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Letter to the Editor

LETTER TO THE EDITOR

Water soluble lipid precursor contaminants in yeast culture medium ingredients

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One sentence summary: The authors report that widely used media ingredients contain trace amounts of inositol and/or choline that are not mentioned on the product label, possibly influencing experimental outcome.

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ABSTRACT

The presence of the water soluble glycerophospholipid precursors choline and inositol in culture media highly affects lipid biosynthesis and regulation thereof. We report that widely used media ingredients contain trace amounts of choline and inositol that are not mentioned on the product label, influencing experimental outcome.

Keywords: lipid metabolism; inositol; choline; *Saccharomyces cerevisiae*; soluble lipid precursors; culture medium ingredients

Saccharomyces cerevisiae is a widely used model organism in research addressing lipid metabolism, lipid transport, and membrane homeostasis (Santos and Riezman 2012; Singh 2017). The biosynthesis of yeast phospholipids (PLs) is tightly regulated, and depends amongst others on the exogenous availability of inositol, ethanolamine and choline, which serve as precursors for the synthesis of phosphatidylinositol (PI), phosphatidylethanolamine and phosphatidylcholine, respectively (Henry, Kohlwein and Carman 2012). In addition to its role as the precursor of PI, inositol plays a key role in the regulation of PL biosynthesis via the regulation of the transcriptional activator complex Ino2/4p (Henry, Kohlwein and Carman 2012). In the absence of inositol, phosphatidic acid (PA) accumulates in the ER membrane retaining the PA sensor protein Opi1p tethered to the ER. Upon addition of inositol to the medium, PA is converted to PI, releasing Opi1p from the ER and allowing it to enter the nucleus. In the nucleus, Opi1p binds the Ino2/4p complex and attenuates the expression of genes activated by Ino2/4p. The sequence bound by Ino2/4p is known as the Upstream Activating Sequence inositol (UAS_{INO}). In addition to the role of inositol in the regulation of lipid metabolism, inositol has been implicated in regulation of various stress responses, including the unfolded protein response (Henry, Gaspar and Jesch 2014). In the presence of inositol, choline strongly influences lipid metabolism and its regulation (Henry, Kohlwein and Carman 2012).

Inositol and choline are known to influence lipid metabolism at very low concentrations. The K_m of the choline transporter Hnm1p was found to be 0.56 μM (58 μg/L) (Hosaka and Yamashita 1980), thus sub mg/L contaminations of choline can already completely satiate choline import. Partial repression of *INO1* (encoding inositol-6-phosphate synthase) by exogenous inositol was detected at 10 μM (≈ 1.8 mg/L; (Hirsch and Henry 1986), and it fully represses *INO1* and co-regulated UAS_{INO}-genes at 75 μM (≈ 13.5 mg/L; Henry, Gaspar and Jesch 2014). Studies addressing aspects of lipid metabolism in yeast require chemically defined culture media that are devoid of water soluble

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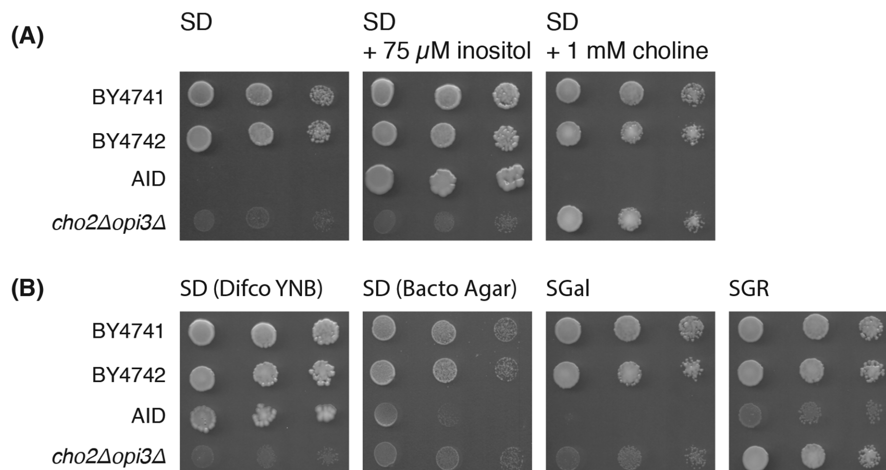

Figure 1. Detection of inositol and/or choline in culture media. Growth of wild type strains BY4741 (*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*), and inositol- (AID; *MAT**a**/α ino1–13/ino1–13 ade1/ade1*; (Greenberg, Reiner and Henry 1982)), and choline- (*cho2Δopi3Δ*; BY4742 *cho2::KanMX opi3::LEU2*; (Boumann *et al.* 2006)) auxotrophic mutants on synthetic defined (SD) media supplemented with 75 μM inositol, or 1 mM choline as indicated **(A)**, and on several commonly used media **(B)**. After pre-culture to early log phase in YPD, cells were washed and serially diluted in sterile water (10^{-1} – 10^{-3} OD_{600} unit/mL); 4 μL aliquots were spotted and incubated at 30°C for 3 days. Synthetic defined media were prepared as described (Griac *et al.* 1996), except for the medium based on Yeast Nitrogen Base (Difco YNB), and contained 2% glucose (SD), 2% galactose (SGal) or 2% galactose and 1% raffinose (SGR) as carbon source. All plates were prepared using Fluka agar (05038), except for the plate prepared with Bacto Agar (as indicated). Data shown is representative of at least three biological replicates.

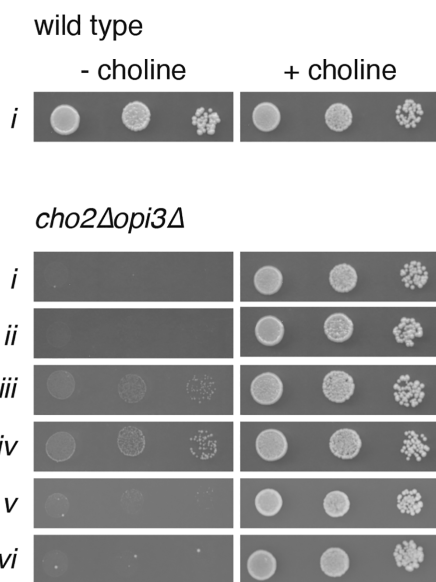

Figure 2. Choline contamination in different agars. Serial dilutions of wild type (BY4742; *MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and the choline auxotrophic mutant (*cho2Δopi3Δ; BY4742 cho2::KanMX opi3::LEU2*) on SD with or without 1 mM choline prepared with the agars specified below. After pre-culture to log phase in SD supplemented with 75 μM inositol and 1 mM choline, serial dilutions (10^{-1}–10^{-3} OD_{600} unit/mL) were spotted and incubated at 30°C for 5 (*i–iv*) or 6 days (*v–vi*). Agars used from Sigma-Aldrich were: (i) A7002, Lot#069K0079; (ii) A7002, Lot#BCBF3876V; (iii) A7002, Lot#BCBR2864V; (*iv*) A7002, Lot#BCBT6029; (*v*) 05038, Lot#BCBT2721; and from PanReac AppliChem: (*vi*) A0917, Lot#7R011016. Data shown is representative of at least three biological replicates.

lipid precursors (see *e.g.* (Griac, Swede and Henry 1996)), that are preferably mixed in house from pure chemicals of highest possible quality. We recently found that several agars used for the preparation of solid media contain contaminations with lipid precursors that may affect experimental outcome. This prompted us to examine the ingredients of our synthetic defined media for the presence of choline and inositol.

The presence of inositol and choline in culture medium is conveniently detected by a spot assay monitoring growth of strains that are auxotrophic for the respective compounds, i.e. *ino1Δ* (AID; Greenberg, Reiner and Henry 1982) and *cho2Δopi3Δ* (Patton-Vogt *et al.* 1997; Boumann *et al.* 2006) (Fig. 1A). Supplementation with exogenous inositol or choline rescued the growth of the auxotrophic strains as expected (Fig. 1A). The agar we used (Fluka 05038) appears to contain a trace amount of choline sufficient for modest growth of the *cho2Δopi3Δ* mutant in supposedly choline-free conditions.

An important commercially available ingredient of synthetic defined medium, Yeast Nitrogen Base (YNB) without amino acids, is known to contain inositol (11 μM, Sherman 1991; Hanscho *et al.* 2012; Fig. 1B, Difco YNB). Therefore, YNB for use in inositol-free media is generally mixed in house (Fig. 1A) based on the recipe described by Sherman (1991). The widely used Bacto Agar (BD Diagnostics, Franklin Lakes, NJ) contains trace amounts of choline and inositol, allowing moderate growth of the inositol and choline auxotrophs (Fig. 1B, Bacto Agar). Synthetic Galactose Raffinose medium (SGR) is often used for overexpressing genes of interest from a *GAL*-promotor. Galactose (150610010) from Acros Organics (Geel, BE) contains trace amounts of choline (Fig. 1B, SGal), whereas raffinose (195675000) from Acros contains a contamination of choline that supports wild type-like growth of the choline auxotroph, in addition to a contamination with inositol (Fig. 1B, SGR).

Of note, the presence of these contaminations can vary between batches, as exemplified by the occurrence of choline contamination in agar (A7002) from Sigma-Aldrich (St. Louis, MO) in Fig. 2 (*i–iv*). Since several batches of agar A7002 we tested contained choline, we searched for an alternative. Agars 05038 from Sigma-Aldrich and A0917 from PanReac AppliChem (Darmstadt, DE) are essentially choline-free (Fig. 2 *v* and *vi*), and do not contain detectable inositol (data not shown).

In addition to testing for contaminations with choline and inositol, the presence of ethanolamine, the water soluble precursor of phosphatidylethanolamine, can be detected using the ethanolamine auxotroph strain *psd1Δpsd2Δdpl1Δ* (Birner *et al.* 2001; Aaltonen *et al.* 2016).

Contaminations in bulk medium components as reported here may account for differences in experimental outcome obtained with similar lipid biosynthetic mutants by different laboratories. To avoid problems that may arise from overlooked contaminants, we recommend the use of completely defined synthetic media based on in house mixed YNB, and regular control experiments to verify the absence of relevant contaminants in culture media. Furthermore, we stress the importance of listing the used media ingredients in detail in the methods section of articles.

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Conflict of interest. None declared.

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