Mushroom Culture Media Mushrooms Compliant with USDA Organic Standards

Consumer demand for 'organic' products is growing rapidly. While certified organic production of mushrooms and of mushroom spawn can add significant value for a producer, it comes with higher input costs, extensive record keeping, and sometimes challenging modifications of production methods. The National Organic Standards Board (NOSB) recommendations[1] developed in 2001 are used by the U.S.D.A. to define when an operation can be certified as an organic mushroom producer. As the prevalence of organic production continues to increase, there are increases in the stringency of the NOSB guidelines for mushroom production. For example, the State of Vermont is extending their interpretation of 'organic spawn production' to the culture media used for storage and growth of primary cultures. Driven by a need to comply with Vermont's standards, we tested the performance of commercial agar-based growth media to 'organic growth media,' in which all components were compliant with U.S.D.A. guidelines for organic production. Cultures of *Pleurotus ostreatus* and *Agaricus bisporus* were cultured on a variety of potato-, malt- and compost-based media to determine which, if any, would sustain healthy growth for commercial production.

Current USDA guidelines dictating organic mushroom production practices require documentation of the origin of spawn as well as the inputs used as substrates for spawn growth and for cultivation of fruit bodies or mycelia. However, there is no explicit regulation regarding the stage of mushroom production that requires culture media. These media are typically solidified with agar, which is allowed under current organic standards, as well as a carbohydrate and/or nitrogen source. The most commonly used media are potato dextrose agar (PDA) and malt extract agar (MEA). As of this writing there were no commercial sources for mushroom media that were compliant with USDA Organic Standards. Contact with a major media supplier indicated that it would not be possible to document that any media were categorically free from irradiation, exposure to sewage, and/or free from GMO ingredients since these are not tracked. Components for the media often come from bulk commodities such as dried potatoes or malt powder which are most likely – but not certainly – free from genetically modified sources. Therefore we designed, tested, and implemented simple media comprised in compliance with organic standards to compare their utility to the 'conventional' media.

Methods for making an organic PDA equivalent and an organic MEA equivalent for growth of *P. ostreatus* were investigated. Organic equivalents to PDA, MEA, and compost extract agar (CEA) were tested with a commercial culture of *A. bisporus*. There are many sources for PDA and MEA and their performance may differ. Different sources of organic inputs may be similarly variable. Obviously different types of potatoes vary in their carbon and nitrogen composition, and the age and storage conditions before use could also introduce variability. With

those caveats in mind, the objective was to determine if non-commercial, "organic" inputs could perform in a satisfactory manner, which proved to be the case.

Materials and methods

To develop an alternative to conventional PDA, organic potatoes were sourced from the organic produce section of popular stores. In place of dextrose, organic table sugar was purchased at a grocery store. To test the addition of a component enriched in nitrogen, organic flax flour was used. The flax flour contained 33% protein and 5% fat. Because dried yeast extract is 68% protein, twice the amount typically used in yeast supplemented media was used.

Development of a organically compliant alternative to MEA was more complicated as most commercially available MEA media contain peptone, usually in the form of a papainic digestion of protein from meat, but occasionally from soybean. An organic source for yeast extract was found (Marroquin International Commodity Services), and they generously supplied a small sample for these tests (see medium composition below).

Compost Extract Agar was made using modification of a method supplied by the Mushroom Culture Collection of Pennsylvania State university. Sources of organic compost were Intervale Germination Mix (Burlington, VT), McEnroe Lite Growing Mix (Millerton, NY), and Vermont Compost potting soil (Montpelier, VT).

Potato Dextrose Agar (P772), Malt Extract Broth (M484), and plant tissue culture micropropagation grade agar (A296) were obtained from Phytotechnology Labs (Shawnee Mission, KS). These media are from a source that specializes in the growth of extremely sensitive plant tissue cultures.

Transfers of *P. ostreatus* mycelia (cloned from a commercial strain) were taken from the growing edge of a mycelium using a 5 mm diameter tissue punch (Fine Science Tools, 18035-5). For PDA/PSA experiments, the transfers were taken from a malt extract medium (O-MEA). Similarly, for MEA experiments transfers were made from a potato based medium (PSA). Plates were grown at 20 degrees Celsius for one week.

A. bisporus culture MC459 was obtained from the Pennsylvania State University Culture collection. Transfers were taken as 5 mm punches directly from the plate sent from the culture collection, which contained compost extract agar medium.

Table 1: Potato-Based Media

PDA Phytotechnology Labs (P772). Per liter, glucose 20.0 g, potato powder 4.0 g, agar 15.0 g.

PSA (1 liter)
300 g Organic potatoes, unpeeled, diced[1]
20 g Organic granulated sugar[2]
15 g agar[3]
+/- 8 g flax flour[4]

Boil potatoes in 1000 ml of water for 30 min. Strain twice through three layers of cheesecloth, adding water to draw out soluble components, but take care to limit total volume. Increase volume to 1000 ml. Add sugar and agar. Autoclave 15 min. at 121 C.

PSA-flax as above, but 8 g/liter flax flour added.

Based upon ATCC medium 336, Potato dextrose agar (PDA)
Diced potatoes.....300.0 g
Glucose.....20.0 g
Agar.....15.0 g
Distilled water.....1.0 L
Boil finely diced potatoes in 500 ml of water until thoroughly cooked; filter through cheesecloth and add water to filtrate to 1.0 L. Heat the filtrate to dissolve the agar. Add the glucose before sterilization. Autoclave at 121 C for 15 minutes.

[1]Whole Foods Certified Organic Golden Potatoes. Certified by Quality Assurance International. Distributed by Whole Foods Market, Austin, TX, 78703. Purchased in San Diego, CA.

[2]Domino Certified Organic Sugar. Certified by Quality Assurance International. Product of Paraguay. Distributed by Domino Foods Inc., Yonkers, NY 10705. (800) 729-4840.

[3]Phytotechnology Laboratories, A296. Agar, Plant TC Micropropagation Grade, CAS# 9002-18-0, Lot 06J29639A. Shawnee Mission, KS 66282. (913) 341-5343.

[4]BakOmega Organic Flax Flour. King Arthur Flour Item #3322. Certified Organic by NOFA-VT.

Table 2: malt extract-based media

P-MEA = Phytotechnology Labs Malt Extract Broth (M484)[1] 20 g/l, agar (A296) 15 g/l. pH = 6.6.

O-MEA (for one liter)

Organic sugar	**4 g**
Organic DME[2]	**10 g**
water	**1 l**
agar	**15 g**
pH = 6.6	

O-MEA-YW = add 4 g per liter of Marroquin international organic yeast autolysate flakes (with yeast flour, item 25107.30.0)[3]. pH = 6.0.

O-MEA-YR = add 4 g per liter of Marroquin international organic yeast autolysate flakes (with rice flour, item 25182.30.0)[3]. pH = 6.2.

Autoclave media for 30 min. at 121°C.

Agar is acceptable as per section 205.605 "Nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as 'organic' or 'made with organic (specified ingredients or food groups(s)).'"

[1]M484 contains 17 g/l malt extract, 3 g/l peptone from meat.

[2]Organic DME is produced by Briess and distributed by Northern Brewer, 1150 Grand Avenue St. Paul, MN 55105.

[3]Generously supplied by Marroquin International Commodity Services, 303 Potrero St. #18, Santa Cruz, CA 95060, certified organic by Lacon.

MEA composition based upon ATCC Medium 196 (Yeast Malt Extract Agar)

Yeast extract	4.0 g
Glucose	4.0 g
Malt extract	10.0 g
Distilled water	1.0 L
Agar	15.0 g

Table 3. Compost Extract Agar

CEA (1 liter)

Add 100 g air dried compost to 1 l water, autoclaved one hour at 121 degrees C.
Filter twice through three layers cheesecloth, increase volume to 1 l.
Add 15 g agar, autoclave 20 min.

Based on the medium used by Vija L. Wilkinson at the Pennsylvania State University Department of Plant Pathology.

RESULTS

Figure 1 shows the growth of *P. ostreatus* mycelia on conventional and organic potato-based media. The organic media performed better than the conventional media, with flax-supplemented PSA performing best. Figure 2 shows the growth of mycelia on conventional and organic malt extract media. The performance of all media was comparable.

A. bisporus M459 grew more slowly than the *P. ostreatus* culture and responded differently to the various media. Of the potato-based media, the commercial PDA performed best (Fig. 3), suggesting that if certified organic media are required, malt extract might be a better choice. Figure 4 shows that the organic MEA performed significantly better than the commercial MEA, though supplements to the organic MEA appeared detrimental to growth rate.

Since organic composts are readily available and a compost extract agar (CEA) was the medium selected by the culture collection at Pennsylvania State University, a variation on their method for CEA was tested versus the best performing medium found for *A. bisporus* (organic MEA). Though the vigor of the starting culture was lower (transfers were taken from the same original plate), all three organic compost extract based media performed significantly better than the organic MEA.

Conclusion

Basal culture media in solid and liquid form can be prepared using readily available ingredients that are complaint with USDA organic standards. These media should perform at least as well as commercial media. In the case of *P.ostreatus*, the best medium proved to be a flax-supplemented potato sucrose agar comprised of baking flax flour and organic sugar added to an extract of organic potatoes. For a rapidly growing strain such as the one used here, the

addition of relatively insoluble flour or other components can be excluded with minimal effect on growth.

A. bisporus grew best on a simple compost extract agar, but also grew well on an organic malt extract medium. Supplementing a compost extraction may be the most direct method to find an optimal medium for other compost-loving basidiomycetes.

The important conclusion from this study is that simple media prepared using ingredients that are compliant with USDA Organic Standards can perform as well as commercially available growth media. While the differences in *P. ostreatus* growth were often statistically significant, increases in growth rate may not be practically significant as growth on commercial media lags by a short time. The differences in exponential growth rate do not result in a significant time savings when using solid media but are desirable for the use of liquid media for primary spawn cultures.

It is relatively simple to test a variety of components by adding them to a boiling or autoclaved extract to quickly identify a good medium for a new or stubborn culture. Organic flours, grains and other food items can be taken advangtage of to optimize growth and maintain media that are compliant with U.S.D.A. organic standards.

[1]205.208 Mushroom practice standard

(a) The producer must maintain a production environment that prevents contact between organically produced mushrooms and prohibited substances throughout the entire growing cycle, harvesting and post harvesting process. The producer must not use lumber treated with arsenate or other prohibited materials for new installations or replacement purposes in contact with the growth substrate.

(b) The producer must use organically produced spawn, Except, that nonorganically produced spawn that that have not been treated with a prohibited substance and have not been raised on GMO substrate may be used when organically produced spawn are not commercially available.

(c) Agricultural materials including grain and straw that are used in production substrate must be organically produced. Sawdust, logs or other materials derived from wood used as a growth substrate must originate from trees that have been grown in areas free of prohibited materials for at least three years, and must not have been treated with a prohibited substance after tree harvest. Producers may include nonsynthetic, nonagricultural materials in substrate used to produce mushrooms.

(d) Manure and any nonorganic agricultural material used as a growth substrate must be composted. Compost used as a growth substrate must be produced in accordance with compost guidelines presented in 205.203 (c) (2)

(e) Sanitizers and disinfectants not on the National List for such purpose may not be applied to crops or growing substrates

Acknowledgements

We acknowledge the help of Vija Wilkinson, who provided invaluable advice and propagation of mushroom cultures as well as information on media compositions.

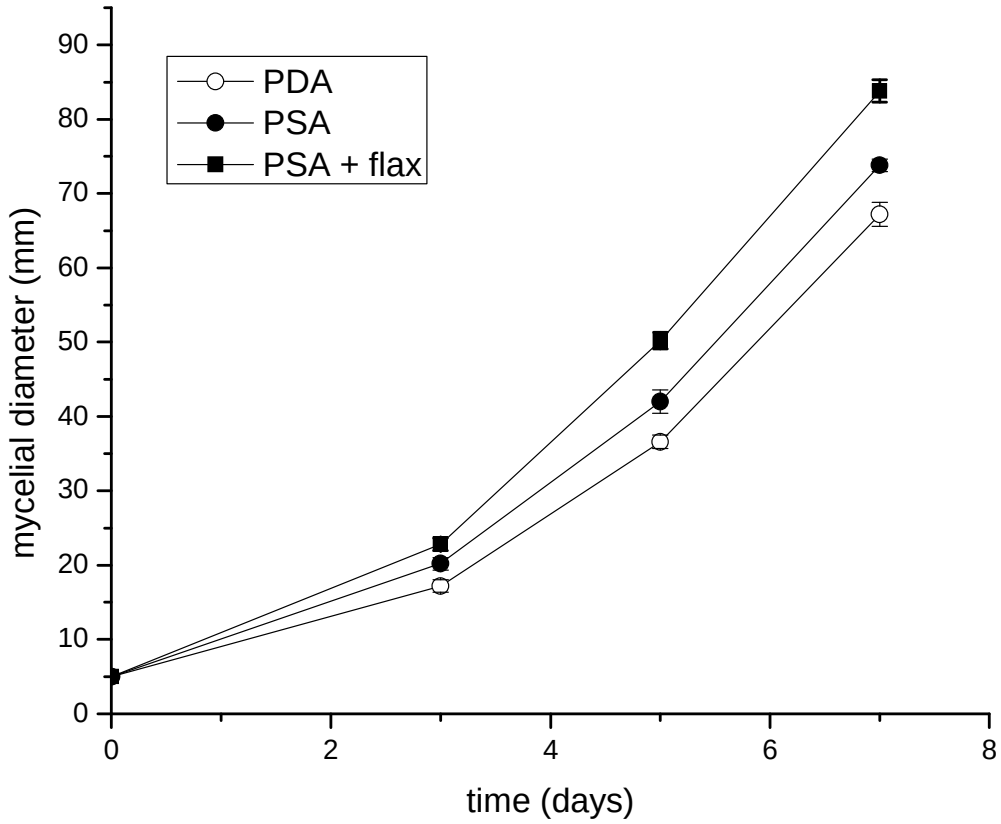

Figure 1. Growth of *P. ostreatus* mycelia on commercial PDA (open circles), organic PSA (closed circles), and organic PSA supplemented with flax flour (solid squares). Data are mean ± standard deviation (n = 5).

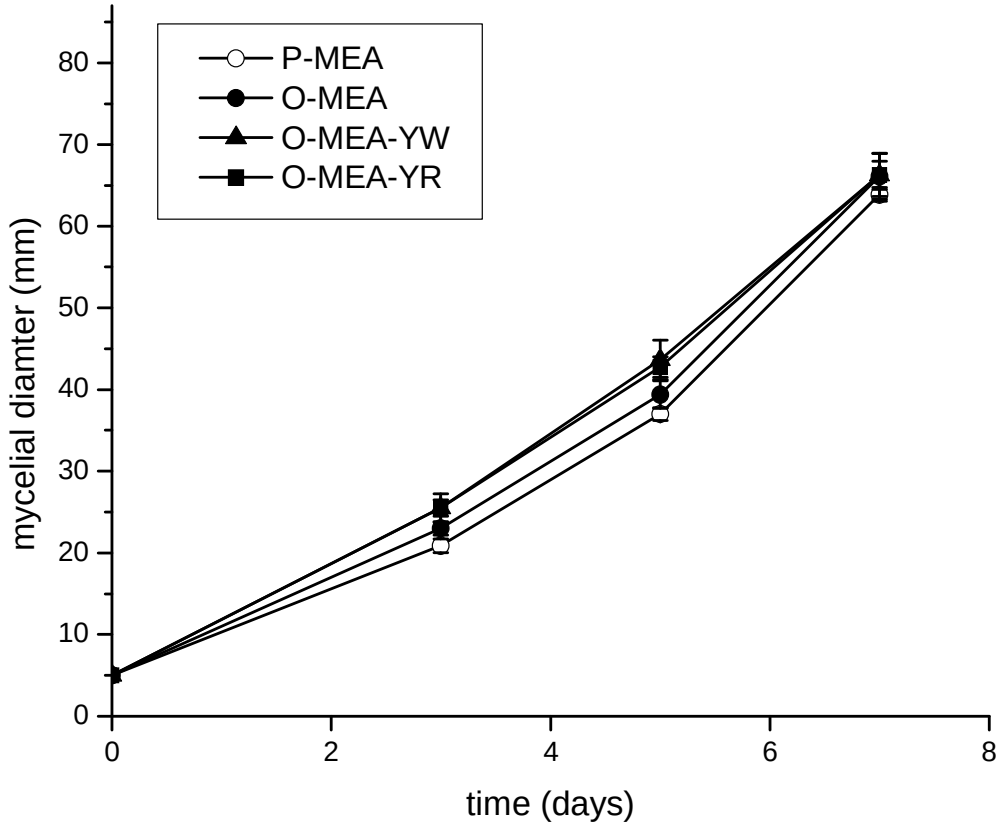

Figure 2. Growth of *P. ostreatus* mycelia on commercial MEA (open circles), organic MEA (solid circles), organic MEA supplemented with yeast extract plus wheat (triangles) or yeast extract plus rice flour (squares). Data are mean ± standard deviation (n = 4).

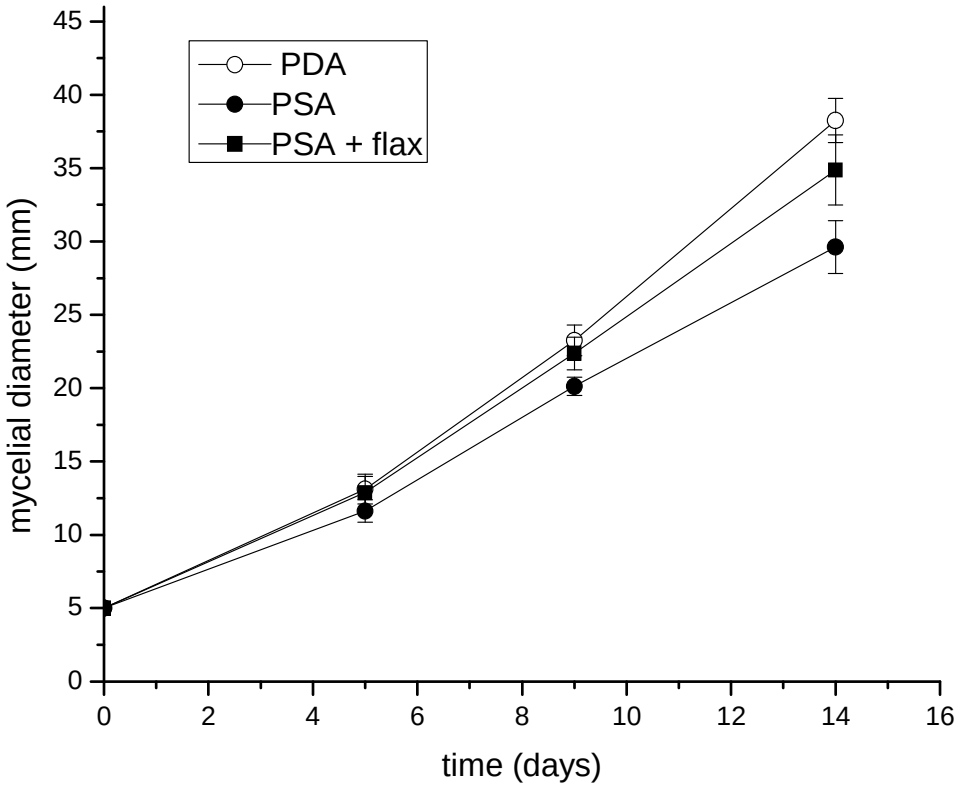

Figure 3. Growth of *A. bisporus* mycelia on commercial PDA (open circles), organic PSA (closed circles), and organic PSA supplemented with flax flour (solid squares). Data are mean ± standard deviation (n = 4).

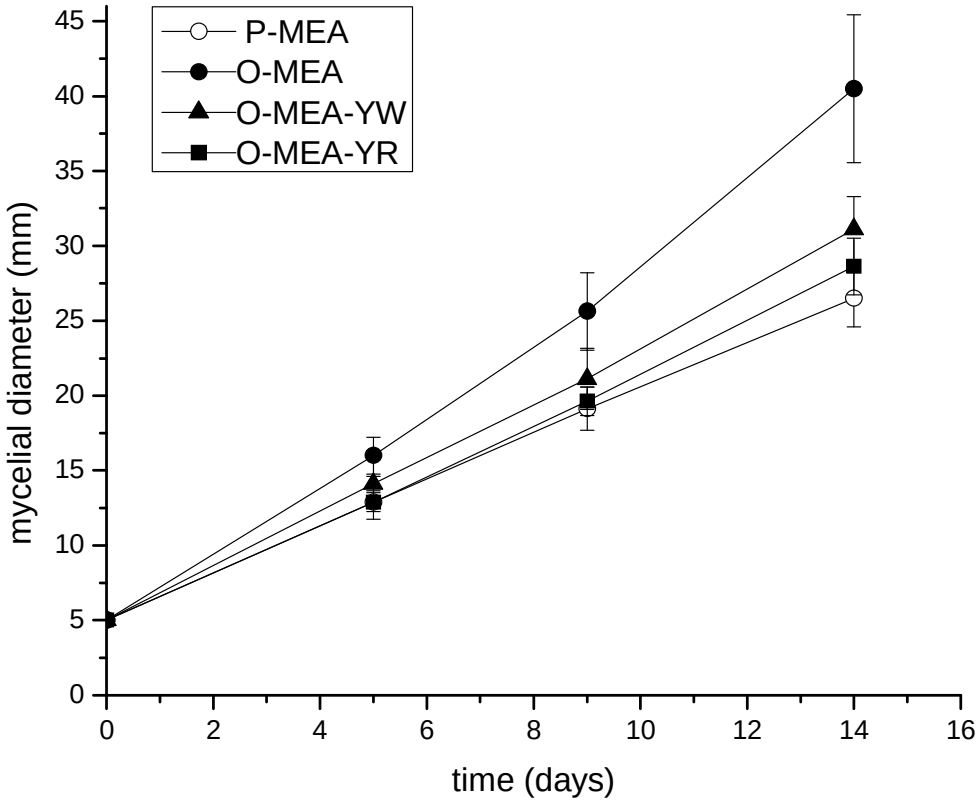

Figure 4. Growth of *A. bisporus* mycelia on commercial MEA (open circles), organic MEA (solid circles), organic MEA supplemented with yeast extract plus wheat (triangles) or yeast extract plus rice flour (squares). Data are mean ± standard deviation (n = 4).

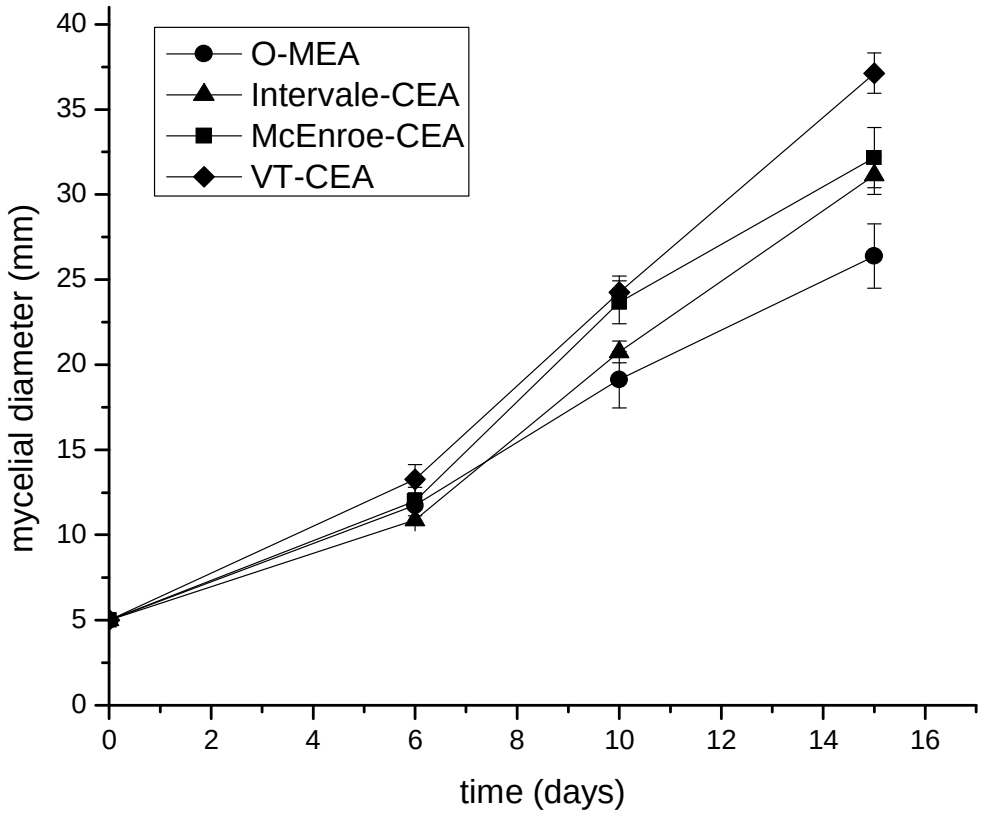

Figure 5. Growth of *A. bisporus* mycelia on organic malt extract agar (closed circles), or media made with extracts of organic compost from Intervale (triangles), McEnroe (squares) or Vermont Compost (diamonds).