Identification of Yeasts Present in Sour Fermented Foods and Fodders

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Abstract:

This paper deals with rapid methods for identification of 50 yeast species frequently isolated from foods and fodders that underwent a lactic acid fermentation. However, many yeast species present in olive brine, alpechin, and other olive products were not treated. The methods required for identification include light microscopy, physiological growth tests (ID32C system of BioMérieux), assimilation of nitrate and of ethylamine as sole nitrogen sources, vitamin requirement, and maximum growth temperature. An identification key to treated yeast species is provided. In another table characteristics of all yeast species treated are listed.

Index Entries: Yeast identification; foods, sour foods.

1.Introduction

Lactic acid fermentation is commonly used for food conservation. The main products of this bacterial process are lactic and acetic acids, which are toxic to many microorganisms, most yeasts included. The low pH achieved by the lactic acid fermentation, together with anerobiosis, provides conditions adverse to spoiling and pathogenic microorganisms. Hence, the fermented commodities are stable and can be stored for a long time without loss of quality. The production of sauerkraut is a good example of this practice. In the dairy industry buttermilk and yogurt are well-known products. This application of the lactic acid fermentation originated from Central Asia in times immemorial and has spread from there to Europe and the Orient. It was unknown in other continents until these were colonized from Europe.

Only a limited number of the approx 700 yeast species known at present are tolerant to lactic and acetic acids at low pH. Many of these inhabit fermented foods and fodders. Some of these are mild pathogens (e.g., *Candida glabrata* and *C. parapsilosis*), others are very harmful when present in silage because of their rapid degradation of lactic and acetic acids under aerobic conditions, which results in loss of nutritive value. *C. milleri*, *Pichia fermentans,* and *Saccharomyces exiguus* are the main causative agents of aerobic spoilage of maize silage ***(1)***. A review of yeast species isolated from various silages has been given by Middelhoven ***(2)***. Many more than the about 50 species treated in this paper have been isolated from fermented olives, alpechin, and other olive products. No attention could be paid to these species, which seem to be specific for habitats rich in salt and fats. Likewise, yeast species known from fruit juices and alcoholic beverages were not dealt with in order to keep this study surveyable.

In this article easy methods for rapid yeast identification are described. They include simple light microscopy, physiological growth tests, and some additional characteristics. The yeast species to be identified are those found in various silages ***(2)*** and

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species mentioned in both recent yeast monographs ***(3,4)*** as inhabitants of commodities like sauerkraut, buttermilk, cucumber brine, and pickles. If identification by the methods proposed in this article is unsuccessful, the methods prescribed in both yeast monographs ***(3,4)*** should be applied. It must be kept in mind that only part of the yeasts present in nature has been described yet. Unidentifiable yeast strains may represent unknown species. They are welcomed by yeast culture collections of which the yeast collection of the Centraalbureau voor Schimmelcultures (CBS) at Utrecht, The Netherlands is the most prominent one.

Before carrying out any of the tests described herein, it must have been proven that the isolated yeast cultures are pure and indeed are yeasts. This can be ascertained by plating the cultures on 1% yeast extract, 1% glucose, 2% agar, and by microscopical examination. Only colonies of one type should develop on the plates and light microscopy (magnification × 1000) should reveal true budding yeast cells, which are considerably bigger than bacteria (usually 3 µm wide or more). However, some species do not propagate by budding but by splitting cells or fragmenting mycelium (e.g., *Dipodascus, Galactomyces* sp.). Some of these yeastlike fungi, which do not show budding, are frequently isolated from sour foods and fodders and are treated here as they are in the most authorative yeast monographies ***(3,4)***.

The colonies of most species treated in the identification key are white or cream; some species are red or orange, but never black. Black yeastlike fungi are not treated here. For their identification a culture collection, e.g., the CBS at Utrecht, The Netherlands, should be consulted. Slant cultures of isolated strains should be incubated at 25°C for at least two weeks, to be sure that no black pigment will develop.

In this article a dichotomous identification key to the yeast species listed in **Table 1** is provided (**Table 2**). Identification is valid only if the isolated strain fits the morphological and physiological properties of the species that are given in **Table 3**. These were taken from recent yeast monographs ***(3,4)*** and from the CBS Yeast Data Base (http://www.cbs.knaw.nl). The nomenclature of the species is according to Kurtzman and Fell ***(4)***. The most current synonyms are presented also (**Table 1**).

2. Materials

1. Yeast identification system ID 32 C of BioMérieux 69280 Marcy-l'Etoile, France or 595 Anglum Drive, Hazelwood, Missouri.
2. Soluble starch (Merck).
3. Growth media from Difco Laboratories, Detroit, Michigan, YM Agar, Yeast Nitrogen Base, Yeast Carbon Base, Yeast Extract, Potato Dextrose Agar, Bacto Vitamin-free Yeast Base.

3.Methods

3.1. Morphology

Cells taken from a young pure slant culture are examined microscopically (magnification ×1000) for presence of budding yeast cells and filaments (mycelium or pseudomycelium). Several species fail to produce mycelium in slant cultures. They should be examined in slide cultures. For this purpose, a Petri dish containing a U-shaped glass rod supporting a glass microscope slide is sterilized by dry heat at 160–180°C for 2 h. A suitable agar, e.g., maize (corn) meal agar or potato dextrose agar (both commercialy available), is melted and poured into a second Petri dish. The glass slide is quickly removed from the glass rod with a flame-sterilized pair of tweezers and dipped into the molten agar, after which it is replaced on the glass rod. After the surface of the agar has solidified, the yeast is lightly inoculated in either one or two lines along the slide and a sterile cover slip is placed over part of it. A little sterile water is poured into the Petri dish to prevent the agar from drying out. The culture is then incubated at 25°C. After 3 d the slide is examined microscopically (magnification ×400) for the formation of filaments along the edges of the streak, both under and around the cover slip. Some genera (e.g., *Arxula, Dipodascus*, *Galactomyces*, *Trichosporon*) are notable for fragmenting of the mycelium into arthroconidia, which often lay in a characteristic zig-zag way.

A flask culture in 2% glucose, 0.5% yeast extract, 1% peptone (GYEP) broth is recommended. In a 100 mL conical flask 50 mL of the broth is put

Table 1
Names and current synonyms of the yeast species treated

1. *Arxula adeninivorans*, synonym: *Trichosporon adeninovorans*
2. *Candida apicola*, synonyms: *Torulopsis apicola*, *Torulopsis bacillaris*
3. *Candida boidinii*, assimilates methanol
4. *Candida glabrata*, synonym: *Torulopsis glabrata*
5. *Candida holmii*, anamorph of *Saccharomyces exiguus*
6. *Candida lactis-condensi*, synonym: *Torulopsis lactis-condensi*
7. *Candida milleri*
8. *Candida parapsilosis*
9. *Candida pseudolambica*
10. *Candida sake*, synonym: *Torulopsis sake*
11. *Candida tenuis*
12. *Candida tropicalis*
13. *Candida versatilis*, synonym: *Torulopsis versatilis*
14. *Candida wickerhamii*, synonym: *Torulopsis wickerhamii*
15. *Debaryomyces etchellsii*, synonyms: *Pichia etchellsii*, *Torulaspora etchellsii*
16. *Debaryomyces hansenii*, synonyms: *Candida famata*, *Torulopsis candida*
17. *Dipodascus capitatus*, synonyms: *Tricho–*sporon *capitatum*, *Geotrichum capitatum*
18. *Galactomyces geotrichum*, synonym: *Geotrichum candidum*
19. *Hanseniaspora uvarum*, synonym: *Kloeckera apiculata*
20. *Hanseniaspora valbyensis*, synonym: *Kloeckera japonica*
21. *Issatchenkia orientalis*, synonym: *Candida krusei*
22. *Kluyveromyces lactis*, synonyms: *Candida sphaerica*, *Kluyveromyces marxianus* var. *lactis*
23. *Kluyveromyces marxianus*, synonym: *Candida kefyr*
24. *Pichia anomala*, synonyms: *Hansenula anomala*, *Candida pelliculosa*
25. *Pichia burtonii*, synonyms: *Hyphopichia burtonii, Endomycopsis burtonii*, *Candida variabilis*
26. *Pichia canadensis*, synonyms: *Hansenula canadensis*, *H. wingei*, *Candida melinii*
27. *Pichia fermentans*, synonym: *Candida lambica*
28. *Pichia holstii*, synonyms: *Hansenula holstii*, *Candida silvicola*
29. *Pichia membranifaciens*, synonyms: *Pichia membranaefaciens*, *Candida valida*
30. *Pichia ohmeri*
31. *Pichia pijperi*, synonym: *Hanseniaspora pijperi*
32. *Pichia subpelliculosa*, synonym: *Hansenula subpelliculosa*
33. *Rhodotorula minuta*, red or pink colonies
34. *Rhodotorula mucilaginosa*, synonym: *Rhodotorula rubra*, red or pink colonies
35. *Saccharomyces barnettii*
36. *Saccharomyces cerevisiae*, synonyms: *S. carlsbergenis*, *S.chevalieri*, *S. ellipsoideus*, *S.italicus*, *S. lindneri*, *S. uvarum*
37. *Saccharomyces dairenensis*, synonym: *S. dairensis*. This species can only be distinguished from *S. castellii* and from *S. unisporus* with certainty by molecular techniques, and from *S. rosinii* by maximum growth temperature
38. *Saccharomyces exiguus*, synonyms: *Candida holmii*, *Torulopsis holmii*
39. *Saccharomyces rosinii*, can be distinguished from *S. dairenensis*, *S. castellii* and *S. unisporus* by molecular methods and by its lower maximum growth temperature
40. *Saccharomyces spencerorum*
41. *Saccharomyces unisporus*. See *S. dairenensis* and *S. rosinii*. This species can also be distinguished by microscopy of the ascospores
42. *Sacharomycopsis fibuligera*, synonym: *Endomycopsis fibuliger*. Tufts of aerial hyphal outgrowths on the colony surface give a diagnostic character that generally allows immediate species identification
43. *Saccharomycopsis selenospora*, synonyms: *Guillermondella selenospora*, *Endomycopsis selenospora*
44. *Stephanoascus ciferrii*, synonym: *Candida ciferrii*
45. *Torulaspora delbrueckii*, synonyms: *Saccharomyces delbrueckii*, *Candida colliculosa*. This species is variable for most of the characteristics studied and hence is difficult to identify. Fortunately, many strains produce globose cells with characteristic protuberances that facilitate species identification
46. *Trichosporon gracile*, fragmenting mycelium, urease positive
47. *Zygosaccharomyces bailii*
48. *Saccharomyces bisporus*
49. *Zygosaccharomyces mrakii*, synonyms: *Saccharomyces mrakii*, *Torulaspora mrakii* Inositol (20 mg/L) stimulates growth.
50. *Zygosaccharomyces rouxii*, synonyms: *Saccharomyces bailii* var. *osmophilus*

Table 2
Identification key

	Positive	Negative
1. Colonies red or pink	**2**	**3**
2. Growth on N-acetyl-D-glucosamine	*Rhodotorula minuta*	*Rhodotorula mucilaginosa*
3. Growth on nitrate	**4**	**14**
4. Growth on ethylamine	**6**	**5**
5. Growth on mannitol	*Pichia anomala*	*Candida lactis-condensi*
6. Growth on inositol	*Arxula adeninivorans*	**7**
7. Growth without vitamins	**8**	**9**
8. 0.01% Cycloheximide tolerated	*Pichia anomala* (D)	*Pichia subpelliculosa* *Pichia anomala*
9. Growth on maltose	**10**	**13** *Pichia canadensis*
10. Growth at 35°ßC	**11**	*Candida versatilis* *Pichia holstii*
11. Gas from glucose	**12** *Candida versatilis* (maybe D)	*Pichia canadensis* *Pichia holstii* (D)
12. Growth on raffinose	*Pichia subpelliculosa*	*Pichia holstii*
13. Growth on erythritol	*Candida boidinii*	*Candida wickerhamii* *Candida versatilis*
14. Growth on ethylamine	**15**	**92**
15. Budding yeast cells	**17**	**16**
16. Growth on xylose	*Galactomyces geotrichum*	*Dipodascus capitatus* *Stephanoascus ciferrii*
17. Growth on inositol	**18**	**20**
18. Growth on xylose	**19**	*Saccharomycopsis fibuligera*
19. Growth on rhamnose	*Stephanoascus ciferrii*	*Trichosporon gracile*
20. Growth on 2-Keto-D-gluconate	**21**	**59**
21. Growth on maltose	**27**	**22**
22. Growth on cellobiose (maybe D)	**23**	**24** *Pichia anomala*
23. Growth on galactose	*Candida sake*	*Hanseniaspora uvarum*
24. Growth at 30°C	**26**	**25**
25. Growth on melibiose	*Zygosaccharomyces mrakii*	**115**
26. Tolerates 1% acetic acid	*Zygosaccharomyces bailii* *Zygosaccharomyces bisporus* (may be D)	**27**
27. Growth on mannitol	**34** *Debaryomyces hansenii* *Pichia anomala*	**28**
28. Growth on erythritol	**29**	**30**
29. Growth on maltose	*Saccharomycopsis fibuligera* *Pichia burtonii*	*Candida boidinii*
30. Growth on N-acetyl-D-glucosamine	**31** *Candida sake* *Pichia burtonii* *Pichia membranifaciens*	**32** *Torulaspora delbrueckii* *Hanseniaspora uvarum*
31. Growth without vitamins	*Issatchenkia orientalis*	*Candida apicola* *Candida boidinii*
32. Growth on galactose	**33** *Candida sake*	*Hansenispora uvarum* *Zygosaccharomyces rouxii*
33. Growth on sucrose	*Candida sake*	*Zygosaccharomyces rouxii* (or D) *Hanseniaspora uvarum*

(continued)

Table 2 *(continued)*
Identification key

	Positive	Negative
34. Growth on N-acetyl-D-glucosamine	**35**	**42** *Saccharomycopsis fibuligera*
35. True mycelium present	**40** *Debaryomyces etchellsii*	**36**
36. Growth on trehalose	**40**	**37**
37. Growth on cellobiose	*Debaryomyces etchellsii*	**38**
38. Fermentation of glucose (strong)	*Torulaspora delbrueckii*	**39**
39. Growth on raffinose	*Candida apicola* (may be D)	*Pichia membranifaciens*
40. Growth on xylose (maybe D)	**41**	*Pichia ohmeri* (or D) *Candida sake*
41. Growth on cellobiose	**42**	**50**
42. Growth on rhamnose	**43**	**44**
43. Growth on raffinose	*Debaryomyces hansenii*	*Candida tenuis* (maybe D)
44. Growth on raffinose (may be D)	**45**	**48** *Candida tropicalis* *Debaryomyces etchellsii*
45. Fragmenting mycelium	*Pichia burtonii*	**46**
46. True mycelium	*Pichia ohmeri* *Saccharomycopsis fibuligera*	**47**
47. Gas from glucose absent or slow	*Debaryomyces hansenii*	*Torulaspora delbrueckii*
48. Growth on L-arabinose	*Debaryomyces etchellsii* (may be D)	**49** *Torulaspora delbrueckii* *Zygosaccharomyces rouxii* *Saccharomycopsis fibuligera*
49. Growth at 40°C	*Candida tropicalis* *Saccharomycopsis fibuligera*	*Candida sake*
50. Growth on L-arabinose	**51**	**52**
51. Growth on galactose	*Candida parapsilosis* *Pichia burtonii* *Candida tropicalis*	*Candida boidinii*
52. Growth on erythritol	**53**	**54**
53. Growth on galactose	*Pichia burtonii*	*Candida boidinii*
54. Growth on N-acetyl-D-glucosamine	**55**	*Torulaspora delbrueckii* *Candida sake*
55. Growth on raffinose (may be D)	**56**	**57** *Pichia membranifaciens*
56. Growth on maltose	*Pichia burtonii*	*Candida apicola*
57. Growth at 40°C	**58**	*Candida sake*
58. Growth on soluble starch (may be D)	*Candida tropicalis*	*Candida parapsilosis*
59. Growth on N-acetyl-D-glucosamine	**60**	**73**
60. Growth on sucrose	**61**	**67**
61. Growth on cellobiose	**62**	**64**
62. True mycelium present	**63**	*Candida sake*
63. Growth on rhamnose	*Candida tenuis*	*Candida tropicalis*
64. True mycelium present	**65**	**66**
65. Growth on soluble starch	*Candida tropicalis* (may be D)	*Candida parapsilosis*
66. Growth on trehalose	*Candida sake*	*Candida apicola*
67. True mycelium present	**68** *Candida sake*	**72** (only pseudomycelium)

(continued)

Table 2 *(continued)*
Identification key

	Positive	Negative
68. Growth on glucitol	**69**	**70**
69. Growth on galactose	*Candida tropicalis* *Candida parapsilosis*	*Candida boidinii*
70. Gas from glucose (strong)	**71**	*Pichia membranifaciens* (D)
71. Growth on xylose	*Pichia fermentans* (may be D)	*Issatchenkia orientalis*
72. Growth on trehalose	*Candida sake*	*Hanseniaspora valbyensis* (may be D) *Pichia membranifaciens*
73. Growth on sucrose	**74**	**84**
74. Growth without vitamins	**75**	**77**
75. Growth on raffinose	**76**	*Candida sake* *Zygosaccharomyces rouxii*
76. Growth on soluble starch	*Pichia anomala*	*Candida sake* *Torulaspora delbrueckii* *Zygosaccharomyces rouxii*
77. Biotin sufficient	**78**	**81**
78. 0.01 % Cycloheximide tolerated	**79** *Candida sake* (D)	**80**
79. Growth on lactate	*Kluyveromyces lactis*	*Zygosaccharomyces bailii*
80. 1% Acetic acid tolerated	*Zygosaccharomyces bailii*	*Candida sake* *Torulaspora delbrueckii* *Zygosaccharomyces rouxii*
81. Thiamine sufficient	*Saccharomyces spencerorum*	**82**
82. Biotin + pantothenate sufficient	*Zygosaccharomyces rouxii*	**83**
83. True mycelium present	*Saccharomycopsis fibuligera*	*Kluyveromuces lactis* *Kluyveromyces marxianus*
84. Growth without vitamins	*Candida pseudolambica* *Candida sake* *Torulaspora delbrueckii* *Zygosaccharomyces rouxii*	**85**
85. Biotin sufficient	**86**	**87**
86. 1% Acetic acid tolerated	*Zygosaccharomycers bailii* *Zygosaccharomyces bisporus* (D)	*Candida sake* *Torulaspora delbrueckii* *Zygosaccharomyces rouxii*
87. Growth on cellobiose	**88**	**90**
88. True mycelium present	*Saccharomycopsis fibuligera*	**89**
89. Growth on glucitol	*Pichia pijperi*	*Hanseniaspora valbyensis*
90. Biotin + thamine sufficient	*Zygosacharomyces bisporus*	**91**
91. Biotin + pantothenate sufficient	*Zygosaccharomyces rouxii*	*Saccharomyces unisporus*
92. Growth without vitamins	**93**	**99**
93. Growth on mannitol	**94**	**96**
94. Gas from glucose	**95**	*Debaryomyces hansenii* (maybe DW)
95. Growth on soluble starch	*Pichia anomala*	**92**
96. Growth on raffinose	**97**	*Candida sake* *Torulaspora delbrueckii* *Pichia anomala*

(continued)

Table 2 *(continued)*
Identification key

	Positive	Negative
97. Growth on trehalose	*Torulaspora delbrueckii* (may be D) *Pichia anomala* (may be D)	**98**
98. Ascospores formed	*Saccharomyces exiguus*	*Candida holmii*
99. Biotin sufficient	**100**	**107**
100. Growth on N-acetyl-D-glucosamine	**101**	**103**
101. Gas from glucose (strong)	**102**	*Debaryomyces hansenii*
102. True mycelium	*Candida parapsilosis*	*Candida sake* *Torulaspora delbrueckii*
103. Growth on mannitol	**101**	**104** *Torulaspora delbrueckii*
104. Growth on raffinose	**105**	*Candida sake*
105. Growth at 30_C	**106**	*Saccharomyces barnettii*
106. Ascospores formed	*Saccharomyces exiguus*	*Candida holmii* *Candida milleri*
107. Growth on galactose	**108**	**113**
108. Growth at 30°C	**109**	*Saccharomyces rosinii*
109. Growth on raffinose	**110**	**111**
110. 0.01% Cycloheximide tolerated	*Candida milleri*	*Saccharomyces cerevisiae* *Candida milleri*
111. True mycelium present	*Saccharomycopsis selenospora*	**112** *Saccharomyces cerevisiae*
112. 0.1% Cycloheximide tolerated	*Saccharomyces unisporus*	*Saccharomyces dairenensis* *Saccharomyces cerevisiae*
113. True mycelium present	*Saccharomycopsis fibuligera*	**114** *Saccharomyces cerevisiae*
114. Growth at 37 or 40_C	*Candida glabrata*	*Hanseniaspora uvarum*
115. 0.01% Cycloheximide tolerated	**116**	**117** (may be D)
116. Biotin sufficient	*Zygosaccharomyces bailii*	*Hanseniaspora uvarum*
117. Fermentation glucose strong	**118**	**119**
118. 1% Acetic acid tolerated	*Zygosaccharomyces bailii*	*Candida sake*
119. Growth on sucrose	*Candida apicola*	*Pichia membranifaciens*

and sterilized at 120°C for 20 min. The yeast is inoculated to the glass wall at the liquid surface. After incubation for three days at 25°C without shaking the culture is examined for the formation of a sediment and a pellicle that may creep onto the glass wall.

3.2. Assimilation of Carbon Compounds

The easiest way to study the pattern of carbon compound utilization, which in many cases is species-specific, is by using the yeast identification system ID 32 C of BioMérieux. For inoculation the manufacturer's instructions should be followed. The test strips are inspected for growth daily, up to 7 d. The test kits should be prevented from drying out. The test kits must be stored at 4°C and should not be used after the expiry date. The following carbon compounds are included in the system:

Monosaccharides: Glucose, Galactose, L-Sorbose, D-Ribose, D-Xylose, L-Arabinose,Rhamnose, α-Methylglucoside

Disaccharides and trisaccharides: Sucrose (Saccharose), Maltose, Trehalose,

Cellobiose, Melibiose, Lactose, Raffinose, Melezitose

Amino sugars: D-Glucosamine, N-acetyl-D-glucosamine

Polyoles: Glycerol, Erythritol, Glucitol (Sorbitol), Mannitol, Inositol

Organic acids: DL-Lactate, D-Gluconate, D-Glucuronate, 2-Keto-D-gluconate

Table 3 a
Characteristics of Individual Yeast Species Inhabiting Sour Foods

Yeast species (nr)	*Arxula adeninivorans*	*Candida apicola*	*Candida boidinii*	*Candida glabrata*	*Candida holmii*	*Candida lactis-condensi*	*Candida milleri*	*Candida parapsilosis*	*Candida pseudolambica*	*Candida sake*
Budding cells	+	+	+	+	+	+	+	+	+	+
True mycelium	+	–	+	–	–	–	–	+	+W	+W
Fragmenting	+	–	–	–	–	–	–	–	–	–
Pellicle	+	V	+	–	–	–	–	–	?	W
D–Glucose	+	+	+	+	+	+	+	+	+	+
D–Galactose	+	V	–	–	+	–	+	+	–	+
L–Sorbose	+	+D	V	–	–	–	–	V	–	V
D–Ribose	+	V	+	–	–	–	–	V	–	V
D–Xylose	+	V	+	–	–	–	–	+	+D	V
L–Arabinose	+	–D	V	–	–	–	–	V	–	–
Rhamnose	D	–	–	–	–	–	–	–	–	–
α–Methylglucoside	+	–	–	–	–	–	–	+D	–	V
Sucrose	+	+	–	–	+	+	+	+	–	V
Maltose	+	–	–	–	–	–	–	+	–	V
Trehalose	+	–	–	–	+	–	+	+	–	+
Cellobiose	+	–	–	–	–	–	–	–	–	V
Melibiose	+	–	–	–	–	–	–	–	–	–
Lactose	V	–	–	V	–	–	–	–	–	–
Raffinose	+	+D	–	–	+	+D	+	–	–	–
Melezitose	+D	–	–	–	–	–	–	+	–	V
D–Glucosamine	+	–	V	–	–	–	–	V	+D	V
Acetyl–D–glucosamine	+	+	+	–	–	?	–	+	–	V
Soluble starch	+	–	–	–	–	–	–	–	–	–
Glycerol	+	+	+D	V	–	–	V	+D	–D	+D
Erythritol	+	–	+	–	–	–	–	–	–	–
Glucitol	+	V	+	–	–	–	–	+	–	V
Mannitol	+	V	+D	–	–	–	–	+	–	V
Inositol	+	–	–	–	–	–	–	–	–	–
DL–Lactate	V	–	+	V	–D	–	V	V	+	V
D–Gluconate	+	V	–	V	–	–	–	+D	–	V
D–Glucuronate	+	–	–	–	–	–	–	–	–	–
2–Keto–D–gluconate	+	V	V	V	–	–	–	V	–	V
Nitrate	+	–	V	–	–	+	–	–	–	–
Fermentation of glucose	+D	D	+	+	+	+	+	+	+	+
Ethylamine (N)	+	+	+	–	–	–	–	V	+	V
Vitamin requirement	OT	BT	B	M	0B	BT	BM	B	0	0B
Urease	–	–	–	–	–	–	–	–	–	–
Max. growth T (°C)	45	V	35	<45	<35	<35	<35	>37	35	V
Cycloheximide (100ppm)	+	–	+	–	V	–D	V	V	–	–D

+, positive; –, negative; W, weak response; D, delayed positive (7 days or more); V, variable results; ?, no data known; 0, no vitamins required; B, biotin required; T, thiamine required; P, pantothenate required; M, more or other vitamins required.

Table 3b
Characteristics of Individual Yeast Species Inhabiting Sour Foods

Yeast species (nr)	*Candida tenuis*	*Candida tropicalis*	*Candida versatilis*	*Candida wickerhamii*	*Debaryomyces etchellsii*	*Debaryomyces hansenii*	*Dipodascus capitalus*	*Galactomyces geotrichum*	*Hanseniaspora uvarum*	*Hanseniaspora valbyensis*
Budding cells	+	+	+	+	+	+	–	–	+	+
True mycelium	+	+	–	–	+W	–W	+	+	–	–
Fragmenting	–	–	–	–	–	–	+	+	–	–
Pellicle	–	W	–	–	–	V	+	+	–	–
D–Glucose	+	+	+	+	+	+	+	+	+	+
D–Galactose	+	+	+	+	+	+	V	+	–	–
L–Sorbose	V	V	–	–	+	V	V	+	–	–
D–Ribose	+	–D	V	V	V	V	–	V	–	–
D–Xylose	+	+	–D	+	+	+D	–	+	–	–
L–Arabinose	V	V	V	+D	+D	V	–	–	–	–
Rhamnose	+	–	–	V	–	V	–	–	–	–
α–Methylglucoside	V	V	–	–	+	V	–	–	–	–
Sucrose	+	V	V	–	+	+	–	–	–	–
Maltose	+	+	V	–	+	+	–	–	–	–
Trehalose	+	+	V	V	V	+	–	–	–D	–D
Cellobiose	+	V	+	+	+	V	–	–	+D	+
Melibiose	–	–	V	–	–	V	–	–	–	–
Lactose	V	–	V	–	–	V	–	–	–	–
Raffinose	–D	–	V	–	–	+	–	–	–	–
Melezitose	V	V	–	–	+	V	–	–	–	–
D–Glucosamine	V	V	–	V	–	V	–	–	–	–
Acetyl–D–glucosamine	+	+	+	+	+	V	?	?	?	?
Soluble starch	V	+D	–	–	–	V	–	–	–	–
Glycerol	+	V	+	+	+D	+D	+	+	–	–
Erythritol	V	–	–	–	–	V	–	–	–	–
Glucitol	+D	+	–	+	+	+	–	+D	–D	–
Mannitol	+	+	V	+	+	+	–	+	–	–
Inositol	–	–	–	–	–	–	–	–	–	–
DL–Lactate	V	V	–W	–D	V	V	+D	V	–	–
D–Gluconate	+D	V	–D	V	–	V	–	V	V	–D
D–Glucuronate	–	–	–	–	–	V	–	?	–	–
2–Keto–D–gluconate	V	+D	V	V	+	+	–	–	+	–
Nitrate	–	–	+	+	–	–	–	–	–	–
Fermentation of glucose	V	+	+D	+	+D	V	–	V	+D	+
Ethylamine (N)	+	+	+	+	+	V	+	+	+W	+
Vitamin requirement	BT	0B	BT	BT	B	0B	?	0	M	M
Urease	–	–	–	–	–	–	–	–	–	–
Max. growth T (°C)	V	>37	<35	<35	<40	V	>37	<37	<35	<35
Cycloheximide (100ppm)	V	+	V	+	–	V	+	?	+	+

+, positive; –, negative; W, weak response; D, delayed positive (7 days or more); V, variable results; ?, no data known; 0, no vitamins required; B, biotin required; T, thiamine required; P, pantothenate required; M, more or other vitamins required.

Table 3c
Characteristics Of Individual Yeast Species Inhabiting Sour Foods

Yeast species (nr)	*Issatchenkia orientalis*	*Kluyveromyces lactis*	*Kluyveromyces marxianus*	*Pichia anomala*	*Pichia burtonii*	*Pichia canadensis*	*Pichia fermentans*	*Pichia holstii*	*Pichia membranifaciens*	*Pichia ohmeri*
Budding cells	+	+	+	+	+	+	+	+	+	+
True mycelium	+	+	+W	V	+	+	+	+	V	+
Fragmenting	–	–	–	–	+	–	–	–	–	–
Pellicle	+	+	V	V	V	–W	+	V	+	+
D–Glucose	+	+	+	+	+	+	+	+	+	+
D–Galactose	–	V	+D	V	+	–	–	V	–	+
L–Sorbose	–	V	V	–	V	–	–	V	V	+D
D–Ribose	V	–	V	V	+D	–D	–	+	–	V
D–Xylose	–	V	V	V	+D	+	+D	+	V	–D
L–Arabinose	–	–D	V	–	V	–	–	+D	–	–
Rhamnose	–	–	–	–	–	+D	–	+	–	–
α–Methylglucoside	–	V	V	V	+D	V	–	V	–	+
Sucrose	–	+	+	+	+	+	–	+D	–	+
Maltose	–	V	V	V	+	+	–	+	–	+
Trehalose	–	V	V	+D	+	+D	–	+	–	+
Cellobiose	–	V	V	V	V	+	–	+	–	+
Melibiose	–	–	–	–	–	–	–	–	–	–
Lactose	–	V	V	V	+D	–	–	–	–	+D
Melezitose	–	V	V	V	V	+	–	V	–	–D
D–Glucosamine	V	–	–	–	–D	–	V	+D	V	V
Acetyl–D–glucosamine	+	–	–	–	+	–	+	+	+	+
Soluble starch	–	–	V	V	V	–	–	V	–	–
Glycerol	+D	+	+D	+	+	+	+D	+	V	+
Erythritol	–	–	–	V	+D	–	–	V	–	–
Glucitol	–	V	V	+D	V	V	–	+	–D	+
Mannitol	–	V	V	+	+D	V	–	+	–	+
Inositol	–	–	–	–	–	–	–	–	–	–
DL–Lactate	+	+D	+	+	–D	V	+	V	V	V
D–Gluconate	–	–	–	V	V	+D	–	V	–	–D
D–Glucuronate	–	–	–	–	–	–	–	–	–	–
2–Keto–D–gluconate	V	–	–	V	+	V	–	V	–D	+
Nitrate	–	–	–	+V	–	+	–	+	–	–
Fermentation of glucose	+	V	+	+	+D	V	+	+D	–D	+
Ethylamine (N)	+	+	+	+V	+	+	+	+	+	+
Vitamin requirement	0	BM	M	0	0B	M	T	BT	OBT	0B
Urease	–	–	–	–	–	–	–	–	–	–
Max. growth T (°C)	<42	V	>37	<37	<37	V	<40	<40	V	<42
Cycloheximide (100ppm)	–	+	+	–D	–	–	–D	+	–D	V

+, positive; –, negative; W, weak response; D, delayed positive (7 days or more); V, variable results; ?, no data known; 0, no vitamins required; B, biotin required; T, thiamine required; P, pantothenate required; M, more or other vitamins required.

Table 3d
Characteristics of Individual Yeast Species Inhabiting Sour Foods

Yeast species (nr)	*Pichia pijperi*	*Pichia subpelliculosa*	*Rhodotorula minuta*	*Rhodotorula mucilaginosa*	*Saccharomyces barnettii*	*Saccharomyces cerevisiae*	*Saccharomyces dairenensis*	*Saccharomyces exiguus*	*Saccharomyces rosinii*	*Saccharomyces spencerorum*
Budding cells	+	+	+	+	+	+	+	+	+	+
True mycelium	–W	V	–	–W	–	–	–	–	–	–
Fragmenting	–	–	–	–	–	–	–	–	–	–
Pellicle	+	V	–	–	–	–	–	–	–	–
D–Glucose	+	+	+	+	+	+	+	+	+	+
D–Galactose	–	V	V	V	+D	V	+	+	+	+
L–Sorbose	+	–	V	V	–	–	–	–	–	–
D–Ribose	–	V	V	+	–	–	–	–	–	–
D–Xylose	+	V	+D	+	–	–	–	–	–	–
L–Arabinose	–	V	V	V	–	–	–	–	–	–
Rhamnose	–	–	–	V	–	–	–	–	–	–
α–Methylglucoside	–	+	–	V	–	V	–	–	–	–
Sucrose	–	+	V	+	+D	V	–	+	–	+D
Maltose	–	+	–	V	–	V	–	–	–	–
Trehalose	–	+	V	+	+	V	–D	+	–	V
Cellobiose	+	V	V	V	–	–	–	–	–	–
Melibiose	–	–	–	–	–	V	–	–	–	–
Lactose	–	–	V	–	–	–	–	–	–	–
Raffinose	–	+	V	+	+	V	–	+	–	–D
Melezitose	–	V	V	V	–	V	–	–	–	–
D–Glucosamine	–	–	–	–	–	–	–	–	–	–
Acetyl–D–glucosamine	–	–	+	–	–	–	–	–	–	–
Soluble starch	–	V	–	–	–	V	–	–	–	–
Glycerol	+	+	+	V	–	V	–	–	–	+D
Erythritol	–	+	–	–	–	–	–	–	–	–
Glucitol	+	+	V	V	–	–D	–	–	–	–
Mannitol	+D	+	V	V	–	–D	–	–	–	–
Inositol	–	–	–	–	–	–	–	–	–	–
DL–Lactate	+	V	V	V	+D	V	–	–	–	–D
D–Gluconate	–	–D	+D	V	–	–	–	–	–	–
D–Glucuronate	–	–	V	–	–	–	–	–	–	–
2–Keto–D–gluconate	–	–D	V	V	–	–	–	–	–	–
Nitrate	–	+	–	V	–	–	–	–	–	–
Fermentation of glucose	+	+	–	–	+	+	+	+	+	+
Ethylamine (N)	+	+	V	V	–	–	–	–	–	+
Vitamin requirement	M	0BT	V	T	B	M	M	0B	M	T
Urease	–	–	+	+	–	–	–	–	–	–
Max. growth T (°C)	<37	<40	V	<40	<30	V	V	<37	<30	<37
Cycloheximide (100ppm)	–	–	V	V	V	–	V	+D	+	–

+, positive; -, negative; W, weak response; D, delayed positive (7 days or more); V, variable results; ?, no data known; 0, no vitamins required; B, biotin required; T, thiamine required; P, pantothenate required; M, more or other vitamins required.

Table 3e
Characteristics of Individual Yeast Species Inhabiting Sour Foods

Yeast species (nr)	*Saccharomyces unisporus*	*Saccharomyces fibuligera*	*Saccharomycopsis selenospora*	*Stephanoascus ciferrii*	*Torulaspora delbrueckii*	*Trichosporon gracile*	*Zygosaccharomyces bailii*	*Zygosaccharomyces bisporus*	*Zygosaccharomyces mrakii*	*Zygosaccharomyces rouxii*
Budding cells	+	+	+	V	+	+	+	+	+	+
True mycelium	–	+	+	V	–W	+	–W	–	–W	–W
Fragmenting	–	–	–	–	–	+	–	–	–	–
Pellicle	–	V	–	+	–	+	–	–	–	–
D–Glucose	+	+	+	+	+	+	+	+	+	+
D–Galactose	+	–	+	+	V	–	V	V	+	V
L–Sorbose	–	–	–	+	V	V	V	V	–	–
D–Ribose	–	V	–D	V	–	V	–	–	–	–
D–Xylose	–	–	+D	+	V	+	–	–D	–D	–
L–Arabinose	–	–	+D	+	–	+	–	–	–	–
Rhamnose	–	–	–	+	–	–	–	–	–	–
α–Methylglucoside	–	+	–	V	V	–	–	–	–	–D
Sucrose	–	V	–	+	V	V	V	–	+	–D
Maltose	–	+	–	+	V	V	–	–	–	V
Trehalose	–	+D	–	+	+D	+D	V	–	–	V
Cellobiose	–	+	–	V	–	+	–D	–	–	–
Melibiose	–	–	–	V	V	–	–	–	+	–
Lactose	–	–	–	–	–	V	–	–	–	–
Raffinose	–	V	–	V	V	–	V	–	+	–
Melezitose	–	–D	–	–	V	–	–	–	–	–
D–Glucosamine	–	–	–	V	–	V	–	–	–	–
Acetyl–D–glucosamine	–	–	–	?	–	–	–	–	–	–
Soluble starch	–	+	+D	V	–	V	–	–	–	–
Glycerol	–	+	–	+	V	+D	V	+D	D	V
Erythritol	–	+	–	+	–	–	–	–	–	–
Glucitol	–	V	–	+	V	V	+D	V	+	V
Mannitol	–	V	–	+	V	V	+D	V	+	V
Inositol	–	V	–	+	–	+	–	–	–	–
DL–Lactate	–	V	–	+	V	+	–	–	–	–
D–Gluconate	–	+D	–D	+	V	+	–	–	–	V
D–Glucuronate	–	+	–	+	–D	+	–	–	–	–
2–Keto–D–gluconate	–	V	–	+	V	+D	–D	V	+	V
Nitrate	–	–	–	–	–	–	–	–	–	–
Fermentation of glucose	+	+D	–W	–	+	–	+	+	+	+
Ethylamine (N)	V	V	–	+	V	+	+	+	+	+
Vitamin requirement	M	M	M	BT	0B	T	B	BT	0	0BP
Urease	–	–	–	–	–	+	–	–	–	–
Max. growth T (°C)	<35	<40	<40	V	V	<35	V	<35	<30	V
Cycloheximide (100ppm)	+	+	+D	+	–	+	V	–	+	–

+, positive; –, negative; W, weak response; D, delayed positive (7 days or more); V, variable results; ?, no data known; 0, no vitamins required; B, biotin required; T, thiamine required; P, pantothenate required; M, more or other vitamins required.

3.3. Assimilation of Nitrogen Compounds

Potassium nitrate (40 m*M*) is dissolved in Difco Yeast Carbon Base. The broth is dispensed (2.5 mL) in culture tubes (15 cm, 16 mm width) and is sterilized at 120°C for 20 min. Ethylamine hydrochloride should not be sterilized in the presence of glucose. A separately sterilized concentrated solution is added aseptically to culture tubes with 2.5 mL sterile Yeast Carbon Base, to a concentration of 40 m*M*. The culture tubes are inoculated with a drop of a young culture in Yeast Carbon Base with 40 m*M* ammonium chloride (separately sterilized). For comparison a culture tube with Yeast Carbon Base without nitrogen source is inoculated. All tubes are incubated at 25°C in a rotary shaker up to two weeks and are inspected for growth daily. Positive growth responses should be confirmed by transfer of a loopful of the culture to a second culture tube with the same growth medium. This should also show growth.

3.4. Fermentation of Glucose

Several yeasts present in foods are able to carry out an alcoholic fermentation. This appears from the production of gas, i.e., carbon dioxide, in Durham tubes. The latter are test tubes with a small inverted tube inserted to collect any gas that may be produced. These tubes contain 10 mL of 2% glucose and 1% yeast extract. They are inoculated with a loopful after sterilization for 20 min at 120°C. The tubes are incubated at 25°C until gas is visible in the insert, or up to 28 d if no gas is produced. The tubes are shaken at intervals of some days.

3.5. Additional Characteristics

3.5.1. Urease

In a 10 m*M* potassium phosphate buffer pH 6.0 phenol red is dissolved (20–50 mg per liter). This solution can be stored indefinitely in the dark at room temperature. Aliquots of 0.5 mL are dispensed in test tubes. Immediately before use a freshly prepared concentrated urea solution is added to a final concentration of 20 g per liter. A loopful of a 1 or 2 d old slant culture is or added to the solution. The test tubes are incubated at 37°C irrespective of the yeast's optimum growth temperature. A dark red color appearing within 5 h demonstrates a pH rise due to hydrolysis of urea to ammonia and carbon dioxide. Comparison with an uninoculated blank is recommended. The reaction is characteristic of basidiomycetous yeasts (in this study the genera *Rhodotorula* and *Trichosporon*). Almost all ascomycetous yeasts are urease-negative.

3.5.2. Maximum Growth Temperature

Slants of appropriate growth media (e.g., malt extract agar, Difco YM agar) are inoculated and incubated at constant temperature, preferably in a thermostated water bath. The slants are inspected for growth after 1, 2, or 3 d.

3.5.3. Tolerance of 1% Acetic Acid

This test is only used to discriminate *Zygosaccharomyces* spp. It is carried out by streaking a young preculture (the same as used in the assimilation tests) on agar plates of the following composition: 10% glucose, 1% tryptone, 1% yeast extract, 2% agar is sterilized for 20 min at 120°C and cooled down to approx 45–50°C. Glacial acetic acid (1 mL per 100 mL) is then added, quickly mixed and the agar is poured in Petri dishes.

3.5.4. Resistance to 100 ppm Cycloheximide

This test is included in the ID 32 C test system for assimilation of carbon compounds.

3.5.5. Assimilation of Starch and Methanol

Merck soluble starch is dissolved (5 g per liter) in Difco Yeast Nitrogen Base. Aliquots of 2.5 mL are dispensed in culture tubes (15 cm, 16 mm width) and sterilized for 20 min at 120°C, immediately before inoculation. A concentrated solution of methanol in sterile water is added to culture tubes containing 2.5 mL sterile Difco Yeast Nitrogen Base. The final methanol concentration should not exceed 5 g per liter. Inoculation and incubation are as described above (assimilation of nitrogen compounds). The test for methanol assimilation is only required for confirmation in case any *Candida* or *Pichia* species listed in **Table 1** is identified. Of these, only *Candida boidinii* assimilates methanol.

3.5.6. Vitamin Requirement

Two procedures are recommended. A 10-fold concentration of Bacto Vitamin-free Yeast base is prepared by dissolving 16.7 grams per 100 mL. This concentrated broth should be filter-sterilized. Aliquots of 0.25 mL are added to culture tubes containing 2.25 mL sterile water. Alternatively, the vitamin-free medium can be prepared (for the composition see Yarrow, 1998, page 99 in ref. 4). If ammonium chloride and glucose are kept separately, the growth medium can safely be sterilized at 120°C without browning and with less risk of airborne infections than during filter sterilization. Add concentrated sterile vitamin solutions after sterilization, at final concentrations: biotin (20 µg per liter) and/or thiamine (400 µg per liter), Ca-pantothenate (2 mg per litre) or myo-inositol (20 mg per liter). More complex vitamin requirements are not specified in this study. For inoculation and incubation see above. Results should be confirmed by transfer of a loopful to a second culture tube with medium of the same composition. This should also show growth.

4. Yeast Species Treated

This chapter deals with yeast species which according to refs. **2**, **3**, and **4** have been isolated from foods and fodders that underwent a lactic acid fermentation (**Table 1**). However, products derived from olives (alpechin, olive brine) are very rich in yeasts not found elsewhere in commodities. These species were not included. Neither were yeast species characteristic of fruit juices and alcoholic beverages.

5. Identification Key

A dichotomous identification key to the treated species is shown in **Table 2**. In some cases the key leads to more than one species name. If so, the data should be compared with those listed in **Table 3** in order to find the right name. Some yeast species show variability for most of the characteristics studied (e.g., *Torulaspora delbrueckii*, *Candida sake*, *Saccharomyces cerevisia*e and *Debaryomyces hansenii*) and hence had to be named more than once. Species showing delayed (D) or weak (W) growth responses for a particular character were treated as both positive (+) and negative (-) in order to facilitate rapid identification.

6. Characteristics of Individual Yeast Species

In **Table 3** characteristics of the treated yeast species, observed in this study, are listed. Some more salient characteristics were mentioned in **Table 1**.

References

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