica* *Candida brumptii* *Candida rugosa* *Trichosporon capitatum* *Debaryomyces* sp.	No pseudo mycelia Mucoid colony Slow glucose fermentation Forms arthrospores Forms ascospores
Green	*Candida curvata* *Candida parapsilosis* *Candida albicans* *Candida catenulata*	 Forms chlamydospores
Blue	*Candida pulcherrima*	Forms pulcherrima (ovoid to ellipsoidal in shape) cells
Increased extracellular opacity		
Green	*Candida tropicalis* *Saccharomyces* sp.	 Forms ascospore
Blue	*Candida macedoniensis* *Candida guilliermondii* *Candida robusta*	

Source: Adapted [11]

Table 3
Characteristics of yeasts according to their growth on WL agar

Macro colonial pigment	Species	Surface	Consistency
Green to cream-colored	*Saccharomyces* sp.	Knoblike, smooth, opaque	Creamy
Intense-green	*Hanseniaspora uvarum*	Flat, smooth, opaque	Buttery
Cream	*Zygosaccharomyces bailii*	Small, elevated dome, smooth	Creamy
Cream-less colonies with a hint of green	*Torulaspora delbrueckii*	Knoblike, smooth, opaque	Creamy
Bright green	*Saccharomycodes ludwigi*	Knoblike, convex, smooth, opaque	Creamy
Intense green	*Schizosaccharomyces pombe*	Small, smooth, opaque	Buttery
Red	*Rhodutorula* sp.	Knoblike, convex smooth, mucoid	Buttery
Cream with hint of red, red-brown from bottom	*Metschnikowia pulcherrima*	Small, convex	Floury
Gray-green with hint of blue	*Pichia membranefaciens*	Elevated, convex, wrinkled	Floury
Cream to blue-gray (blue after 8 days)	*Pichia anomala*	Flat, smooth	Creamy
Cream, appears after 8 days	*Brettanomyces intermedius*	Small, elevated to a dome, smooth	Creamy

Source: Adapted [15]

monitor yeast growth dynamics by enumeration of viable cells [6, 8, 13, 14]. Aspects of yeast colonies grown on WL agar can be seen in Table 3 and Fig. 2.

3.1.4 CHROMagar Candida

CHROMagar Candida can be used for the isolation and differentiation of *Candida albicans*, *C. krusei*, and *C. tropicalis*, yeasts that may be present in food (*C. albicans* present as a contaminant). The chromogenic substrates confirm yeast hexosaminidase and alkaline phosphatase activity, so the yeast cells were differentiated according to the color and the morphology of colonies on CHROMagar Candida [16]. The characteristics of yeasts grown on the CHROMagar Candida are described in Table 4.

3.2 Direct Microscopy

3.2.1 Wet Mount Slides

1. Make the dye solution (*see* **Note 8**).
2. If you want to analyze culture in a solid medium, place a drop of the dye solution on the slide (with an inoculating loop). This procedure is unnecessary if the sample is liquid. In this case, mix the dye solution with an equal volume of suspension containing yeasts, place a drop of the mixture on a slide and proceed to **step 5**.

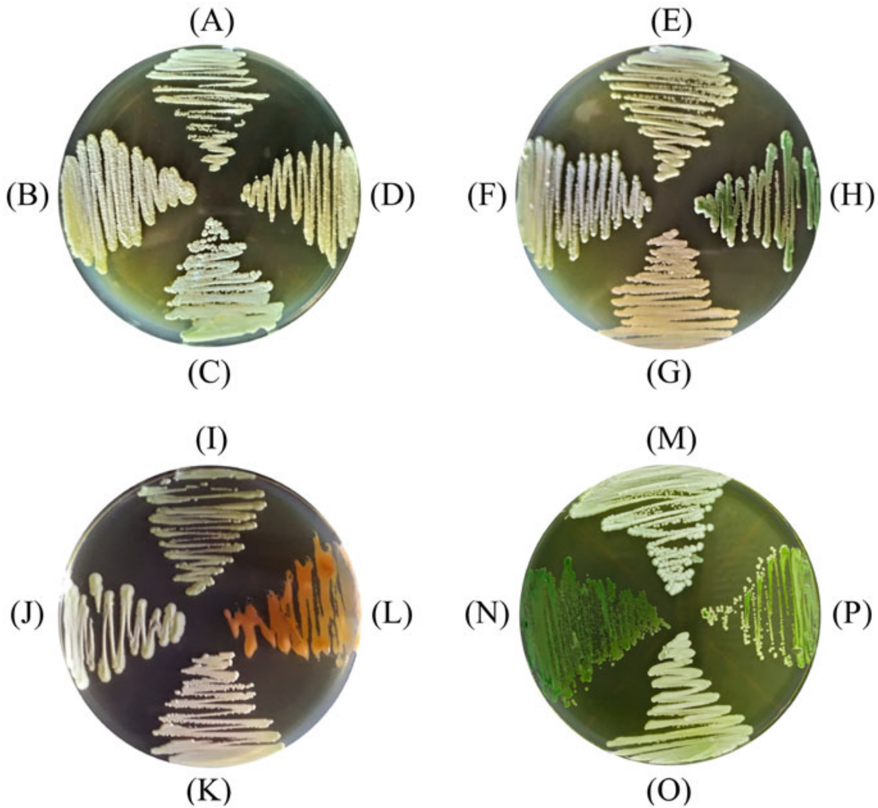

Fig. 2 Yeast colony morphology on WL agar (**a**) *Cyberlindnera saturnus*; (**b**) *Schwanniomyces vanrijiae*; (**c**) *Wickerhamomyces anomalus*; (**d**) *Yarrowia lipolytica*; (**e**) *Saturnispora* sp.; (**f**) *Candida tropicalis*; (**g**) *Candida rugosa*; (**h**) *Kazachstania exigua*; (**i**) *Torulaspora delbrueckii*; (**j**) *Pichia anomala*; (**k**) *Pichia membranifaciens*; (**l**) *Rhodotorula mucilaginosa*; (**m**) *Saccharomyces cerevisiae*; (**n**) *Hanseniaspora uvarum*; (**o**) *Zygosaccharomyces bailii*; (**p**) *Schizosaccharomyces pombe*

3. Sterilize the inoculating loop, let it cool, touch the top of a colony.
4. Mix the cells on the slide with the dye drop.
5. Place the coverslip over the drop (*see* **Note 9**).
6. Take to the optical microscope (*see* **Note 10**).
7. Examine approximately 1000 cells (*see* **Note 11**).
8. Consider viability as the percentage of unstained cells (*see* **Note 12**).
9. For differentiation, carefully analyze the cell morphology.

3.2.2 Neubauer Chamber

1. Mix 100 μL sample with the same volume as the methylene blue solution (*see* **Note 13**).
2. Homogenize the mixture by shaking and let it stand for 1 min.
3. Place the coverslip over the Neubauer chamber (*see* **Note 14**).

Table 4
Visual and microscopic aspects of yeasts grown on CHROMagar Candida

Macro colonial pigment	Species	Other properties
Apple green	*Candida albicans*	Chlamydospores; abundant pseudo and true hyphae
Dull blue, to purple color with pale pink edges	*Candida tropicalis*	Abundant pseudo hyphae with blastoconidia
White to pale pink	*Candida parapsilosis*	Clusters of blastospores
Pale pink colonies	*Candida krusei*	Large, flat, spreading, matt surfaces, pseudo mycelium
White large glossy pale pink to violet	*Candida glabrata*	No pseudo hyphae
Pink to purple	*Candida guilliermondii*	Small with Pseudo hyphae and blastospores
Pink gray purple	*Candida lusitaniae*	Branched pseudo hyphae present
White to light pink	*Candida famata*	No pseudo hyphae
Dark green	*Trichosporon* spp.	
Pink to off white cream	*Candida kefyr*	
Dark pink/violet	*Saccharomyces* sp.	Very small, pinpoint colonies

Source: Adapted [16, 17]

4. Homogenize again and collect an aliquot of the solution (sample diluted in Methylene Blue).
5. Fill the camera using a micropipette (*see* **Note 15**).
6. Wait 2 min for the cells to settle in the chamber.
7. Perform the count on the four quadrilaterals located on the sides and the one in the middle of the chamber (*see* **Note 16**) (Fig. 3).
8. Calculate the cell concentration (Cel/mL) from the equation:

$$\text{Cel/mL} = \sum \text{cells} \times 25 \times \text{FD} \times 10^4/N.$$

where

Σ cells: the sum of the number of viable (or total) cells counted in the *n* grid (*see* **Note 17**).

25: total number of chamber squares.

FD: dilution factor (*see* **Note 18**).

10^4: chamber constant (*see* **Note 19**).

N: number of squares counted among the 25 available (5 recommended, **step** 7) [4].

Yeast cells have about 5 μm diameter, and most important features can be seen in a light microscope, it is a quick practice that allows enumerating and differentiating yeasts, observing the

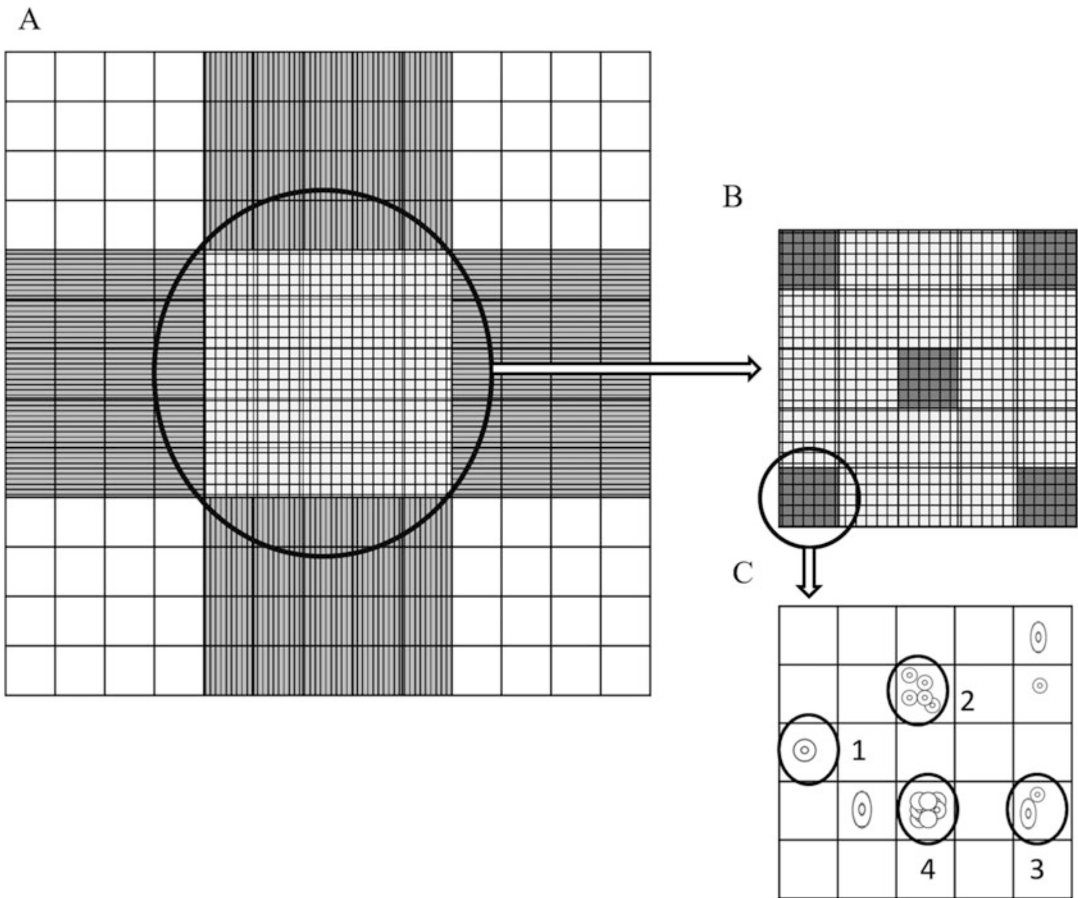

Fig. 3 Schematic drawing of the Neubauer chamber. (**a**) Details of the central quadrant; (**b**) Details of the 25 squares of the central quadrant used for yeast counting, the extreme four ones and the central squares (dark gray) are suggested as sampling during the count; (**c**) Details of one of the quadrants to be counted, 1: single-cell; 2: cluster with cells easily distinguishable by their nuclei and cytoplasm; 3: yeast budding; 4: cluster, whose cells are difficult to distinguish from each other

physiological state of the cells, evidence of contamination, and the presence of yeasts of interest in food [18]. The visual features most used to differentiate yeast in microscopic observations are shown in Table 5.

4 Notes

1. Ideal media for detecting and enumerating yeasts should suppress the bacterial and filamentous fungi growth, so antibiotics and some antifungal compounds (chloramphenicol, rose Bengal, dichloran, propionate, and others) can be added to the yeast media to inhibit bacterial and filamentous fungi presence.

Table 5
Yeast cell morphology by microscope observation

Yeast species	Shape of the cell	Sexual structure	Other properties
Brettanomyces bruxellensis	Ovoid; ellipsoidal; cylindrical to elongate	Multilateral budding	Pseudo hyphae abundantly
Candida zemplinina	Ellipsoid to elongated multilateral	Budding	Cells occur singly or in pairs
Kloeckera apiculata	Apiculate; ovoid; elongate	Bipolar budding	Cells occur singly or in pairs
Lachancea thermotolerans	Spherical to ellipsoidal	Multilateral Budding	Spherical ascospores 1–4 spores per ascus
Metschnikowia pulcherrima	Globose to ellipsoid	Multilateral Budding	Acicular to filiform Ascospore (sphaeropedunculate) 1–2 spores per peduncle
Candida stellata	Ovoid to elongate	Multilateral Budding	Spherical ascospores (1 per ascus)
Saccharomyces cerevisiae	Globose; ovoid; elongate	Multilateral Budding	Globose to short ellipsoidal ascospores 1–4 spores per ascus
Saccharomyces ludwigii	Lemon shaped, elongated with a swelling in the middle	Bipolar budding	Spherical and smooth ascospores 4 or 2 spores per ascus
Schizosaccharomyces pombe	Globose; ellipsoidal; cylindrical	Fission	Globose to ellipsoidal ascospores 2–4 spores per ascus
Torulaspora delbrueckii	Spherical to ellipsoidal	Multilateral Budding	1–4 spores per ascus Conjugation tubes
Wickerhamomyces anomalus	Spherical to elongate	Multilateral Budding	Hat-shaped ascospores (1–4 per ascus), Asci are deliquescent

Source: Adapted [19]

2. For the Lysine agar: first, dissolve the potassium lactate in distilled water and, then, add the other ingredients. For the Molybdate agar: first, it is necessary to make a phosphomolybdic acid solution that will not go into the autoclave: Add 12.5 g of P_2O_5'$20MoO_3$ (phospho-12-molybdic acid) to 100 mL of sterile distilled/deionized water. All other components of the base agar must be autoclaved before adding 15 mL of phosphomolybdic acid solution in 985 mL of the cooled sterile base.
3. Heating can be done in a steam flow using an autoclave with temperature not exceeding 100 °C, or in a microwave oven (set the microwave oven power to 60% for a safer operation). After the initial boil, remove the medium from the autoclave or the oven, rock gently with your hands (use thermal gloves), and

then return to the autoclave or oven. Repeat bursts of heating until the complete fusion of the agar (visibly large bubbles will replace the foam).

4. Adjust pH final before pouring into petri dishes to 4.8 ± 0.2 (Lysine agar and CHROMagar Candida); 5.3 ± 0.2 (Molybdate agar); Adjust pH before autoclaving to 6.5 ± 0.1 (WL agar).
5. To remove surface moisture of prepared medium drying at 37 °C, store the prepared medium at 2–8 °C. The chromogenic medium must be stored in the dark.
6. In the Lysine agar, the number of cells in the inoculum is essential, small numbers of cells (approximately 100 to 1000) still grow to a limited extent on the medium. When the number of yeast cells exceeds 10,000, a count of the colonies in the medium provides a direct measure of the yeasts count.
7. Incubation at 30 °C for 48–72 h for Lysine agar, WL agar, Molybdate agar, and at 37 °C for 48 h in CHROMagar Candida.
8. Dissolve the methylene blue in distilled water. Add sodium citrate (dihydrate) and stir until completely dissolved. Filter with a filter paper and add 100 mL of distilled water to the filtered. Other dyes can be used, for example, trypan blue and violet methylene.
9. Take care not to press the material biological, and this can make visualization difficult or damaged cells.
10. For microscopic yeast count, daughter cells less than half the size of the parent cell are not counted. The concentration of cells to be analyzed should be in the range of 40–60 cells per microscope field. Make the respective dilutions if necessary.
11. Place the preparation under the microscope and focus with the lowest magnifying lens first, using the coarser adjustment and then use the fine adjustment. Move successively to the highest magnification, use the 100× objective lens, if necessary, place on top of the area to observe a drop of immersion oil, and adjust the focus. Do not use immersion oil with any other objective lens.
12. The methylene blue dye (vital dye) stains nonviable cells in blue. To precisely evaluate the cell viability, it is recommended to perform plate counting since the methylene blue method is based on the presence of specific enzymes, and these enzymes may be present in cells that do not reproduce so fast or even some non-viable cells not dead indeed. For a better and reliable result, the counting of cells stained with methylene blue must be performed after 60 s so that the dye is absorbed, and up to 10 min after contact to avoid cell toxicity.

13. If necessary, dilute the sample. Ideally, between 20 and 60 cells per chamber quadrant favor the counting process. The dilution can be done with the dye solution itself or previously in sterile distilled water. Take the dilution in account to do the math.
14. For the fixation moisten the two lateral channels existing among the counting area in the chamber (quadrilateral).
15. Place the pipette or capillary close to the space between the Neubauer chamber and the coverslip and fill the entire area corresponding to the chamber's quadrilateral.
16. Perform the count with 400× magnification, counting the cells (total and viable) present in 5 of the 25 squares, in the central quadrant of the chamber (Fig. 3a), the four quadrilaterals located on the sides of the chamber and from the middle one (Fig. 3b). Adopt the technique of always counting in one direction, observing the existence of cells located in the line that divides the smaller squares. Viable cells in the sample show little or no staining, while nonviable cells are stained in blue.
17. Enumerate cells with a very visible nucleus (Fig. 3c): count isolated cells as one cell (1); counting cluster made up of cells easily distinguishable by their nuclei and cytoplasm as groups of isolated cells and counting each cell (2); daughter cells less than half of the size of the parent cell are not counted (3), cluster, whose cells are difficult to distinguish from each other, should be counted as a single group (4).
18. Example of calculated dilution factor (FD): 100 μL of sample added with 900 μL of sterile distilled water (tenfold dilution), then 100 μL of this dilution is mixed with 100 μL of dye solution (twofold dilution), obtaining a dilution factor equal to 20.
19. The value of the chamber constant refers to the inverse of the central quadrant volume used for counting; the quadrant volume is 0.1 mm^3, which is equivalent to 0.0001 mL (10^{-4} mL).

References

1. Barnett JA, Payne RW, Yarrow D (2000) Yeast. In: Characteristics and identification, 3rd edn. Cambridge University Press, England
2. Zara S, Mannazzu I (2019) Detection, quantification, and identification of yeast in Winemaking. In: Yeasts in the production of wine. Springer, New York, pp 81–115. https://doi.org/10.1007/978-1-4939-9782-4
3. Iris L, Antonio M, Antonia BM, Antonio SLJ (2020) Isolation, selection, and identification techniques for non-saccharomyces yeasts of oenological interest. In: Biotechnological progress and beverage consumption. . The science of beverages, vol 19. Elsevier, pp 467–508. https://doi.org/10.1016/B978-0-12-816678-9.00015-1
4. Dias DR, Schwan RF (2010) Isolamento e identificação de leveduras. In: FMA M, Huising EJ, Bignell DE (eds) Manual de biologia dos solos tropicais: amostragem e caracterização da biodiversidade. Editora da UFLA, Lavras, pp 227–277
5. Fowell RR (1965) The identification of wild yeast colonies on lysine agar. J Appl Bacteriol 28(3):373–383

6. Polizzotto G, Barone E, Ponticello G, Fasciana T, Barbera D, Corona O et al (2016) Isolation, identification and oenological characterization of non-*Saccharomyces* yeasts in a Mediterranean island. Lett Appl Microbiol 63:131–138. https://doi.org/10.1111/lam.12599
7. Li H, Jiang D, Liu W, Yang Y, Zhang Y, Jin C, Sun S (2020) Comparison of fermentation behaviors and properties of raspberry wines by spontaneous and controlled alcoholic fermentations. Food Res Int 128:108801. https://doi.org/10.1016/j.foodres.2019.108801
8. Maoloni A, Blaiotta G, Ferrocino I, Mangia NP, Osimani A, Milanović V et al (2020) Microbiological characterization of Gioddu, an Italian fermented milk. Int J Food Microbiol 323:108610. https://doi.org/10.1016/j.ijfoodmicro.2020.108610
9. Bovill R, Bew J, Robinson S (2001) Comparison of selective media for the recovery and enumeration of probiotic yeasts from animal feed. Int J Food Microbiol 67:55–61. https://doi.org/10.1016/S0168-1605(01)00448-2
10. Rale V (1984) A note on an improved molybdate agar for the selective isolation of yeasts from tropical fruits. J Appl Bacteriol 56:409–413. https://doi.org/10.1111/j.1365-2672.1984.tb01368.x
11. Holland ML, Kunz LJ (1961) Evaluation of molybdate agar as a selective, differential medium for yeasts. J Bacteriol 81(6):869
12. Pallmann CL, Brown JA, Olineka TL, Cocolin L, Mills DA, Bisson LF (2001) Use of WL medium to profile native flora fermentations. Am J Enol Vitic 52:198–203
13. Russo P, Englezos V, Capozzi V, Pollon M, Segade SR, Rantsiou K et al (2020) Effect of mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* on management of malolactic fermentation. Food Res Int 134:109246. https://doi.org/10.1016/j.foodres.2020.109246
14. Binati RL, Junior WJL, Luzzini G, Slaghenaufi D, Ugliano M, Torriani S (2020) Contribution of non-Saccharomyces yeasts to wine volatile and sensory diversity: a study on *Lachancea thermotolerans, Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. Int J Food Microbiol 318:108470. https://doi.org/10.1016/j.ijfoodmicro.2019.108470
15. Cavazza A, Grando MS, Zini C (1992) Rilevazione della flora microbica di mosti e vini. Vignevini 9:17–20
16. Nadeem SG, Hakim ST, Kazmi SU (2010) Use of CHROMagar Candida for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings Libyan. J Med 5(1):2144. https://doi.org/10.3402/ljm.v5i0.2144
17. Yücesoy M, Marol S (2003) Performance of CHROMAGAR candida and BIGGY agar for identification of yeast species. Ann Clin Microbiol Antimicrob 2(1):8. https://doi.org/10.1186/1476-0711-2-8
18. Atlas RM (ed) (2010) Handbook of microbiological media. CRC Press
19. Kurtzman C, Fell JW, Boekhout T (eds) (2011) The yeasts: a taxonomic study. Elsevier