

# Soleris® pre-enrichment protocol for the detection of yeast and molds in yogurt

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## INTRODUCTION

As varieties of yogurt continue to expand and trends toward higher probiotic levels and no preservatives increase, finding a single solution to test the entire range of yogurts for yeasts and molds becomes increasingly challenging.

Traditional “peel and look” yogurt testing methods, where the product incubates in a warm room for several days and is then analyzed for any organoleptic changes, have a multitude of limitations. Certain yeast and mold contaminants are “flat” so no gas is generated (bulging of the cup is not observed), may be light in color, or odorless thus resembling the appearance and smell of yogurt itself.

Further, low levels of yeast or mold contamination, or certain slow growing fungal contaminants, will not visually manifest their presence for quite some time after manufacturing and product release.

As a result, the need in the yogurt industry for a highly sensitive, easy to use, and rapid microbial detection system for the presence of yeast and molds in yogurts has become more imperative than ever before.

## SUMMARY

To meet this important need, Neogen Corporation (Lansing, Mich.) developed a rapid protocol using the Soleris rapid microbial detection system and its Direct Yeast and Mold vial (DYM-109C), which is sensitive enough to detect less than 1 colony-forming unit (CFU) of yeast or mold per 10 grams in yogurt.

The reference test protocol for detecting yeast and mold in yogurt has a minimum test duration of five days. The new test protocol that was developed as a result of this study has a test duration of three days — two days less than the standard protocol. The Soleris DYM-109C vial was shown to be an appropriate method for the detection of yeast and molds at less than 1 CFU/10g when used with this novel pre-enrichment protocol.

## SOLERIS ASSAY PRINCIPLES

Soleris technology monitors changes in the chemical characteristics of microbial liquid growth medium and detects microorganisms with carbon dioxide (CO<sub>2</sub>) sensitive reagents. The reagents change their optical patterns as the metabolic process takes place. These changes are detected photometrically by an optical reader and monitored every six minutes.

The Soleris Direct Yeast and Mold Medium vial allows for a shorter detection time of yeast and molds. When CO<sub>2</sub> is released by growing yeast and molds, it diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris instrument. The membrane layer also serves as a barrier, eliminating product interference with the reading frame.

For the standard protocol, the DYM-109C vial can be used in a specification monitoring application (or threshold manner) in which the result is positive or negative around a desired cutoff (in CFU/g) determined by the dilution and volume of sample homogenate added to the vial. However, for the 48-hour

pre-enrichment with 24-hour vial yogurt protocol used for this study, 1 mL of pre-enriched sample was added to the vial post-incubation. It is assumed that 1 CFU of the target organism introduced into the Soleris vial will lead to a positive result.

## STUDY DESIGN

The new 72-hour protocol uses a pre-enrichment step that includes incubating a pre-enrichment bottle of yeast and mold growth medium to which 10 grams of yogurt has been added. Then, 1 mL from the pre-enrichment bottle is inoculated into the DYM-109C vial, inserted into the Soleris unit and incubated for 24 hours.

The pre-enrichment protocol was compared to the inoculation of the DYM-109C vial without the pre-enrichment and incubation step, as well as the standard plating method (inoculation onto Potato Dextrose Agar or Sabouraud Dextrose Agar). Sixteen different yeast and mold spoilage organisms were used in the study, some of which were natural isolates from a yogurt facility. Fourteen different flavors of Low-fat, Greek and Probiotic yogurt were used.

## MATERIALS AND METHODS

### Materials required:

Direct Yeast and Mold vial (DYM-109C)  
Yeast and Mold Supplement, rehydrated (YI-110C)  
Sterile deionized water  
YM-EBY Enrichment Broth for Yogurt, 90 mL

### Yeast and Mold Supplement preparation:

Add 10 mL of sterile, deionized water to a vial of YI-110C supplement. Mix well. Store in the refrigerator up to seven days after rehydration. For more information, please see the YI-110C product insert.

### Soleris yogurt pre-enrichment protocol:

Add 2.5 mL of rehydrated Yeast and Mold Supplement (YI-110C) to the 90 mL bottle of YM Broth. **NOTE:** The addition of this supplement to the bottle may be optional but required for high probiotic yogurts. Gently invert to mix thoroughly.

### Sample preparation:

Add 10 g of test sample to the YM-EBY Enrichment Broth bottle. Cap bottle. Gently invert contents of bottle to mix thoroughly. Slightly loosen the cap of the pre-enrichment bottle and place in a 25°C incubator for 48 hours.

### Vial preparation:

Add 0.60 mL of Yeast and Mold Supplement (YI-110C) directly to the DYM-109C vial liquid. Gently invert to mix thoroughly.

**NOTE:** Add the test sample to the vial within two hours of the addition of YI-110C.



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## Inoculation of the vial:

With the cap of the YM-EBY Enrichment Broth bottle tightly closed, vigorously shake the contents of the incubated bottle for 10 seconds to mix thoroughly.

Inoculate the DYM-109C vial containing 0.60 mL of Yeast and Mold Supplement (YI-110C) with no more than 1 mL of the incubated pre-enrichment sample.

Cap the vial and gently invert three times to mix the sample. Slightly loosen the cap to permit air and gas exchange.

Insert the vial into the Soleris instrument set at 25°C.

**CAUTION:** Products containing CO<sub>2</sub>-releasing compounds, such as ascorbic acid, calcium carbonate, or calcium ascorbate need to be carefully validated as reactions with the vial chemistry may occur, which may cause false positives.

## Soleris parameters:

Test	Threshold	Skip	Shuteye	Duration	Temperature
DYM-109C	8	2	70	24	25°C

## RESULTS

Using the 48-hour pre-enrichment protocol, detections less than 1 CFU/10g of yeast and mold contamination in yogurt were possible in the Soleris instrument within 24 hours. Inoculation of the DYM-109C vial without pre-enrichment did not consistently detect yeast or mold at a contamination level of 1 cfu/10g. The pre-enrichment protocol provides improved sensitivity with the 48-hour pre-enrichment for low levels of yeast and mold detection in yogurt.

**NOTE:** Potato Dextrose Agar is denoted as PDA and ND denotes no detection in the Soleris instrument.

**Table 1:**

Organism	Type of Yogurt	CFU/gram inoculated into YM-EBY Bottle	YM-EBY Bottle #1 without YI-110C supplement		YM-EBY Bottle #2 without YI-110C supplement	
			Vial #	DT ^ (hrs)	Vial #	DT ^ (hrs)
<i>Candida kefyr</i> ATCC 8553	Regular/Low-fat Vanilla	157	1	6.4	1	6.4
			2	6.4	2	6.4
		19	1	6.4	1	6.4
			2	6.4	2	6.4
<i>Candida parapsilopsis</i> (yogurt isolate)	Regular/Low-fat Vanilla	3	1	6.4	1	6.8
			2	6.4	2	6.4
		2	1	12.0	1	15.6
			2	11.0	2	15.6
<i>Geotrichum candidum</i> ATCC 34614	Regular/Low-fat Vanilla	1	1	6.4	1	6.4
			2	6.4	2	6.4
		0	1	ND	1	ND
			2	ND	2	ND
<i>Saccharomyces cerevisiae</i> MYA-658	Regular/Low-fat Vanilla	32	1	6.4	1	6.4
			2	6.4	2	6.4
		3	1	6.4	1	6.4
			2	6.4	2	6.4
<i>Aspergillus brasiliensis</i> ATCC 16404	Regular/Low-fat Vanilla	0	1	ND	1	ND
			2	29.8	2	ND
		0	1	ND	1	ND
			2	ND	2	ND
<i>Cladosporium cladosporioides</i> ATCC 16022	Regular/Low-fat Vanilla	0	1	ND	1	ND
			2	ND	2	ND
		0	1	ND	1	ND
			2	ND	2	ND
<i>Mucor circinelloides</i> ATCC 56647	Regular/Low-fat Vanilla	2	1	6.4	1	6.4
			2	6.4	2	6.4
		0	1	6.4	1	ND
			2	6.4	2	ND

^ Detection time of 6.4 hours indicates a 'Shut-eye' detection time. Actual test detection time is less than 6.4 hours.



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**Table 2:**

Organism	Type of Yogurt	CFU/gram inoculated into YM-EBY Bottle	YM-EBY Bottle with YI-110C supplement			YM-EBY Bottle without YI-110C supplement		
			CFU/gram POST Incubation	Vial #	DT * (hrs)	CFU/gram POST Incubation	Vial #	DT * (hrs)
<i>Candida albicans</i> ATCC 10231	Regular/Low-fat Strawberry	1	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
		0	0	1	30.4	TNTC	1	9.4
				2	ND		2	9.2
<i>Aspergillus brasiliensis</i> ATCC 16404	Regular/Low-fat Strawberry ^	0	0	1	ND	0	1	ND
				2	ND		2	ND
		1	0	1	ND	0	1	ND
				2	ND		2	ND
<i>Candida kefyr</i> ATCC 8553	Greek, Plain	25	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
		0	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
<i>Aureobasidium pullulans</i>	Greek, Plain	15	TNTC	1	6.4	TNTC	1	8.2
				2	6.4		2	6.4
		0	TNTC	1	ND	TNTC	1	ND
				2	ND		2	ND
<i>Mucor racemosus</i> ATCC 42647	Greek, Plain	3	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
		0	2	1	12.8	0	1	ND
				2	11.8		2	ND
<i>Saccharomyces cerevisiae</i> MYA-658	Probiotic, Plain	2	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
		2	TNTC	1	6.4	TNTC	1	6.4
				2	6.4		2	6.4
<i>Penicillium roqueforti</i> ATCC 10110	Probiotic, Plain	14	5	1	26	0	1	ND
				2	19.6		2	ND
		2	12	1	ND	0	1	ND
				2	ND		2	ND

\* Detection time of 6.4 hours indicates a 'Shut-eye' detection time. Actual test detection time is less than 6.4 hours.

^ Low-fat Strawberry yogurt contains low level of preservatives.



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**Table 3:**

Organism	Type of Yogurt	Data Set #	YM-EBY Bottle with YI-110C supplement		
			CFU/gram inoculated into YM-EBY Bottle	CFU/gram POST incubation	DT * (hrs)
<i>Penicillium viticola</i>	Greek/ Plain	1	190	1.88 X 10 <sup>3</sup>	6.6
			19	2.74 X 10 <sup>3</sup>	6.6
			1.9	4.9 X 10 <sup>2</sup>	6.7
			0.19	1 X 10 <sup>2</sup>	15.6
<i>Aureobasidium pullulans</i>	Greek/ Strawberry	1	7.3 X 10 <sup>2</sup>	1.48 X 10 <sup>8</sup>	6.6
			730	5.7 X 10 <sup>7</sup>	6.6
			73	1.2 X 10 <sup>7</sup>	6.6
	Greek/ Plain	2	90	2.4 X 10 <sup>7</sup>	6.6
			9	1.7 X 10 <sup>7</sup>	6.6
		3	130	2.4 X 10 <sup>7</sup>	6.6
<i>Penicillium corylophilum</i>	Greek/ Black Cherry	1	2.6	5.9 X 10 <sup>3</sup>	6.6
			0.26	2.9 X 10 <sup>2</sup>	6.6
	Greek/ Cherry	2	2.16	2.1 X 10 <sup>3</sup>	6.6
			0.216	2.7 X 10 <sup>3</sup>	6.6
<i>Cladosporium spp.</i>	Greek/ Lemon	1	0.54	6.5 X 10 <sup>2</sup>	6.4
			0.054	1.3 X 10 <sup>2</sup>	8.1
Yogurt mold isolate (not identified)	Greek/ Peach and Passion Fruit	1	130	2.5 X 10 <sup>3</sup>	7.3
			13	8 X 10 <sup>1</sup>	9.9
	Greek/ Coconut	2	60	3.1 X 10 <sup>3</sup>	6.4
			6	4.1 X 10 <sup>2</sup>	6.4
<i>Penicillium crustosum</i> - Isolate one	Greek/ Blueberry	1	1.7 X 10 <sup>2</sup>	3.9 X 10 <sup>3</sup>	6.4
			170	6.6 X 10 <sup>3</sup>	6.4
<i>Penicillium crustosum</i> - Isolate two	Greek/ Strawberry	1	40	5.1 X 10 <sup>3</sup>	6.4
			4	9.4 X 10 <sup>3</sup>	6.4
		2	10	1.88 X 10 <sup>3</sup>	6.4
			1	6.6 X 10 <sup>2</sup>	6.4
<i>Candida parapsilopsis</i>	Greek/ Peach	1	4.1	6.5 X 10 <sup>7</sup>	6.4
			0.41	7.6 X 10 <sup>6</sup>	6.4
		2	2.1	1.8 X 10 <sup>7</sup>	6.4

\* Detection time of 6.4 hours indicates a 'Shut-eye' detection time. Actual test detection time is less than 6.4 hours.

## CONCLUSION

Using this novel pre-enrichment protocol, the Soleris rapid detection system, in conjunction with the DYM-109C vial, successfully detected <1CFU/10 grams of yeast and molds in Low-fat, Greek, and Probiotic yogurts in as little as three days, which is significantly faster than the five day reference testing method. As demonstrated with the above results, it may be possible to leave out the YI-110C supplement from the YM-EBY bottle for certain yogurt types. To clearly determine the appropriate protocol for your yogurt product, please contact Neogen Technical Services.



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