Comparison study between CHROMagar™ Candida and CHROMagar™ Candida Plus media versus Dichloran Rose Bengale Chloramphenicol and Glucose and Chloramphenicol agar media

CONFIDENTIAL

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Table of contents

1. Introduction 3
2. Materials and methods 3
   2.1. Materials 3
      2.1.1. Devices 3
      2.1.2. Culture media 3
      2.1.3. Strains 3
         2.1.3.1. Mold strains 3
         2.1.3.1. Yeast strains 4
   2.2. Methods 5
      2.2.1. Mold and yeast study protocol 5
3. Results 6
   3.1. CHROMagar™ Candida medium 6
      3.1.1. Raw results with mold strains 6
      3.1.2. Raw results with yeast strains 7
   3.2. CHROMagar™ Candida Plus medium 9
      3.2.1. Raw results with mold strains 9
      3.2.2. Raw results with yeast strains 11
   3.3. Interpretation 13
      3.3.1. CHROMagar™ Candida medium 13
      3.3.2. CHROMagar™ Candida Plus medium 13
      3.3.3. Supplementary tests with Glucose + Chloramphenicol agar medium 14
4. Conclusion 14

Appendix A: Pictures of molds
Appendix B: Pictures of yeasts
Appendix C: Supplementary tests Glucose + Chloramphenicol agar medium versus CHROMagar™ Candida
Appendix D: Supplementary tests Glucose + Chloramphenicol agar medium versus CHROMagar™ Candida Plus

1. Introduction

The aim of this study is to verify the analytical performance of the CHROMagar™ Candida media and CHROMagar™ Candida Plus media versus the Dichloran Rose Bengale Chloramphenicol agar medium for the enumeration of yeast and mold strains at different incubation times.
In order to compare the performances of the three-culture media, twenty-seven molds and twenty-three yeasts were tested.

2. Materials and methods

2.1. Materials

2.1.1. Devices

The devices used in this study are the ones usually encountered in a laboratory. All tests were carried out in accordance with good laboratory practices.

2.1.2. Culture media

The culture media used were as follows:

- Sabouraud Dextrose Broth - BIOKAR – reference: BK026HA (in-house preparation)
- Peptone Salt Broth – BioMérieux – reference: AEB111499
- Dichloran Rose Bengale Chloramphenicol (DRBC) – BIOKAR – reference: BK198HA (in-house preparation)
- CHROMagar™ Candida medium - CHROMagar™ - reference: CA222
- CHROMagar™ Candida Plus medium - CHROMagar™ - reference: CA242
- Glucose + chloramphenicol – BIOKAR – reference: BK0007HA (in-house preparation)

2.1.3. Strains

2.1.3.1. Mold strains

Twenty-seven molds were tested in the study. The majority of them are of wild origin and representative of the different categories of food encountered or of the environment.

The list is shown in table 1.

Table 1 : mold strains

#	Strain	Origin	Code
1	*Aspergillus niger*	Collection strain – ATCC 6275	ASP.1.1
2	*Aspergillus brasiliensis*	Collection strain – ATCC 16404	ASP.1.2
3	*Aspergillus versicolor*	Collection strain – ATCC 11730	ASP.2.1
4	*Eurotium herbariorum*	Pet food	DNGZ69
5	*Penicillium rubrum*	Food coloring	DNLX29
6	*Penicillium spp.*	Food	DQDT96
7	*Phaeosphaeria avenaria*	Pet food	DQZL88
8	*Fusarium fujikuroi*	Water	FZGZ89
9	*Penicillium glandicola*	Environment food powder	GBHY87
10	*Penicillium viridicatum*	Dehydrated shallot	GBJQ26
11	*Rhizomucor pusillus*	Lemon balm	GBXP24
12	*Talaromyces atroroseus*	Dried date	GDDU72
13	*Aspergillus foetidus*	Thyme leaf	GDFD73
14	*Talaromyces amestolkiae*	Pineapple chunks	GFEQ48
15	*Penicillium speluncae*	Environment food powder	GFFK87
16	*Cladosporium colombiae*	Flour	GHQX36
17	*Boeremia exigua var. exigua*	Infusion	GJXY77
18	*Talaromyces islandicus*	Organic flour	GKQU22
19	*Aspergillus melleus*	Thyme leaf	GLEW92
20	*Aspergillus costaricensis*	Food supplement	GLHZ64
21	*Didymella glomerata*	Organic thyme	GLRU94
22	*Sarocladium strictum*	Flour	GMCY72
23	*Cladosporium cladosporioides*	Flour	GMRU52
24	*Mucor circinelloides*	Dried verbena	GMZX64
25	*Xenoacremonium falcatum*	Coconut	GNMF62
26	*Coprinus domesticus*	Water	GRFZ54
27	*Trichoderma virens*	DSM 1963	HJUC32

2.1.3.1. Yeast strains

Twenty-three yeasts were tested in the study. The majority of them are of wild origin and representative of the different categories of food encountered or of the environment.

The list is shown in table 2.

Table 2: yeast strains

#	Strain	Origin	Code
28	*Rhodotorula mucilaginosa*	Food	FSUU25
29	*Rhodotorula mucilaginosa*	Dried artichoke leaf	FVYW49
30	*Rhodotorula mucilaginosa*	Rinse water vegetables	FWXA29
31	*Rhodotorula minuta*	Food	FSMR25
32	*Candida albicans*	Food	CAN 1.1
33	*Candida spherica 1*	Food	FRRT96
34	*Zygosaccharomyces rouxii*	Collection strain – DSM 2531	HFYF47
35	*Zygosaccharomyces bailii*	Food	DRDL94
36	*Candida tropicalis*	Collection strain – DSM 1346	HGSC29
37	*Candida colliculosa*	Food	DMTL37
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62
39	*Filobasidium oeirense*	Dietary supplement	EAQZ59
40	*Sporidiobolus metaroseus*	Leaf thyme	EBJX53
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28
42	*Rhodosporidiobolus colostri*	Sugar	EDKJ77
43	*Candida sake*	Pineapple chunks	EETR26
44	*Holtermanniella takashimae*	Flour	EFKN77
45	*Zygosaccharomyces mellis*	Honey	EHTT27
46	*Apiotrichum montevideense*	Dried date	FVSJ97
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87
48	*Naganishia albida*	Rinse water vegetables	GLWF74
49	*Geotrichum candidum*	Collection strain – DSM 1240	HHDC88
50	*Candida parapsilosis*	Dairy environment	GVNH48

2.2. Methods

2.2.1. Mold and yeast study protocol

Fifty pure strains were inoculated in 9 mL of Sabouraud Dextrose Broth and incubated for 5 days at 25±1°C.

Dilutions were carried out in peptone salt 9 mL and enumerated by surface spreading inoculation with:

- Dichloran Rose Bengale Chloramphenicol (DRBC),
- CHROMagar™ Candida Plus agar medium,
- CHROMagar™ Candida agar medium.

DRBC plates were incubated 5 days at 25±1°C before reading.

CHROMagar™ Candida Plus agar and CHROMagar™ Candida agar media were incubated at 25±1°C and enumerated at three different time points: 48 hours, 72 hours and 5 days.

The dilutions offering an interpretable result were chosen for each strain. For seventeen strains, no growth was observed with DRBC medium on the first dilution inoculated. In this case, a second test was carried out and the results appear in the tables following the initial test.

3. Results

3.1. CHROMagar™ Candida medium

3.1.1. Raw results with mold strains

The raw results are given in table 1.

Table 1: raw results with mold strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida					
							Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C	
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
1	*Aspergillus niger*	Collection strain	ASP.1.1	-2	>150	3 700	>150	19 000	>150	19 000	>150	19 000
				-3	34		19		19		19	
				-4	7		1		1		1	
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	-2	0	<100	1	100	1	100	1	100
				-3	0		0		0		0	
				-4	0		0		0		0	
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	P	0	<1	4	4	6	6	6	6
				-1	0		0		0		0	
				-2	0		0		0		0	
3	*Aspergillus versicolor*	Collection strain	ASP.2.1	-2	>150	49 000	>150	38 000	>150	43 000	>150	44 000
				-3	51		40		45		45	
				-4	3		2		2		4	
4	*Eurotium herbariorum*	Pet food	DNGZ69	-1	0	<10	0	<10	0	<10	26	250
				-2	0		0		0		1	
				-3	0		0		0		0	
4	*Eurotium herbariorum*	Pet food	DNGZ69	P	3	3	0	<1	1	1	2	2
				-1	0		0		0		0	
				-2	0		0		0		0	
5	*Penicillium rubrum*	Food coloring	DNLX29	-2	>150	150 000	>150	120 000	>150	120 000	>150	130 000
				-3	153		>150		>150		>150	
				-4	11		12		12		13	
6	*Penicillium spp.*	Food	DQDT96	-3	>150	170 000	>150	220 000	>150	230 000	>150	230 000
				-4	17		22		22		22	
				-5	0		2		3		4	
7	*Phaeosphaeria avenaria*	Pet food	DQZL88	-3	61	64 000	34	35 000	35	36000	35	36 000
				-4	10		5		5		5	
				-5	0		1		1		1	
8	*Fusarium fujikuroi*	Water	FZGZ89	-4	0	<10 000	0	<10000	16	170000	17	190 000
				-5	0		0		3		4	
				-6	0		0		0		0	
				-7	0		0		0		0	
8	*Fusarium fujikuroi*	Water	FZGZ89	P	88	90	>150	>150 000	>150	>150 000	>150	>150 000
				-1	8		>150		>150		>150	
				-2	0		>150		>150		>150	
				-3	0		>150		>150		>150	
9	*Penicillium glandicola*	Environment food powder	GBHY87	-4	4	40 000	4	40 000	4	40000	5	50 000
				-5	0		1		1		1	
				-6	0		0		0		0	
				-7	0		0		0		0	
10	*Penicillium viridicatum*	Dehydrated shallot	GBJQ26	-4	5	50 000	7	70 000	7	70000	8	80 000
				-5	0		0		0		0	
				-6	0		0		0		0	
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	-3	0	<1000	0	<1000	35	33000	43	44 000
				-4	0		0		1		6	
				-5	0		0		0		0	
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	P	0	<1	2	2	>150	5 800	>150	5 900
				-1	0		0		>150		>150	
				-2	0		0		53		53	
				-3	0		0		11		12	
12	*Talaromyces atroroseus*	Dried date	GDDU72	-1	2	20	0	<10	4	40	5	50
				-2	0		0		0		0	
				-3	0		0		0		0	
13	*Aspergillus foetidus*	Thyme leaf	GDFD73	-3	11	10 000	13	13 000	13	13000	13	13 000
				-4	0		1		1		1	
				-5	0		0		0		0	
14	*Talaromyces amestolkiae*	Pineapple chunks	GFEQ48	-2	>150	29 000	>150	17 000	>150	17000	>150	17 000
				-3	30		17		17		17	
				-4	1		1		1		1	

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida					
							Surface spreading -		Surface spreading -		Surface spreading -	
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
15	*Penicillium speluncae*	Environment food powder	GFFK87	-4	1	10 000	1	10 000	1	10000	1	10 000
				-5	0		0		0		0	
				-6	0		0		0		0	
16	*Cladosporium colombiae*	Flour	GHQX36	-1	0	<10	0	<10	1	10	1	10
				-2	0		0		0		0	
				-3	0		0		0		0	
16	*Cladosporium colombiae*	Flour	GHQX36	P	0	<1	1	1	1	1	1	1
				-1	0		0		0		0	
				-2	0		0		0		0	
				-3	0		0		0		0	
17	*Boeremia exigua var. exigua*	Infusion	GJXY77	p	>150	220	29	35	35	40	36	40
				-1	22		6		8		8	
				-2	2		1		1		1	
18	*Talaromyces islandicus*	Organic flour	GKQU22	-3	3	3 000	4	4 000	5	5000	6	6 000
				-4	0		0		0		0	
				-5	0		0		0		0	
19	*Aspergillus melleus*	Thyme leaf	GLEW92	p	50	55	37	40	37	40	37	40
				-1	9		7		8		8	
				-2	0		1		1		1	
20	*Aspergillus costaricensis*	Food supplement	GLHZ64	-5	7	700 000	5	500 000	5	500 000	5	500 000
				-6	0		1		1		1	
				-7	0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	-1	0	<10	0	<10	1	10	1	10
				-2	0		0		0		0	
				-3	0		0		0		0	
				-4	0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	P	0	<1	>150	440	>150	470	>150	470
				-1	0		44		47		47	
				-2	0		4		4		4	
				-3	0		0		0		0	
22	*Sarocladium strictum*	Flour	GMCY72	p	>150	10 000	>150	10 000	>150	11 000	>150	16 000
				-1	>150		>150		>150		>150	
				-2	>150		>150		>150		>150	
				-3	10		10		11		16	
23	*Cladosporium cladosporioides*	Flour	GMRU52	-1	0	<10	0	<10	0	<10	7	70
				-2	0		0		0		0	
				-3	0		0		0		0	
23	*Cladosporium cladosporioides*	Flour	GMRU52	P	>150	1 200	>150	1 400	>150	2 100	>150	2 700
				-1	>150		>150		>150		>150	
				-2	12		14		21		30	
				-3	1		0		0		0	
24	*Mucor circinelloides*	Dried verbena	GMZX64	-1	3	30	>150	3 000	>150	5 500	>150	>15 000
				-2	0		30		55		>150	
25	*Xenoacremonium falcatum*	Coconut	GNMF62	-4	1	10 000	0	<10 000	39	400 000	45	540 000
				-5	0		0		5		14	
				-6	0		0		0		0	
				-7	0		0		0		0	
26	*Coprinus domesticus*	Water	GRFZ54	-1	0	<10	>150	1 100	>150	1 400	>150	1 400
				-2	0		11		14		14	
				-3	0		1		1		1	
				-4	0		0		0		0	
26	*Coprinus domesticus*	Water	GRFZ54	P	0	<1	>150	200	>150	1 500	>150	1 500
				-1	0		20		142		143	
				-2	0		2		20		20	
				-3	0		0		2		2	
27	*Trichoderma virens*	DSM 1963	HJUC32	p	19	20	>150	800	>150	800	>150	1 000
				-1	4		>150		>150		>150	
				-2	0		8		8		10	

3.1.2. Raw results with yeast strains

The raw results are given in table 2.

Table 2: raw results with yeast strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida								
							Surface spreading - 48 h at 25°C			Surface spreading - 72 h at 25°C			Surface spreading - 5 days at 25°C		
					CFU/mL	Result	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies
28	*Rhodotorula mucilaginosa*	Food	FSUU25	-3	2	2 000	24	22 000	light pink colonies	30	27 000	pink colonies	30	27 000	pink colonies
				-4	0		0			0			0		
				-5	0		0			0			0		
29	*Rhodotorula mucilaginosa*	Dried artichoke leaf	FVYW49	-2	>150	300 000	>150	350 000	pink colonies	>150	350 000	pink colonies	>150	350 000	pink colonies
				-3	>150		>150			>150			>150		
				-4	30		35			35			35		
30	*Rhodotorula mucilaginosa*	Rinse water vegetebales	FWXA29	-2	>150	720 000	>150	830 000	pink colonies	>150	830 000	pink colonies	>150	830 000	pink colonies
				-3	>150		>150			>150			>150		
				-4	72		83			83			83		
31	*Rhodotorula minuta*	Food	FSMR25	-3	0	<1000	0	<1000	/	0	<1000	/	>150	5 200 000	white colonies
				-4	0		0			0			>150		
				-5	0		0			0			48		
				-6	0		0			0			9		
				-7	0		0			0			0		
31	*Rhodotorula minuta*	Food	FSMR25	P	>150	34 000	>150	120	white colonies	>150	150 000	white colonies	>150	150 000	white colonies
				-1	>150		12			>150			>150		
				-2	>150		1			>150			>150		
				-3	34		0			146			146		
32	*Candida albicans*	Food	CAN 1.1	-5	0	<100 000	>150	28 000 000	blue colonies	>150	28 000 000	blue-green colonies	>150	28 000 000	blue-green colonies
				-6	0		29			29			29		
				-7	0		2			2			2		
32	*Candida albicans*	Food	CAN 1.1	P	>150	>150 000	>150	>150 000	green colonies	>150	>150 000	green colonies	>150	>150 000	green colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
33	*Candida spherica 1*	Food	FRRT96	-4	0	<10 000	33	330 000	pink colonies	36	360 000	purple colonies	36	360 000	purple colonies
				-5	0		3			4			4		
				-6	0		0			0			0		
				-7	0		0			0			0		
33	*Candida spherica 1*	Food	FRRT96	P	>150	35 000	>150	56 000	brown colonies	>150	56 000	brown colonies	>150	56 000	brown colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	35		56			58			58		
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	-5	0	<100 000	3	300 000	small white colonies	36	3 900 000	small white colonies	36	3 900 000	white colonies
				-6	0		0			7			7		
				-7	0		0			0			0		
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	P	>150	1 400	0	<1	/	>150	52 000	white colonies	>150	59 000	white colonies
				-1	>150		0			>150			>150		
				-2	14		0			>150			>150		
				-3	1		0			52			59		
35	*Zygosaccharomyces bailii*	Food	DRDL94	-5	>150	11 000 000	>150	24 000 000	small white colonies	>150	26 000 000	small white colonies	>150	27 000 000	small white colonies
				-6	12		20			22			22		
				-7	0		7			7			8		
36	*Candida tropicalis*	Collection strain	HGSC29	-5	11	1 200 000	41	4 400 000	green colonies	41	4 400 000	green colonies	41	4 400 000	green colonies
				-6	2		8			8			8		
				-7	0		0			0			0		
37	*Candida colliculosa*	Food	DMTL37	-5	0	<100 000	23	2 300 000	pink colonies	33	3 000 000	pink colonies	33	3 000 000	pink colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
37	*Candida colliculosa*	Food	DMTL37	P	>150	>150 000	>150	>150 000	yellow colonies	>150	>150 000	yellow colonies	>150	>150 000	yellow colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	-5	0	<100 000	>150	31 000 000	pink colonies	>150	31 000 000	purple colonies	>150	31 000 000	purple colonies
				-6	0		32			32			32		
				-7	0		2			2			2		
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	P	>150	>150 000	>150	>150 000	green colonies	>150	>150 000	green colonies	>150	>150 000	green colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
39	*Filobasidium oeirense*	Dietary supplement	EAQZ59	-5	2	200 000	4	400 000	small white colonies	4	400 000	white colonies	5	500 000	pink colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
40	*Sporidiobolus metaroseus*	Leaf thyme	EBJX53	p	1	1	0	<1	/	>150	38 000	purple colonies	>150	38 000	purple colonies
				-1	0		0			>150			>150		
				-2	0		0			>150			>150		
				-3	0		0			38			38		
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	-4	0	<10 000	107	1 100 000	small white colonies	109	1 100 000	pink and white colonies	109	1 100 000	pink and white colonies
				-5	0		13			13			13		
				-6	0		0			0			0		
				-7	0		0			0			0		
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	P	>150	8 300	>150	74 000	pink colonies	>150	74 000	pink colonies	>150	74 000	pink colonies
				-1	>150		>150			>150			>150		
				-2	83		>150			>150			>150		
				-3	9		74			74			74		
42	*Rhodosporidiobolus colostri*	Sugar	EDKJ77	-1	140	1 400	>150	1 000	purple colonies	>150	1000	purple colonies	>150	1 000	purple colonies
				-2	10		10			10			10		
				-3	0		0			0			0		
43	*Candida sake*	Pineapple chunks	EETR26	-5	>150	200 000 000	>150	90 000 000	blue colonies	>150	130 000 000	blue colonies	>150	190 000 000	purple colonies
				-6	>150		>150			>150			>150		
				-7	20		9			13			19		
				-8	2		0			0			1		
44	*Holtermanniella takashimae*	Flour	EFKN77	-2	0	<100	0	<100	/	0	<100	/	>150	10 000	pink colonies
				-3	0		0			0			10		
44	*Holtermanniella takashimae*	Flour	EFKN77	P	23	27	0	<1	/	>150	1 400	white colonies	>150	2 200	white colonies
				-1	7		0			>150			>150		
				-2	0		0			14			22		
				-3	0		0			2			2		
45	*Zygosaccharomyces mellis*	Honey	EHTT27	-5	19	2 000 000	0	<100 000	/	0	<100 000	/	0	<100 000	/
				-6	3		0			0			0		
				-7	1		0			0			0		

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida								
							Surface spreading - 48 h at 25°C			Surface spreading - 72 h at 25°C			Surface spreading - 5 days at 25°C		
					CFU/mL	Result	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies
46	*Apiotrichum montevideense*	Dried date	FVSJ97	-5	8	800 000	58	5 700 000	small white colonies	58	5 700 000	white colonies	58	5 700 000	light pink colonies
				-6	0		5			5			5		
				-7	0		0			0			1		
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	-5	0	<100 000	19	1 700 000	small white colonies	19	1 700 000	pink colonies	21	1 900 000	pink colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	P	>150	14 000	>150	56 000	pink colonies	>150	71 000	pink colonies	>150	73 000	pink colonies
				-1	>150		>150			>150			>150		
				-2	131		>150			>150			>150		
				-3	26		56			71			73		
48	*Naganishia albida*	Rinse water vegetebales	GLWF74	-5	9	900 000	30	3 200 000	white colonies	31	3 200 000	pink colonies	31	3 200 000	purple colonies
				-6	5		5			5			5		
				-7	0		0			0			0		
49	*Geotrichum candidum*	Collection strain	HHDC88	-5	1	100 000	9	900 000	white colonies	9	900 000	pink colonies	9	900 000	purple colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
50	*Candida parapsilosis*	Dairy environment	GVNH48	-5	10	1 000 000	27	2 900 000	white colonies	28	3 000 000	pink colonies	30	3 200 000	purple colonies
				-6	1		5			5			5		
				-7	0		1			1			1		

3.2. CHROMagar™ Candida Plus medium

3.2.1. Raw results with mold strains

The raw results are given in table 3.

Table 3: raw results with mold strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida Plus					
							Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C	
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
1	*Aspergillus niger*	Collection strain	ASP.1.1	-2	>150	3 700	>150	16 000	>150	16 000	>150	16 000
				-3	34		16		16		16	
				-4	7		1		1		1	
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	-2	0	<100	0	<100	0	<100	1	100
				-3	0		0		0		0	
				-4	0		0		0		0	
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	P	0	<1	4	4	4	4	4	4
				-1	0		0		0		0	
				-2	0		0		0		0	
3	*Aspergillus versicolor*	Collection strain	ASP.2.1	-2	>150	49 000	>150	53 000	>150	53 000	>150	57 000
				-3	51		58		58		61	
				-4	3		0		2		2	
4	*Eurotium herbariorum*	Pet food	DNGZ69	-1	0	<10	0	<100	1	10	1	10
				-2	0		0		0		0	
				-3	0		0		0		0	
4	*Eurotium herbariorum*	Pet food	DNGZ69	P	3	3	0	<1	1	1	2	2
				-1	0		0		0		0	
				-2	0		0		0		0	
5	*Penicillium rubrum*	Food coloring	DNLX29	-2	>150	150 000	>150	180 000	>150	200 000	>150	200 000
				-3	153		>150		>150		>150	
				-4	11		18		20		20	
6	*Penicillium spp.*	Food	DQDT96	-3	>150	170 000	>150	330 000	>150	340 000	>150	340 000
				-4	17		35		35		35	
				-5	0		1		3		3	
7	*Phaeosphaeria avenaria*	Pet food	DQZL88	-3	61	64 000	58	57 000	58	57 000	58	57 000
				-4	10		5		5		5	
				-5	0		0		0		0	
8	*Fusarium fujikuroi*	Water	FZGZ89	-4	0	<10 000	9	110 000	17	190 000	17	190 000
				-5	0		3		4		4	
				-6	0		0		0		0	
				-7	0		0		0		0	
8	*Fusarium fujikuroi*	Water	FZGZ89	P	88	90	>150	>150 000	>150	>150 000	>150	>150 000
				-1	8		>150		>150		>150	
				-2	0		>150		>150		>150	
				-3	0		>150		>150		>150	
9	*Penicillium glandicola*	Environment food powder	GBHY87	-4	4	40 000	5	50 000	6	60 000	6	60 000
				-5	0		1		2		2	
				-6	0		0		0		0	
				-7	0		0		0		0	
10	*Penicillium viridicatum*	Dehydrated shallot	GBJQ26	-4	5	50 000	2	20 000	4	40 000	4	40 000
				-5	0		0		1		1	
				-6	0		0		0		0	

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida Plus					
							Surface spreading -		Surface spreading -		Surface spreading -	
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	-3	0	<1000	0	<1000	30	27 000	33	34 000
				-4	0		0		0		4	
				-5	0		0		0		0	
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	P	0	<1	3	3	>150	23 000	>150	27 000
				-1	0		0		>150		>150	
				-2	0		0		>150		>150	
				-3	0		0		23		27	
12	*Talaromyces atroroseus*	Dried date	GDDU72	-1	2	20	0	<10	4	40	4	40
				-2	0		0		0		0	
				-3	0		0		0		0	
				-4	0		0		0		0	
				-5	0		0		0		0	
				-6	0		0		0		0	
13	*Aspergillus foetidus*	Thyme leaf	GDFD73	-3	11	10 000	15	15 000	15	15 000	15	15 000*
				-4	0		2		2		2	
				-5	0		0		0		0	
14	*Talaromyces amestolkiae*	Pineapple chunks	GFEQ48	-2	>150	29 000	>150	19 000	>150	19 000	>150	19 000*
				-3	30		17		17		17	
				-4	1		4		4		4	
15	*Penicillium speluncae*	Environment food powder	GFFK87	-4	1	10 000	1	10 000	1	10 000	1	10 000
				-5	0		0		0		0	
				-6	0		0		0		0	
16	*Cladosporium colombiae*	Flour	GHQX36	-1	0	<10	0	<10	0	<10	2	20
				-2	0		0		0		0	
				-3	0		0		0		0	
16	*Cladosporium colombiae*	Flour	GHQX36	P	0	<1	1	1	1	1	1	1
				-1	0		0		0		0	
				-2	0		0		0		0	
				-3	0		0		0		0	
17	*Boeremia exigua var. exigua*	Infusion	GJXY77	p	>150	220	>150	300	>150	360	>150	360
				-1	22		30		36		36	
				-2	2		0		3		4	
18	*Talaromyces islandicus*	Organic flour	GKQU22	-3	3	3 000	0	<1000	1	1 000	1	1 000
				-4	0		0		0		0	
				-5	0		0		0		0	
19	*Aspergillus melleus*	Thyme leaf	GLEW92	p	50	55	102	110	109	120	110	120
				-1	9		19		20		20	
				-2	0		1		1		1	
20	*Aspergillus costaricensis*	Food supplement	GLHZ64	-5	7	700 000	3	300 000	3	300 000	3	300 000
				-6	0		1		1		1	
				-7	0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	-1	0	<10	0	<10	2	20	3	30
				-2	0		0		0		0	
				-3	0		0		0		0	
				-4	0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	P	0	<1	>150	680	>150	700	>150	700
				-1	0		72		74		74	
				-2	0		3		3		3	
				-3	0		0		0		0	
22	*Sarocladium strictum*	Flour	GMCY72	p	>150	10 000	>150	12 000	>150	14 000	>150	18 000
				-1	>150		>150		>150		>150	
				-2	>150		>150		>150		>150	
				-3	10		12		14		18	
23	*Cladosporium cladosporioides*	Flour	GMRU52	-1	0	<10	0	<10	0	<10	18	160
				-2	0		0		0		0	
				-3	0		0		0		0	
				-4	0		0		0		0	
23	*Cladosporium cladosporioides*	Flour	GMRU52	P	>150	1 200	>150	1 400	>150	2 700	>150	3 700
				-1	>150		>150		>150		>150	
				-2	12		14		27		37	
				-3	1		1		2		4	
24	*Mucor circinelloides*	Dried verbena	GMZX64	-1	3	30	>150	4 600	>150	4 600	>150	>15 000
				-2	0		46		46		>150	
25	*Xenoacremonium falcatum*	Coconut	GNMF62	-4	1	10 000	0	<10 000	49	540 000	52	580 000
				-5	0		0		10		12	
				-6	0		0		0		0	
				-7	0		0		0		0	
26	*Coprinus domesticus*	Water	GRFZ54	-1	0	<10	0	<10	>150	1 700	>150	1 700
				-2	0		0		15		15	
				-3	0		0		4		4	
				-4	0		0		0		0	
26	*Coprinus domesticus*	Water	GRFZ54	P	0	<1	>150	450	>150	700	>150	700
				-1	0		45		>150		>150	
				-2	0		5		7		7	
				-3	0		0		1		1	
27	*Trichoderma virens*	DSM 1963	HJUC32	p	19	20	17	20	>150	350	>150	370
				-1	4		5		35		37	
				-2	0		0		3		7	

3.2.2. Raw results with yeast strains

The raw results are given in table 4.

Table 4: raw results with yeast strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida Plus								
							Surface spreading - 48 h at 25°C			Surface spreading - 72 h at 25°C			Surface spreading - 5 days at 25°C		
					CFU/mL	Result	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies
28	*Rhodotorula mucilaginosa*	Food	FSUU25	-3	2	2 000	16	18 000	light pink colonies	21	24 000	pink colonies	21	24 000	pink colonies
				-4	0		4			5			5		
				-5	0		0			0			0		
				-6	0		0			0			0		
				-7	0		0			0			0		
29	*Rhodotorula mucilaginosa*	Dried artichoke leaf	FVYW49	-2	>150	300 000	>150	280 000	pink colonies	>150	280 000	pink colonies	>150	280 000	pink colonies
				-3	>150		>150			>150			>150		
				-4	30		28			28			28		
30	*Rhodotorula mucilaginosa*	Rinse water vegetebales	FWXA29	-2	>150	720 000	>150	720 000	pink colonies	>150	740 000	pink colonies	>150	740 000	pink colonies
				-3	>150		>150			>150			>150		
				-4	72		72			74			74		
31	*Rhodotorula minuta*	Food	FSMR25	-3	0	<1000	0	<1000	/	0	<1000	/	>150	1 000 000	pink colonies
				-4	0		0			0			>150		
				-5	0		0			0			10		
				-6	0		0			0			0		
				-7	0		0			0			0		
31	*Rhodotorula minuta*	Food	FSMR25	P	>150	34 000	>150	1 800	white colonies	>150	>150 000	white colonies	>150	>150 000	pink colonies
				-1	>150		>150			>150			>150		
				-2	>150		20			>150			>150		
				-3	34		0			>150			>150		
32	*Candida albicans*	Food	CAN 1.1	-5	0	<100 000	>150	24 000 000	blue colonies	>150	24 000 000	blue-green colonies	>150	24 000 000	blue-green colonies
				-6	0		22			22			22		
				-7	0		5			5			5		
32	*Candida albicans*	Food	CAN 1.1	P	>150	>150 000	>150	>150 000	blue colonies	>150	>150 000	blue colonies	>150	>150 000	blue colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
33	*Candida spherica 1*	Food	FRRT96	-4	0	<10 000	38	380 000	pink colonies	39	390 000	pink colonies	39	400 000	pink colonies
				-5	0		4			4			5		
				-6	0		0			0			0		
				-7	0		0			0			0		
33	*Candida spherica 1*	Food	FRRT96	P	>150	35 000	>150	58 000	pink colonies	>150	62 000	pink colonies	>150	62 000	pink colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	35		58			62			62		
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	-5	0	<100 000	0	<100 000	/	35	3 600 000	small white colonies	35	4 000 000	small white colonies
				-6	0		0			5			9		
				-7	0		0			0			0		
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	P	>150	1 400	0	<1	/	>150	76 000	white colonies	>150	90 000	white colonies
				-1	>150		0			>150			>150		
				-2	14		0			>150			>150		
				-3	1		0			76			90		
35	*Zygosaccharomyces bailii*	Food	DRDL94	-5	>150	11 000 000	>150	27 000 000	small white colonies	>150	35 000 000	small white colonies	>150	36 000 000	small white colonies
				-6	12		29			35			36		
				-7	0		1			3			3		
36	*Candida tropicalis*	Collection strain	HGSC29	-5	11	1 200 000	61	6 100 000	pink colonies	61	6 100 000	purple colonies	61	6 100 000	purple colonies
				-6	2		6			6			6		
				-7	0		0			0			0		
37	*Candida colliculosa*	Food	DMTL37	-5	0	<100 000	12	1 600 000	pink colonies	15	1 900 000	pink colonies	18	2 200 000	pink colonies
				-6	0		6			6			6		
				-7	0		0			0			0		
37	*Candida colliculosa*	Food	DMTL37	P	>150	>150 000	>150	>150 000	pink colonies	>150	>150 000	pink colonies	>150	>150 000	pink colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	-5	0	<100 000	>150	35 000 000	pink colonies	>150	36 000 000	purple colonies	>150	36 000 000	purple colonies
				-6	0		37			38			38		
				-7	0		2			2			2		
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	P	>150	>150 000	>150	>150 000	pink colonies	>150	>150 000	purple colonies	>150	>150 000	purple colonies
				-1	>150		>150			>150			>150		
				-2	>150		>150			>150			>150		
				-3	>150		>150			>150			>150		
39	*Filobasidium oeirense*	Dietary supplement	EAQZ59	-5	2	200 000	0	<100 000	/	3	300 000	small white colonies	4	400 000	pink colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
40	*Sporidiobolus metaroseus*	Leaf thyme	EBJX53	p	1	1	0	<1	/	>150	42 000	purple colonies	>150	42 000	purple colonies
				-1	0		0			>150			>150		
				-2	0		0			>150			>150		
				-3	0		0			42			42		
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	-4	0	<10 000	106	1 010 000	blue colonies	106	1 010 000	blue colonies	106	1 010 000	blue colonies
				-5	0		6			6			6		
				-6	0		1			1			1		
				-7	0		0			0			0		
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	P	>150	8 300	>150	67 000	blue colonies	>150	67 000	blue colonies	>150	67 000	blue colonies
				-1	>150		>150			>150			>150		
				-2	83		>150			>150			>150		
				-3	9		67			67			67		

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida Plus								
							Surface spreading - 48 h at 25°C			Surface spreading - 72 h at 25°C			Surface spreading - 5 days at 25°C		
					CFU/mL	Result	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies	CFU/mL	Result	Colour of the colonies
42	*Rhodosporidiobolus colostri*	Sugar	EDKJ77	-1	140	1 400	>150	1 800	purple colonies	>150	1 800	purple colonies	>150	1 800	purple colonies
				-2	10		18			18			18		
				-3	0		0			0			0		
43	*Candida sake*	Pineapple chunks	EETR26	-5	>150	200 000 000	>150	120 000 000	blue colonies	>150	190 000 000	blue colonies	>150	240 000 000	blue colonies
				-6	>150		>150			>150			>150		
				-7	20		12			19			24		
				-8	2		1			1			2		
44	*Holtermanniella takashimae*	Flour	EFKN77	-2	0	<100	0	<100	/	>150	11 000	small white colonies	>150	11 000	pink colonies
				-3	0		0			11			11		
44	*Holtermanniella takashimae*	Flour	EFKN77	P	23	27	0	<1	/	>150	2 200	white colonies	>150	2 300	white colonies
				-1	7		0			>150			>150		
				-2	0		0			20			21		
				-3	0		0			4			4		
45	*Zygosaccharomyces mellis*	Honey	EHTT27	-5	19	2 000 000	0	<100 000	/	0	<100 000	/	0	<100 000	/
				-6	3		0			0			0		
				-7	1		0			0			0		
46	*Apiotrichum montevideense*	Dried date	FVSJ97	-5	8	800 000	42	4 400 000	small white colonies	44	4 600 000	white colonies	44	4 600 000	light pink colonies
				-6	0		6			7			7		
				-7	0		1			1			1		
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	-5	0	<100 000	12	1 500 000	small white colonies	14	1 800 000	pink colonies	15	2 000 000	pink colonies
				-6	0		5			6			7		
				-7	0		0			0			0		
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	P	>150	14 000	>150	68 000	white colonies	>150	86 000	white/pink colonies	>150	86 000	white/pink colonies
				-1	>150		>150			>150			>150		
				-2	131		>150			>150			>150		
				-3	26		68			86			86		
48	*Naganishia albida*	Rinse water vegetebales	GLWF74	-5	9	900 000	22	2 300 000	blue colonies	25	2 500 000	blue colonies	27	2 700 000	purple colonies
				-6	5		3			3			3		
				-7	0		0			1			1		
49	*Geotrichum candidum*	Collection strain	HHDC88	-5	1	100 000	4	400 000	blue green colonies	4	400 000	blue green colonies	4	400 000	blue colonies
				-6	0		0			0			0		
				-7	0		0			0			0		
50	*Candida parapsilosis*	Dairy environment	GVNH48	-5	10	1 000 000	49	4 600 000	white colonies	54	5 100 000	pink colonies	54	5 100 000	purple colonies
				-6	1		2			2			2		
				-7	0		0			0			0		

3.3. Interpretation

3.3.1. CHROMagar™ Candida medium

The distribution of the positive results obtained with the CHROMagar™ Candida medium versus the DRBC medium of the ISO 21527 standard, is expressed for each incubation time in table 5. For seventeen strains that were reanalyzed, it was the result of the retest that was taken into account.

Table 5: distribution of positive results using CHROMagar™ Candida medium

#	Total	%	ISO 21527 (DRBC)		CHROMagar™ Candida - 48h		CHROMagar™ Candida - 72h		CHROMagar™ Candida - 5 days	
			Total +ve	%	Total +ve	%	Total +ve	%	Total +ve	%
Mold	27	54%	22	81%	25	93%	27	100%	27	100%
Yeast	23	46%	23	100%	20	87%	22	96%	22	96%
Mold & Yeast	**50**	**100%**	**45**	**90%**	**45**	**90%**	**49**	**98%**	**49**	**98%**

The ISO 21527 method using the DRBC agar medium allowed the growth of 22 mold strains out of the 27 tested (i.e., 81%) and 23 yeast strains out of the 23 tested (i.e.,100%). A total of 45 strains of yeasts and molds were detected out of the 50 strains tested by the ISO 21527 method (i.e., 90%).

The CHROMagar™ Candida medium allowed the growth of 25 mold strains out of the 27 tested after 48 hours of incubation (i.e., 93%). This percentage increases to 100% (27 strains) after 72 hours and 5 days of incubation.
The CHROMagar™ Candida medium allowed the growth of 20 yeast strains out of the 23 tested after 48 hours of incubation (i.e., 87%). This percentage increases to 96% (22 strains) after 72 hours and 5 days of incubation.
On a panel of 50 strains of yeasts and molds tested, the CHROMagar™ Candida medium allowed a recovery of 90% after 48 hours of incubation and 98% after 72 hours and 5 days of incubation at 25±1°C.

3.3.2. CHROMagar™ Candida Plus medium

The distribution of the positive results obtained with the CHROMagar™ Candida Plus medium versus the DRBC medium of the ISO 21527 standard, is expressed for each incubation time in table 6. For strains that were reanalyzed, it was the result of the retest that was taken into account.

Table 6: distribution of positive results using CHROMagar™ Candida Plus medium

#	Total	%	ISO 21527 (DRBC)		CHROMagar™ Candida Plus - 48h		CHROMagar™ Candida Plus - 72h		CHROMagar™ Candida Plus - 5 days	
			Total +ve	%	Total +ve	%	Total +ve	%	Total +ve	%
Mold	27	54%	22	81%	23	85%	27	100%	27	100%
Yeast	23	46%	23	100%	19	83%	22	96%	22	96%
Mold & Yeast	**50**	**100%**	**45**	**90%**	**42**	**84%**	**49**	**98%**	**49**	**98%**

The ISO 21527 method using the DRBC agar medium allowed the growth of 22 mold strains out of the 27 tested (i.e., 81%) and 23 yeast strains out of the 23 tested (i.e.,100%). A total of 45 strains of yeasts and molds were detected out of the 50 strains tested by the ISO 21527 method (i.e., 90%).

The CHROMagar™ Candida Plus medium allowed the growth of 23 mold strains out of the 27 tested after 48 hours of incubation (i.e., 85%). This percentage increases to 100% (27 strains) after 72 hours and 5 days of incubation.
The CHROMagar™ Candida Plus medium allowed the growth of 19 yeast strains out of the 23 tested after 48 hours of incubation (i.e., 83%). This percentage increases to 96% (22 strains) after 72 hours and 5 days of incubation.
On a panel of 50 strains of yeasts and molds tested, the CHROMagar™ Candida Plus medium allowed a recovery of 84% after 48 hours of incubation and 98% after 72 hours and 5 days of incubation at 25±1°C.

3.3.3. Supplementary tests with Glucose + Chloramphenicol agar medium

In this study, a number of strains were found to have undercounts with ISO DRBC medium compared to CHROMagar™ Candida media. Additional tests were carried out using the 4-culture media DRBC, Glucose + chloramphenicol, CHROMagar™ Candida and CHROMagar™ Candida Plus. The results are presented in Appendices C and D. Better recovery of the different cells tested was observed with Glucose + Chloramphenicol agar medium compared to DRBC agar medium and equivalent to the two CHROMagar™ media.

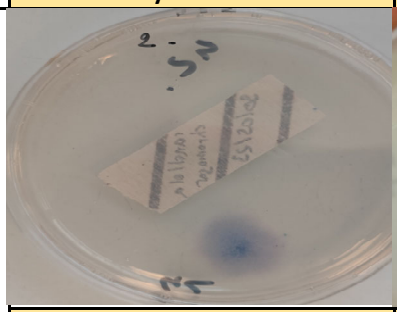
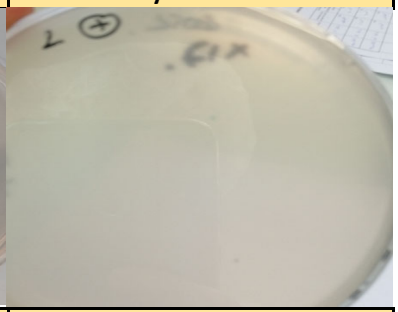
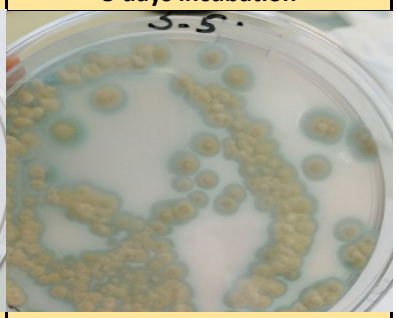
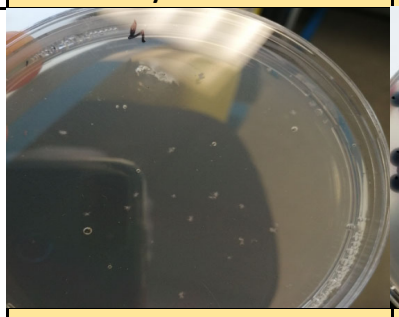
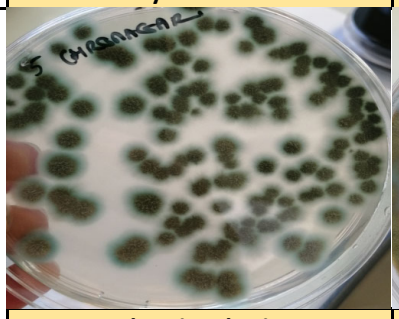
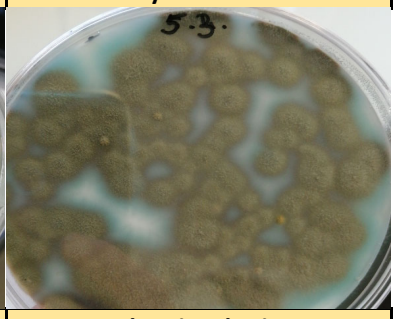
4. Conclusion

On 50 strains tested (27 molds and 23 yeasts), the recovery rates of CHROMagar™ Candida and CHROMagar™ Candida Plus media are respectively 90% and 84% from 48 hours of incubation while that of DRBC used in the ISO standard 21527 (5 days incubation) is 90%. These rates increase to 98% after 72 hours and 5 days of incubation. The use of CHROMagar™ Candida media can reduce the incubation time to 72 hours compared to 5 days with the application of the ISO 21527 method. CHROMagar™ Candida media allow easy distinction of yeast and mold colonies on the same Petri dish thanks to its chromogenic formula.

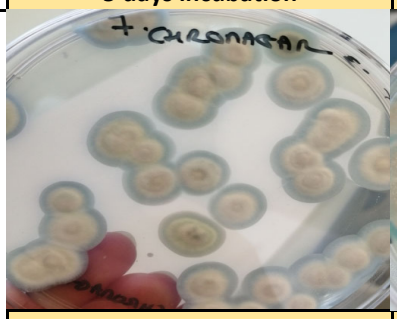
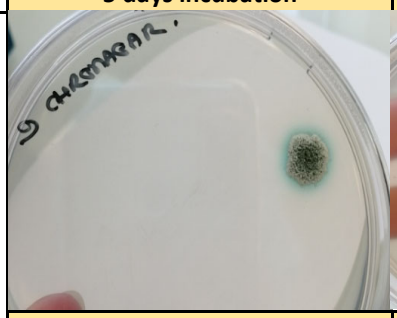
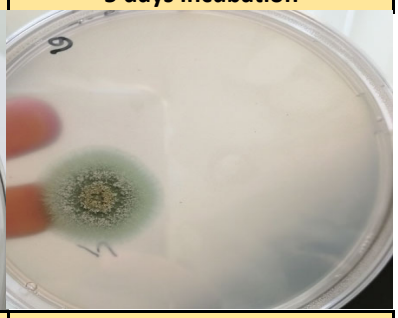
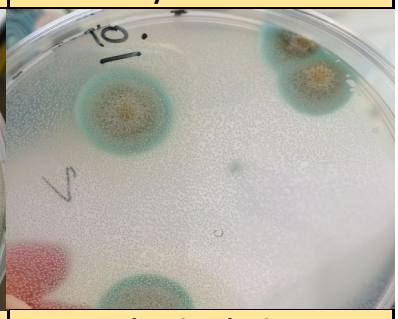
Le Lion d'Angers, January 11, 2024
Guillaume MESNARD,
Deputy Manager of Validation and R&D department



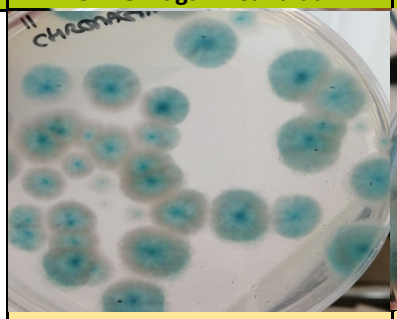
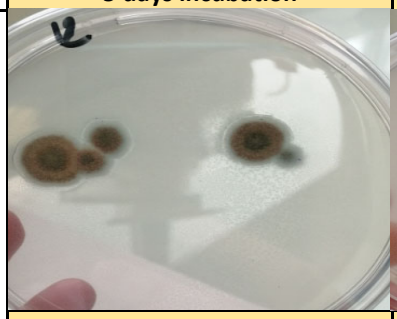
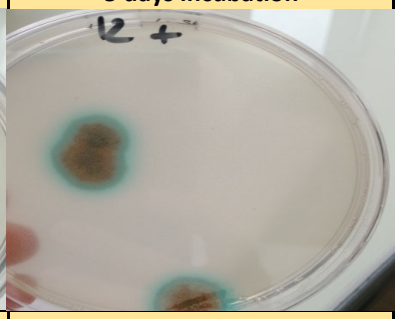
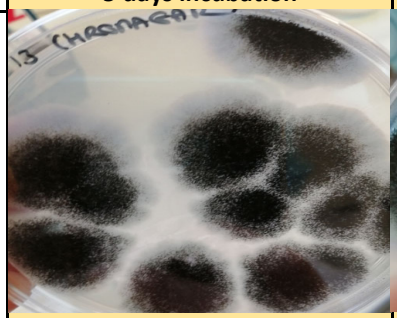
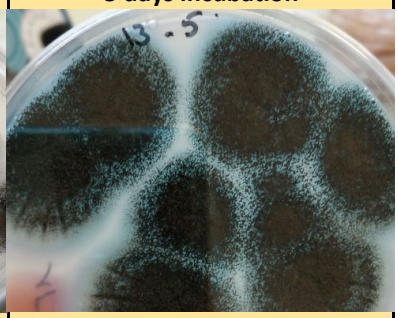
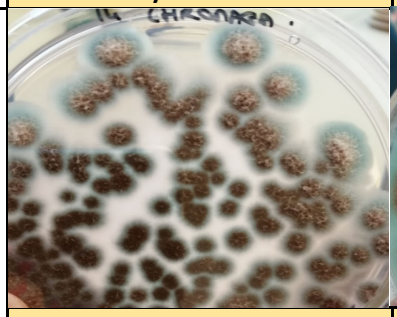
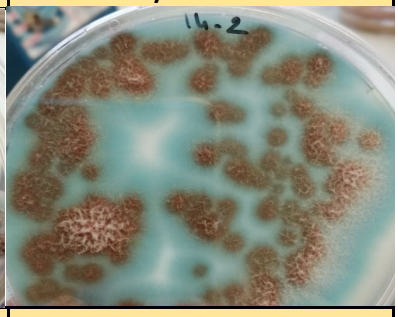
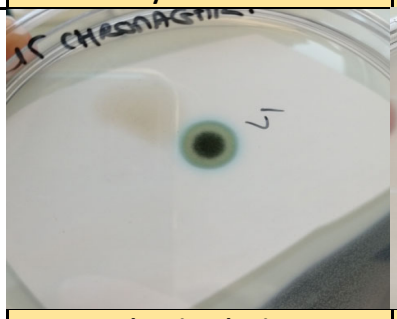
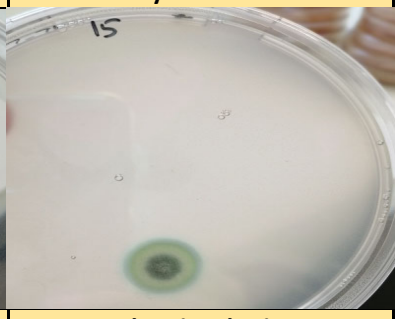
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
1	*Aspergillus niger*		
		5 days incubation	5 days incubation
2	*Aspergillus brasiliensis*		
		72 hours incubation	5 days incubation
3	*Aspergillus versicolor*		
		5 days incubation	5 days incubation
4	*Eurotium herbariorum*		
		5 days incubation	5 days incubation
5	*Penicillium rubrum*		
		5 days incubation	5 days incubation

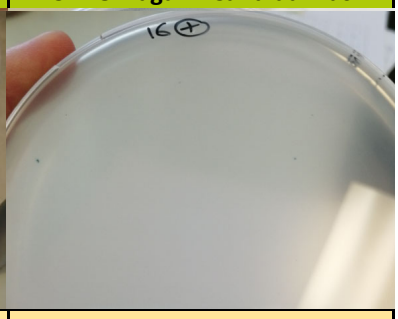
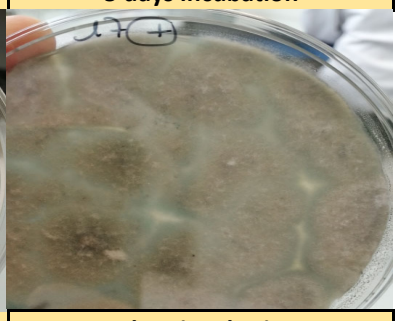
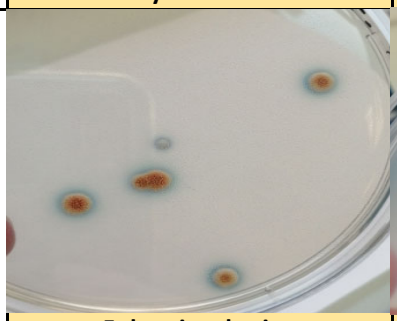
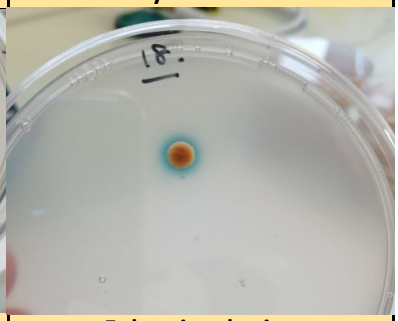
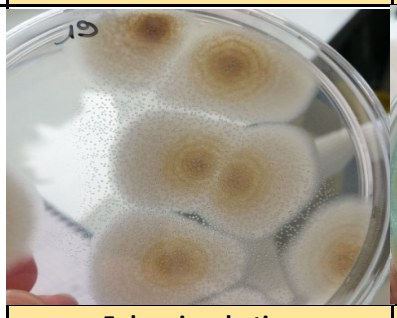
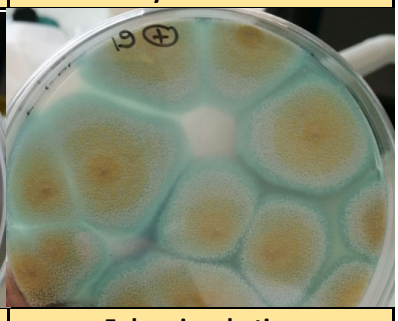
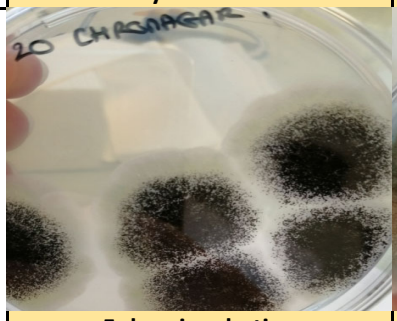
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
6	*Penicillium spp.*		
		5 days incubation	5 days incubation
7	*Phaeosphaeria avenaria*		
		5 days incubation	5 days incubation
8	*Fusarium fujikuroi*		
		5 days incubation	5 days incubation
9	*Penicillium glandicola*		
		5 days incubation	5 days incubation
10	*Penicillium viridicatum*		
		5 days incubation	5 days incubation

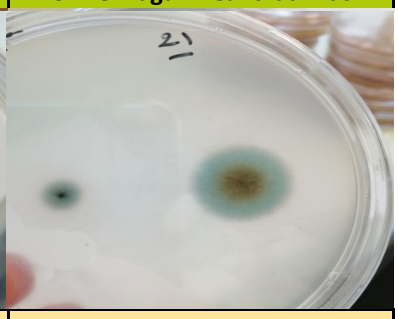
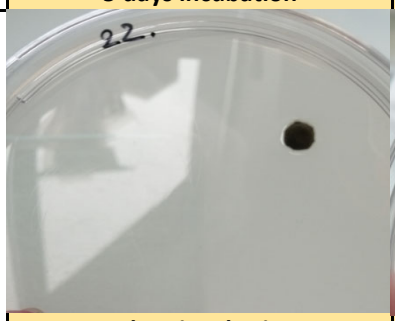
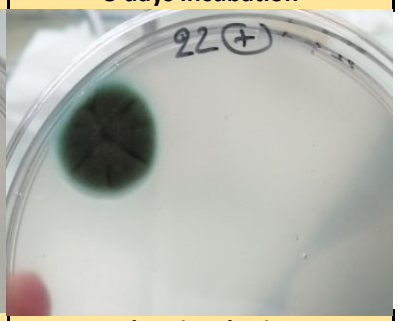
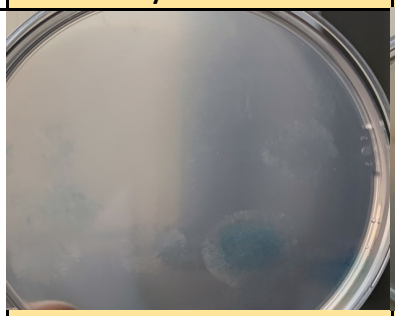
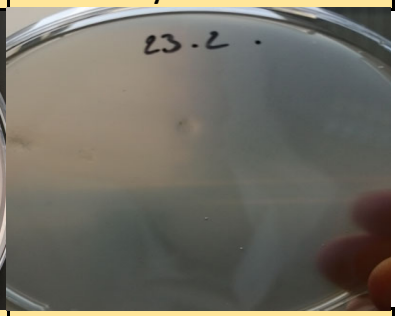
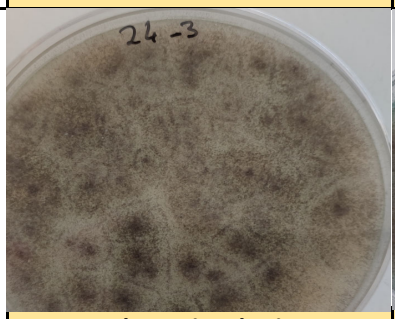
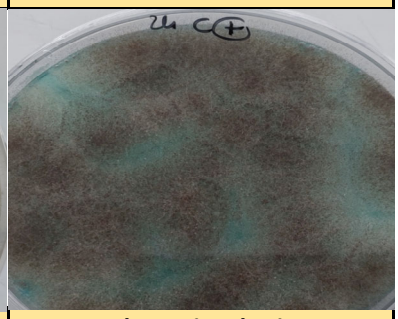
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
11	*Rhizomucor pusillus*		
		5 days incubation	5 days incubation
12	*Talaromyces atroroseus*		
		5 days incubation	5 days incubation
13	*Aspergillus foetidus*		
		5 days incubation	5 days incubation
14	*Talaromyces amestolkiae*		
		5 days incubation	5 days incubation
15	*Penicillium speluncae*		
		5 days incubation	5 days incubation

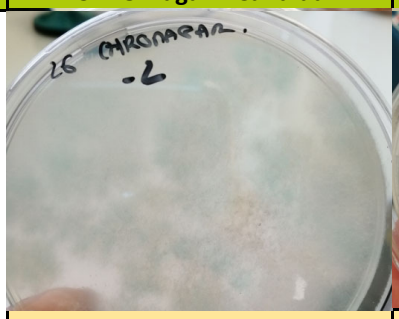
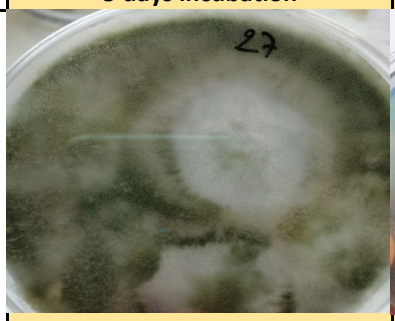
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
16	*Cladosporium colombiae*		
		5 days incubation	5 days incubation
17	*Boeremia exigua var. exigua*		
		5 days incubation	5 days incubation
18	*Talaromyces islandicus*		
		5 days incubation	5 days incubation
19	*Aspergillus melleus*		
		5 days incubation	5 days incubation
20	*Aspergillus costaricensis*		
		5 days incubation	5 days incubation

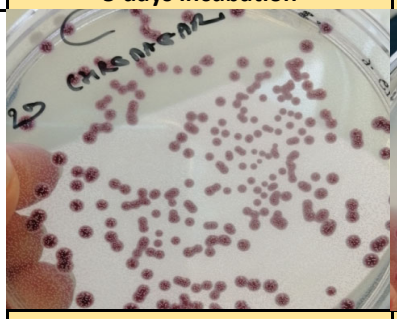
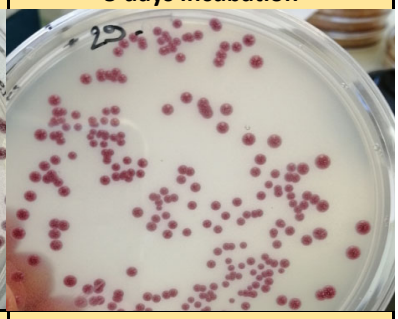
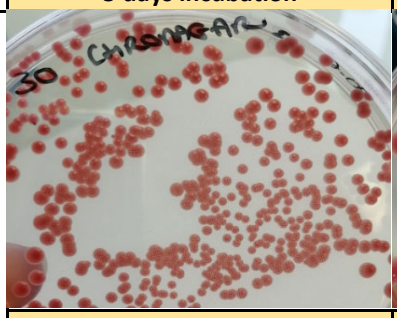
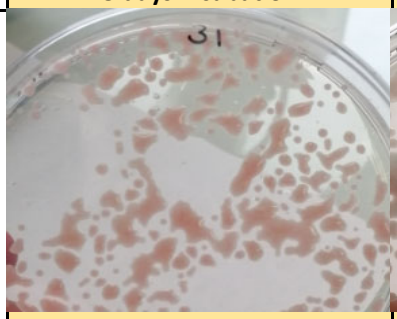
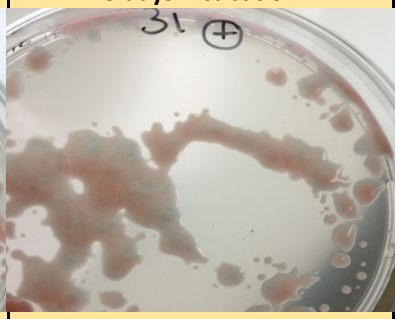
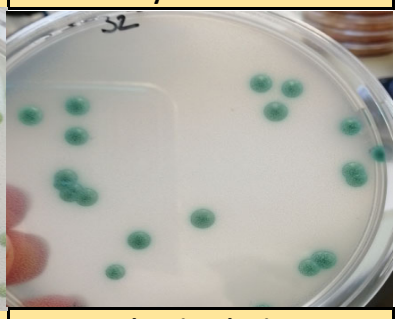
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
21	*Didymella glomerata*		
		5 days incubation	**5 days incubation**
22	*Sarocladium strictum*		
		5 days incubation	**5 days incubation**
23	*Cladosporium cladosporioides*		
		72 hours incubation	**72 hours incubation**
24	*Mucor circinelloides*		
		72 hours incubation	**72 hours incubation**
25	*Xenoacremonium falcatum*		
		5 days incubation	**5 days incubation**

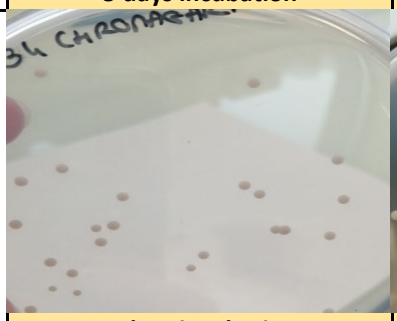
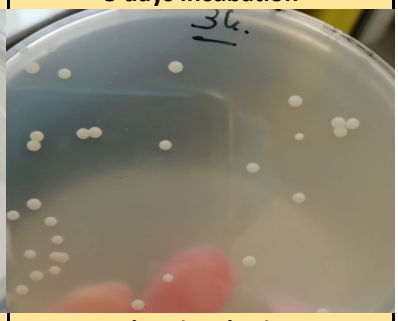
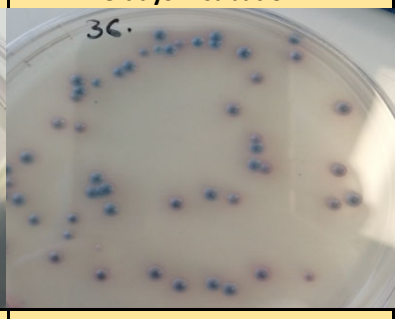
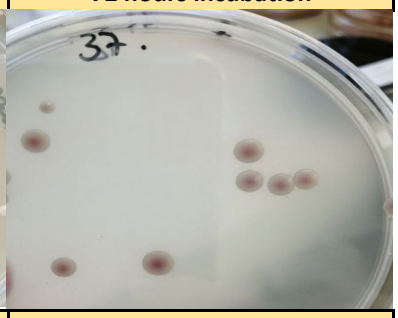
Appendix A - Pictures of molds

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
26	*Coprinus domesticus*		
		5 days incubation	5 days incubation
27	*Trichoderma virens*		
		5 days incubation	5 days incubation

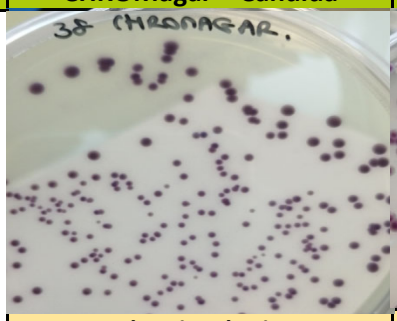
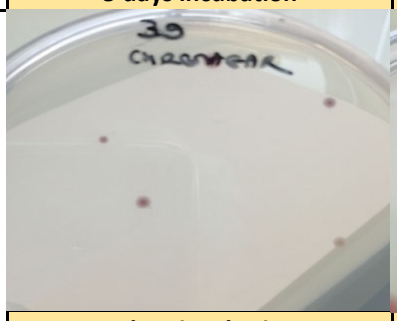
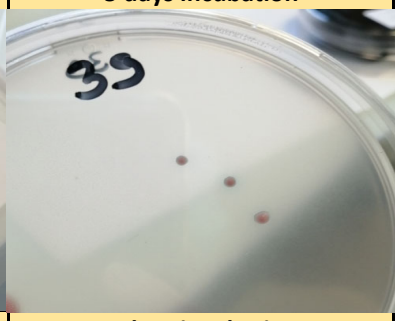
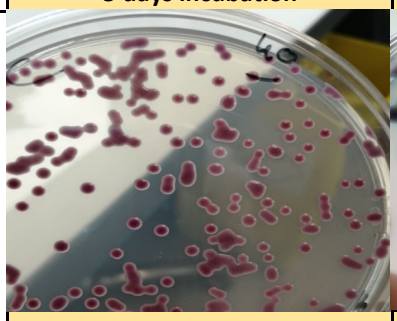
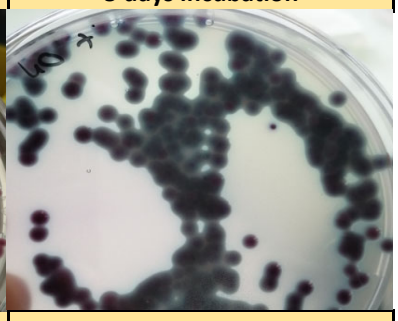
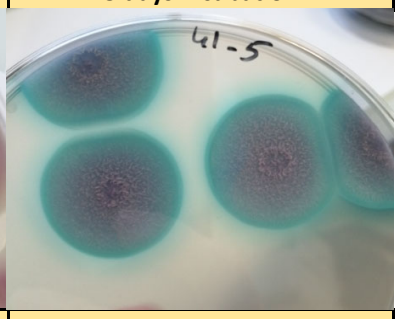
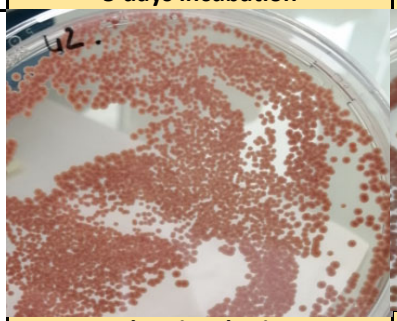
Appendix B - Pictures of yeasts

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
28	*Rhodotorula mucilaginosa*		
		5 days incubation	5 days incubation
29	*Rhodotorula mucilaginosa*		
		5 days incubation	5 days incubation
30	*Rhodotorula mucilaginosa*		
		5 days incubation	5 days incubation
31	*Rhodotorula minuta*		
		5 days incubation	5 days incubation
32	*Candida albicans*		
		5 days incubation	5 days incubation

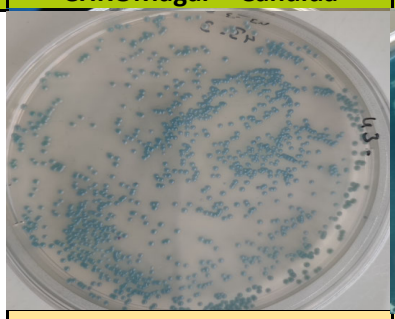
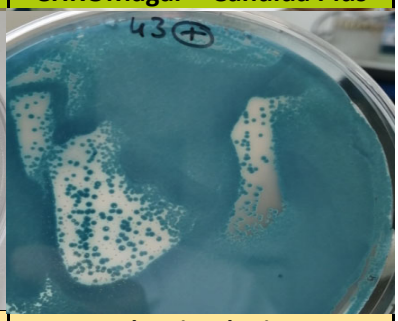
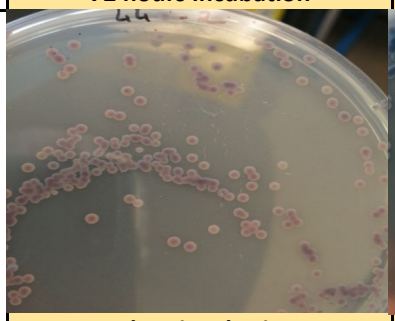
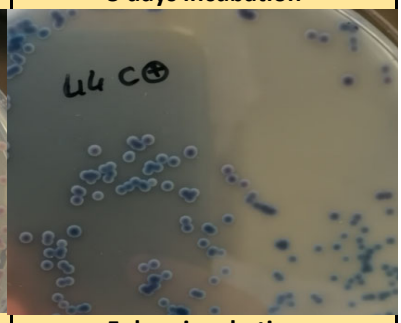
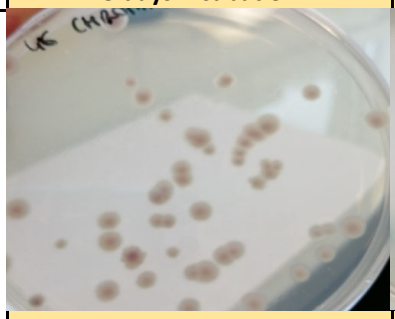
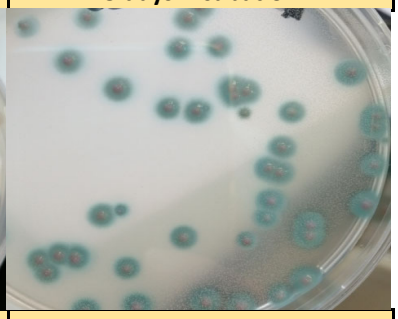
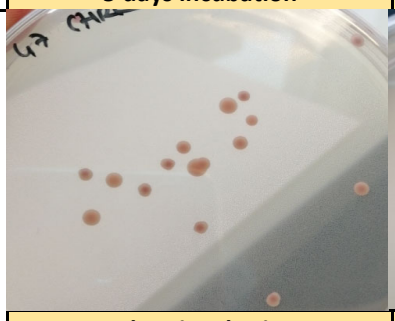
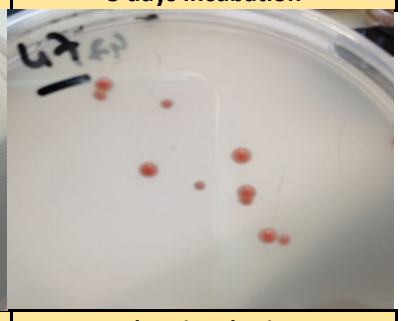
Appendix B - Pictures of yeasts

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
33	*Candida spherica 1*		
		5 days incubation	5 days incubation
34	*Zygosaccharomyces rouxii*		
		5 days incubation	5 days incubation
35	*Zygosaccharomyces bailii*		
		5 days incubation	5 days incubation
36	*Candida tropicalis*		
		5 days incubation	72 hours incubation
37	*Candida colliculosa*		
		72 hours incubation	5 days incubation

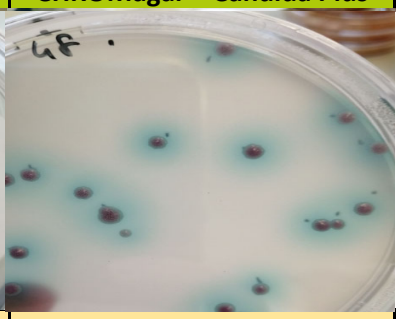
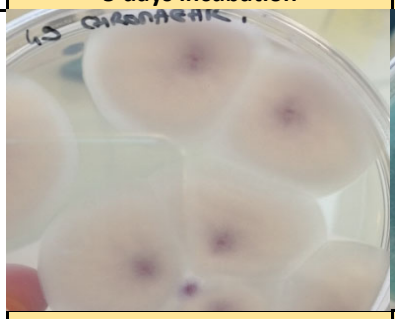
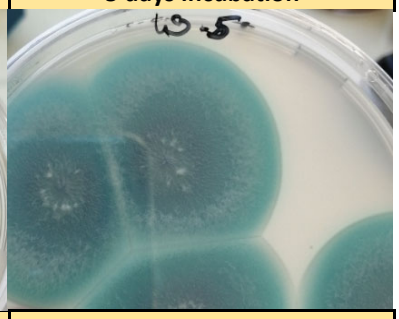
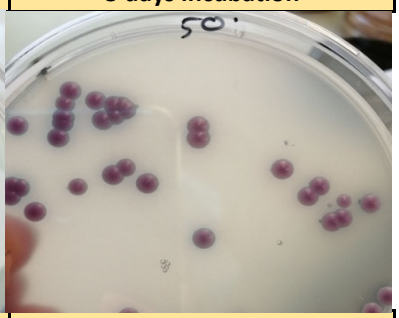
Appendix B - Pictures of yeasts

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
38	*Saccharomyces cerevisiae*		
		5 days incubation	5 days incubation
39	*Filobasidium oeirense*		
		5 days incubation	5 days incubation
40	*Sporidiobolus metaroseus*		
		5 days incubation	5 days incubation
41	*Dipodascus geotrichum*		
		5 days incubation	5 days incubation
42	*Rhodosporidiobolus colostri*		
		5 days incubation	5 days incubation

Appendix B - Pictures of yeasts

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
43	*Candida sake*		
		72 hours incubation	**5 days incubation**
44	*Holtermanniella takashimae*		
		5 days incubation	**5 days incubation**
45	*Zygosaccharomyces mellis*	/	/
		5 days incubation	**5 days incubation**
46	*Apiotrichum montevideense*		
		5 days incubation	**5 days incubation**
47	*Cystofilobasidium infirmomoniatum*		
		5 days incubation	**5 days incubation**

Appendix B - Pictures of yeasts

#	Strain	CHROMagar™ Candida	CHROMagar™ Candida Plus
48	*Naganishia albida*		
		5 days incubation	5 days incubation
49	*Geotrichum candidum*		
		5 days incubation	5 days incubation
50	*Candida parapsilosis*		
		5 days incubation	5 days incubation

Appendix C - supplementary tests with glucose + chloramphenicol agar medium - CHROMagar Candida

Retests with mold strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida						Glucose + chloramphénicol	
					CFU/mL	Result	Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C		CFU/mL	Result
							CFU/mL	Result	CFU/mL	Result	CFU/mL	Result		
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	P	0	<1	4	4	6	6	6	6	5	5
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
4	*Eurotium herbariorum*	Pet food	DNGZ69	P	3	3	0	<1	1	1	2	2	5	5
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
8	*Fusarium fujikuroi*	Water	FZGZ89	P	88	90	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	8		>150		>150		>150		>150	
				-2	0		>150		>150		>150		>150	
				-3	0		>150		>150		>150		>150	
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	P	0	<1	2	2	>150	5 800	>150	5 900	>150	4 000
				-1	0		0		>150		>150		>150	
				-2	0		0		53		53		35	
				-3	0		0		11		12		9	
16	*Cladosporium colombiae*	Flour	GHQX36	P	0	<1	1	1	1	1	1	1	1	1
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
				-3	0		0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	P	0	<1	>150	440	>150	470	>150	470	>150	420
				-1	0		44		47		47		39	
				-2	0		4		4		4		7	
				-3	0		0		0		0		0	
23	*Cladosporium cladosporioides*	Flour	GMRU52	P	>150	1 200	>150	1 400	>150	2 100	>150	2 700	>150	2 100
				-1	>150		>150		>150		>150		>150	
				-2	12		14		21		30		20	
				-3	1		0		0		0		3	
26	*Coprinus domesticus*	Water	GRFZ54	P	0	<1	>150	200	>150	1 500	>150	1 500	>150	1 100
				-1	0		20		142		143		>150	
				-2	0		2		20		20		11	
				-3	0		0		2		2		1	

Retests with yeast strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida						Glucose + chloramphénicol	
					CFU/mL	Result	Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C		CFU/mL	Result
							CFU/mL	Result	CFU/mL	Result	CFU/mL	Result		
31	*Rhodotorula minuta*	Food	FSMR25	P	>150	34 000	>150	120	>150	150 000	>150	150 000	>150	>150 000
				-1	>150		12		>150		>150		>150	
				-2	>150		1		>150		>150		>150	
				-3	34		0		146		146		>150	
32	*Candida albicans*	Food	CAN 1.1	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
33	*Candida spherica 1*	Food	FRRT96	P	>150	35 000	>150	56 000	>150	56 000	>150	56 000	>150	37 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	35		56		58		58		37	
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	P	>150	1 400	0	<1	>150	52 000	>150	59 000	>150	71 000
				-1	>150		0		>150		>150		>150	
				-2	14		0		>150		>150		>150	
				-3	1		0		52		59		71	
37	*Candida colliculosa*	Food	DMTL37	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	P	>150	8 300	>150	74 000	>150	74 000	>150	74 000	>150	82 000
				-1	>150		>150		>150		>150		>150	
				-2	83		>150		>150		>150		>150	
				-3	9		74		74		74		82	
44	*Holtermanniella takashimae*	Flour	EFKN77	P	23	27	0	<1	>150	1 400	>150	2 200	>150	140 000
				-1	7		0		>150		>150		>150	
				-2	0		0		14		22		>150	
				-3	0		0		2		2		135	
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	P	>150	14 000	>150	56 000	>150	71 000	>150	73 000	>150	92 000
				-1	>150		>150		>150		>150		>150	
				-2	131		>150		>150		>150		>150	
				-3	26		56		71		73		92	

Appendix D - supplementary tests with glucose + chloramphenicol agar medium - CHROMagar Candida Plus

Retests with mold strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO 21527 - DRBC		Alternative method CHROMagar™ Candida Plus						Glucose + chloramphénicol	
							Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C			
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
2	*Aspergillus brasiliensis*	Collection strain	ASP.1.2	P	0	<1	4	4	4	4	4	4	5	5
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
4	*Eurotium herbariorum*	Pet food	DNGZ69	P	3	3	0	<1	1	1	2	2	5	5
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
8	*Fusarium fujikuroi*	Water	FZGZ89	P	88	90	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	8		>150		>150		>150		>150	
				-2	0		>150		>150		>150		>150	
				-3	0		>150		>150		>150		>150	
11	*Rhizomucor pusillus*	Lemon balm	GBXP24	P	0	<1	3	3	>150	23 000	>150	27 000	>150	4 000
				-1	0		0		>150		>150		>150	
				-2	0		0		>150		>150		35	
				-3	0		0		23		27		9	
16	*Cladosporium colombiae*	Flour	GHQX36	P	0	<1	1	1	1	1	1	1	1	1
				-1	0		0		0		0		0	
				-2	0		0		0		0		0	
				-3	0		0		0		0		0	
21	*Didymella glomerata*	Organic thyme	GLRU94	P	0	<1	>150	680	>150	700	>150	700	>150	420
				-1	0		72		74		74		39	
				-2	0		3		3		3		7	
				-3	0		0		0		0		0	
23	*Cladosporium cladosporioides*	Flour	GMRU52	P	>150	1 200	>150	1 400	>150	2 700	>150	3 700	>150	2 100
				-1	>150		>150		>150		>150		>150	
				-2	12		14		27		37		20	
				-3	1		1		2		4		3	
26	*Coprinus domesticus*	Water	GRFZ54	P	0	<1	>150	450	>150	700	>150	700	>150	1 100
				-1	0		45		>150		>150		>150	
				-2	0		5		7		7		11	
				-3	0		0		1		1		1	

Retests with yeast strains

#	Strain	Origin	Code	Decimal dilution of the initial suspension	Reference method- ISO		Alternative method CHROMagar™ Candida Plus						Glucose + chloramph.	
							Surface spreading - 48 h at 25°C		Surface spreading - 72 h at 25°C		Surface spreading - 5 days at 25°C			
					CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result	CFU/mL	Result
31	*Rhodotorula minuta*	Food	FSMR25	P	>150	34 000	>150	1 800	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		20		>150		>150		>150	
				-3	34		0		>150		>150		>150	
32	*Candida albicans*	Food	CAN 1.1	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
33	*Candida spherica 1*	Food	FRRT96	P	>150	35 000	>150	58 000	>150	62 000	>150	62 000	>150	37 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	35		58		62		62		37	
34	*Zygosaccharomyces rouxii*	Collection strain	HFYF47	P	>150	1 400	0	<1	>150	76 000	>150	90 000	>150	71 000
				-1	>150		0		>150		>150		>150	
				-2	14		0		>150		>150		>150	
				-3	1		0		76		90		71	
37	*Candida colliculosa*	Food	DMTL37	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
38	*Saccharomyces cerevisiae*	Freeze-dried kefir grains	DZWW62	P	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000	>150	>150 000
				-1	>150		>150		>150		>150		>150	
				-2	>150		>150		>150		>150		>150	
				-3	>150		>150		>150		>150		>150	
41	*Dipodascus geotrichum*	Frangipane shortbread	ECZP28	P	>150	8 300	>150	67 000	>150	67 000	>150	67 000	>150	82 000
				-1	>150		>150		>150		>150		>150	
				-2	83		>150		>150		>150		>150	
				-3	9		67		67		67		82	
44	*Holtermanniella takashimae*	Flour	EFKN77	P	23	27	0	<1	>150	2 200	>150	2 300	>150	140 000
				-1	7		0		>150		>150		>150	
				-2	0		0		20		21		>150	
				-3	0		0		4		4		135	
47	*Cystofilobasidium infirmomoniatum*	Soft wheat	FWXC87	P	>150	14 000	>150	68 000	>150	86 000	>150	86 000	>150	92 000
				-1	>150		>150		>150		>150		>150	
				-2	131		>150		>150		>150		>150	
				-3	26		68		86		86		92	