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Research Article

Evaluation of Using Amylopectin of White Rice (*Oryza sativa*) Endosperm Powder in Substituting Agar for the Preparation of Growth Media for Culturing Microorganisms

Mona W Abdalla[1], Yassir Mohammed Abdelrahim[2] and Mutaman AA Kehail[3]*

[1]Department of Food Engineering and Technology, Faculty of Engineering and Technology, University of Gezira, Sudan

[2]Department of Molecular Biology, Faculty of Science, University of Gezira, Sudan

[3]Department of Zoology, Faculty of Science, University of Gezira, Sudan

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***Corresponding author:** Mutaman AA Kehail, Department of Zoology, Faculty of Science, University of Gezira, Sudan, Email: k.mutaman@yahoo.com

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Abstract

Agar is usually mixed with other nutrients to make media, in which microorganisms can be cultured and tested. This study aimed to formulate white rice powder to substitute agar for the preparation of growth media to culture some bacterial and fungal strains. Following the standard methods, nutrient agar, potato dextrose agar, and blood agar media were carefully prepared. Following the same methods, rice-based media were prepared by adding rice powder instead of agar. Pure strains of *E. coli, Staphylococcus aureus, Rhizopus stolonifer,* and *Aspergillus niger* were used to evaluate growth rates (colony formation for bacteria) and mycelial radial growth (for fungi) within agar-based media and rice-based media. There was no significant difference between the growth rate within agar-based media and rice-based media for bacterial strains, but fungal strains were well flourished better within the rice-based media than bacterial strains. Although rice is a potential material for the solidification of nutrient media instead of agar, it can also add important nutritional components, and the amount of rice sufficient to result in the required solidification should be considered, which involves rice varieties.

Introduction

The Asian rice (*Oryza sative* L.) is a monocot plant, and its grains are long, medium, and short-seeded. Long-seed rice is characterized by a high amount of amylose (the sticky substance), while the medium-size rice grain is characterized by a high amount of amylopectin, which makes it stickier than the long-seed rice [1]. The world production of rice-grain during 2022 was 756.7 million metric tons [2].

Before cooking, most of the starch from the rice grain will be lost when rinsed, hence, the ability of rice grain to sticking together will be reduced [2], but when cooked, high amount of water (about 68%) will be added to its nutritional contents, hence, the other components will be decreases (*e.g.*, carbohydrates will be (*ca.* 28%), and proteins (*ca.* 3%)) and it provided about 130 kilocalories per 100 g [3,4]. The nutritional contents for the non-cooked grains show carbohydrates as a main constituent (*ca.* 89%), protein (*ca.* 8%), and few amount of fiber (*ca.* 1.5%) and fat (less than 1%). Several important minerals (Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn, and Se), vitamins (B1, B2, B3, B5, B6, B9, Vitamin E, and Vitamin K), and fatty acids (saturated, monounsaturated, and polyunsaturated) are also detected [5]. Some bacterial spores (*e.g.*, *Bacillus cereus*) were detected in rice, and they can produce toxins if rice is stored within 4–60 °C, though rapid cooling of the cooked rice will reduce toxin production [6].

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Agar is usually obtained from some red algae species, and it is a polysaccharide that acquires gelatinous properties [2]; hence, it is usually added according to certain formulas so as to produce a certain level of solidification in media [7]. It consists of 70% agarose and 30% agaropectin. Agarose is made of D-galactose and 3,6-anhydro-L-galactopyranose, while agaropectin is made of D-galactose and L-galactose [8]. Agar can be solidified as a gel-like substance when mixed with water during the preparation of media of certain types: nutrient and nutrient-rich (for bacterial growth), or selective agar (by adding certain materials, as in tissue culture). Agar media can be melted over 85 °C [9,10].

The agar culture media are usually prepared for most species of bacteria (*e.g.*, nutrient, plate count, trypticase soy), while minimal media is generally prepared without amino acids, whereas selective media is used to culture antibiotic-resistant microbes and in cell culture. Blood agar media is usually used for *Staphylococcus* or *Streptococcus* bacteria, while MacConkey agar is used for lactose fermenter bacteria. Transport media contains only buffers and salt and is used for temporarily storing specimens [11,12].

The bread mold *Rhizopus stolonifer* (*Rhizopodaceae)* is a saprotrophic fungus with a global distribution, and it can be detected everywhere (*e.g.*, soil, air, and organic matter). It can colonize high sugar-content plant tissue and cause rotting. It has sexual (by means of sporangiophores) and asexual (by means of the stolon, which can spread vertically and horizontally) manner of reproduction [13-15].

The common mold, *Aspergillus niger* (ascomycota), is an aerobic fungus distributed within plants, soil, and water. It can grow and germinate rapidly and can withstand a range of 6 - 47 °C and 1.5 – 9.8 pH. It usually grows on potato dextrose agar. It produces a wide variety of mycotoxins that cause plant, animal, and human diseases [16-18].

Escherichia coli is a gram-negative, anaerobic, coliform bacterium that is commonly found in the lower intestine (colon) of humans, and it is considered a part of the normal microbiota. It can survive outside the human body for a limited time. It can be used as a bio-indicator for fecal contamination. Some strains can cause serious health problems to humans, and even tissue death [19].

Staphylococcus aureus is a bacillus, anaerobic, gram-positive, and spherical-shaped bacterium. It is usually found in the upper respiratory tract and on the skin, and can become an opportunistic pathogen. It causes skin infections and is widely associated with antibiotic resistance [20,21].

The current work aimed to evaluate the growth rate of some microorganisms on agar-based media and on white rice-based media.

Materials and methods

Materials

The medium-size white rice grains (trade name: Alfrasha) were brought from the local market of Wad Medani City, Gezira State, Sudan, while the materials and devices required for preparation, culture and incubation of the selected microorganisms were provided by the Biosciences and Biotechnology Center, University of Gezira, whereas the pure strains of *E. coli* (O25:H4) and *S. aureus* (SO-1977) bacteria, *Aspergillus niger* (ATCCÂ®6275TM) and *Rhizopus stolonifer* (19A–0010) fungi were brought from the Central Medial Laboratory, University of Gezira.

Preparation of white rice powder

The white rice grains were cleaned manually and ground using an electrical grinder, then sieved by a fine plastic mesh to about 450 μm particle size. This powder was served in a clean plastic container.

Preparation of agar-based culture media

Potato-dextrose agar media for A. niger and R. stolonifera: From 100 g boiled potato slices, 3 g infused potato was prepared first, and mixed with 10 g dextrose, 10 g agar, then boiled with 500 ml distilled water at 121 °C and 15 lbs for 20 minutes, cooled to about 60 °C, and then poured into sterile plastic petri dishes (100 mm × 15 mm). The plates were allowed to cool and solidify at room temperature and pH of 5.6 ± 0.2 [22].

Nutrient agar media for *E. coli*: A mixture of 5 g peptone, 2.5 g yeast extract, 2.5 g NaCl, and 7.5 g agar was boiled with 500 ml distilled water at 121 °C and 15 lbs for 30 minutes, cooled to about 60 °C, and then poured into sterile plastic petri dishes (100 mm × 15 mm). The plates were allowed to cool and solidify at room temperature and a pH of 7.0 [19].

Blood agar media for S. aureus: A mixture of nutrient agar powder (5 g of peptone, 2.5% yeast extract, 5 g agar, and 2.5 g NaCl) and 500 ml distilled water was heated, stirred to fully dissolve all components, and autoclaved at 121 °C and 15 lbs for 15 minutes, cooled to 45-50 °C. 5% (v/v) sterile defibrinated blood was added and mixed well, then the prepared media was poured into sterile plates (100 mm × 15 mm) before solidifying. pH was adjusted to 7.2 – 7.6 [20].

Preparation of white rice-based culture media

The agar component within BDA, nutrient, and blood agar media was substituted with a certain amount of white rice fine powder (25 g for BDA media, 20 g for nutrient agar, and 12.5 g for the blood agar media). The periods for autoclaving media were extended (50 minutes for BDA and nutrient agar, and 35 minutes for blood agar media) to allow the nutrient component to dissolve and distribute. The rice-based media, when poured onto the Petri-dishes, were stored at 4 °C for 30 minutes before further steps.

Culture and incubation of microorganisms

The prepared petri-dishes that were provided with agar-based media and rice-based media were streaked with the appropriate microorganism strains. A Biosafety Level 2 (BSL-2) cabinet, provided with a fan, ultraviolet lamp, and flame, was used. The petri-dishes were incubated at 37 °C under aerobic conditions for 48 hours.

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Determination of growth parameter

Colony-forming unit (CFU): After 48 hours, each of the culture media that contained *E. coli* or *S. aureus* was transferred to a glass bottle (stock) containing 0.1% peptone solution (composed of 0.5 g peptone and 500 ml distilled water). A series of 6 test tubes, each containing 9 ml of 0.1% peptone solution, was set. 1.0 ml of the stock solution was pipetted to the first tube and shaken well to form the concentration of (10^{-1}), and the same process was repeated to form (10^{-2} to 10^{-6}) concentrations. 1.0 ml of each of the 4th, 5th, and 6th tubes was added to the count plate to estimate the number of the formed colony which was calculated according to the formula:

CFU/ml = No. of colony X dilution factor/volume of culture plate

Mycelial growth: The radial growth of *A. niger* and *R. stolonifer* within their culture plates was measured in mm/h. The diameter of the growth was measured every 8 hours for 48 hours.

Data analysis

Each test was triplicated. For the data of the mycelial growth (diameter (in mm) and time (hours), regression analysis was used to estimate the growth in mm/h. After the growth parameters were measured for the tested microorganisms within the agar-based and rice-based media, an ANOVA analysis for significant differences in growth level within these media was conducted.

Results

Appears that one of the prepared rice-based media

The prepared rice-based media show the same level of transparency, thickness, and viscosity (their resistance to flow when flipped vertically), as same as agar-based media. Blood rice media appeared in a light red or pink color, while Potato-dextrose rice and nutrient rice media were a pale white color (Plate 1). All media did not exceed 2-3 mm thick.

Growth parameter within the agar-based and rice-based media

Bacterial strains: Table 1 and Plate 2 showed that the means of cfu/ml of *E. coli* colonies were 64.33 x 10^{-6} on the agar-based media, and 60.33 x 10^{-6} on the rice-based media, while that of *S. aureus* colonies were 73.67 x 10^{-6} on the agar-based media, and 68.0 x 10^{-6} cfu/ml on the rice-based media. It was also noticed that *S. aureus* counted colonies were relatively more than those of *E. coli*, also the counted colonies in the agar-based media were relatively more than those formed on rice-based media, but ANOVA analysis accurate a non-significant difference between the growth rate of these species within the tested media (p-value = 0.25; which is more than 0.05).

Fungal strains: Table 2 and Plate 3 showed that the means of mycelial radial growth of *A. niger* were 0.104 (mm/h) on the agar-based media, and 0.153 on the rice-based media, while that of *R. stolonifer* was 0.46 on the agar-based media, and 0.56 (mm/h) on the rice-based media. It was also noticed that *R. stolonifer* mycelial growth was relatively more than that of *A. niger*; also, the counted colonies in the agar-based media were relatively fewer than those formed on rice-based media. ANOVA analysis shows a non-significant difference between mycelial growth on the different media (p-value is more than 0.05), but there a clear evidence that *R. stolonifer* is growing faster than *A. niger* (mean±SE did not interfere).

Plate 1: Nutrient-rice media for *E. coli* (left) and Blood-rice media for *Staph aureus* (right).

Table 1: No. Of colony-forming formed (CFU) of *E. coli* and *S. aureus* on agar-based and white rice-based media after 48 hours.

Species	*E. coli* (10^9/ml)		*S. aureus* (10^9/ml)	
Media	Agar-based	White rice-based	Agar-based	White rice-based
Replicates	56	65	80	69
	73	67	79	70
	64	61	62	65
ANOVA analysis				
Mean	64.33	60.33	73.67	68.0
Standard error	4.91	4.06	5.84	1.53
Variance	72.33	49.33	102.33	7.0
F	F-cal. = 1.668; F-crit. = 4.066			
p-value	0.250			

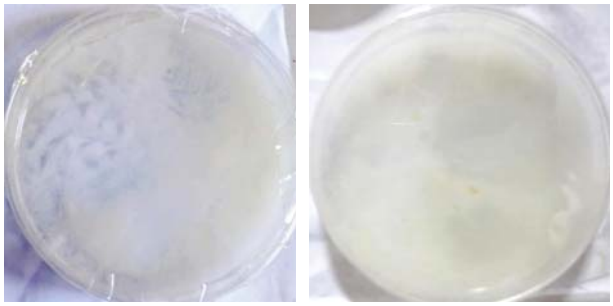

Plate 2: *E. coli* formed colonies (left) and *Staph. Aureus* formed colonies (right).

Table 2: Estimated mycelial radial growth (mm/h) of *A. niger* and *R. stolonifer* on agar-based and white rice-based media after 48 hours.

Species	*A. niger* (mm/h)		*R. stolonifer* (mm/h)	
Media	Agar-based	White rice-based	Agar-based	White rice-based
Replication	0.083	0.125	0.42	0.54
	0.104	0.208	0.51	0.63
	0.125	0.125	0.45	0.51
ANOVA analysis				
Mean	0.104	0.153	0.460	0.560
Standard error	0.012	0.027	0.026	0.036
Variance	0.0004	0.0023	0.0621	0.0031
F	F-cal. = 2.596; F-crit. = 7.709		F-cal. = 5.0; F-crit. = 7.709	
p -value	0.182		0.089	

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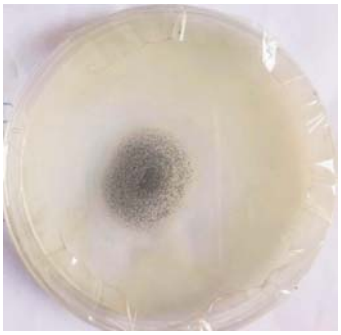

Plate 3: *R. stolonifer* formed hyphae.

Discussion

The proximate analysis of white rice grains determined 6.3% moisture, 2.2% ash, 0.6% oil, 7.8% protein, 3.5% fiber, and 79.6% carbohydrates. This data did not differ greatly from that determined by USDA [5], in which carbohydrate was about 89%, protein was 8%, fat was 0.8% and fiber was 1.5%. According to the obtained data, the energy units (in cal) that can be estimated from 100 g of white rice grain were 355.

For the preparation of white rice-based media, rice was ground to a fine powder so as to increase the surface area, which allows the dissolution of all possible components. This powder is used directly during preparation in as same way as agar. The powder did not rinse because rinsing rice before cooking removes much of the starch, thereby reducing the sticky property [2].

Usually, agar within media is not digested by many organisms, and it remains stable (*i.e.*, unconsumed material), unlike what was expected from most components of white rice powder within the media. Agar and nutrients are mixed as the formulation described for producing certain levels of viscosity for microorganism media [7]. The medium-grain rice contains the same properties because of the amylopectin content (the sticky agent) [1].

As in Table 1, the formed colonies of *E. coli* were 60.33 × 10^{-6} on the rice-based media, while those of *S. aureus* colonies were 68.0 × 10^{-6}. Also, *S. aureus* formed colonies were relatively more than those of *E. coli*, and the counted colonies in the agar-based media were relatively more than those formed on rice-based media, but with a non-significant difference. *In a study conducted by Son and Taylor [19], the estimated growing colonies of E. coli* were more than 10^{9} cfu/ml. In this study, although the count was less than 10^{9} but it may be attributed to the difference in strains, materials, and test conditions.

The same was noticed on the growth rate of *S. aureus* colonies, which is affected by the source of blood. The average formed colony unit was 128 within sheep blood media, but it was only 31 within human B-blood type, and 80 within human O-blood type [20].

Although *E. coli* is anaerobic, it can survive in the air for a limited time [19]. This may explain why its growth rate was less than that of *S. aureus* with respect to the preparation conditions mentioned in the preparation of media section. *S. aureus* is also an anaerobic microorganism, but it can be found in the aerial areas of human skin [20], and it seemed to be more tolerant to withstand the aerobic condition than *E. coli*, as was noticed in the results of this study.

Table 2 showed that the mean mycelial radial growth of *A. niger* was 0.153 mm/h (3.7 mm/d) on the rice-based media, while that of *R. stolonifer* was 13.44 mm/d on the rice-based media. It was also clear that *R. stolonifer* mycelial growth was significantly more than that of *A. niger*; also, the growth rate in the agar-based media was significantly similar to that on rice-based media.

In a recent study, the radial growth rate of *R. stolonifer* (Ah-Rs-01 strain) was found to be affected by temperature. Low growth (8 (mm/d) was noticed at 13 °C, while a higher growth rate (18.9 (mm/d) was noticed at 25 °C, but the growth rate was decreased greatly (0.6 mm/d) at 35 °C [23]. It was also noticed that *R. stolonifer* can invade plant tissues with higher sugar content [14]. Although white rice contains more than 75% carbohydrates, this may explain why this fungus flourishes within the rice-based media than agar-based media, and even, than *A. niger*. In another study, the growth rate of *A. niger* on lime fruits was estimated to be 3.3 (mm/d) at 25 °C, 3.6 at 30 °C, 4.1 at 35 °C and decreased to 1.9 (mm/d) at 37 °C [24]. These growth rates did not differ from those obtained in this study (2.5 – 3.7 mm/d).

A. niger is found throughout the environment and can withstand temperatures ranging from 6 to 47 °C, and pH ranging from 1.5 to 9.8. It can withstand extreme acidic conditions, and usually grows on PDA media [16,18].

Some studies mentioned the trial tests to use rice as a component of the media. During 1985, Rice Extract Agar (5 g white rice extract and 20 g agar) was used for *Candida albicans* and *C. stellatoidea* to differentiate them from other *Candida* species [25]. Later in 2018, rice husk was used for the formulation of media for cultivating fungi. The media was found to be good for isolation and cultivation of *Saccharomyces carlsbergensis* and *Penicillium* species [26]. In the same year, rice water-based crude medium (4%) gave effective growth of *Rhizopus* and *Aspergillus* [27].

The use of rice powder (which is cheap and nutritious) to prepare different microbiological media did not require an extraction or separation process because the whole components of the white rice powder contained other nutrient constituents besides the gelatinizing agent (amylopectin). Other natural vitamin-rich fresh materials can be added to improve the rice-based media with additional constituents that are favored by many microorganisms. Further studies should test the growth rate of other microorganisms using different formulas.

Conclusion

It is the first time ever to introduce white rice powder (as a source of amylopectin) to substitute agar in the preparation of agar-based media to culture some bacterial and fungal strains. Promising results were obtained; hence, further formulations should be tested for the other bacterial and fungal species. The

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rice-based media is very useful for fungal culture, although it gives comparable growth to agar-based media on bacterial strains.

Although agar was usually mixed with nutrients in a certain amount to produce certain levels of viscosity for certain microorganisms, the amount of rice required to result in the same solidification should be considered. Carrot (*Daucus carota*) contains vitamin A, C, and K (which are not found in white rice) among other nutrient components, and its dried powder or fresh juice may improve the growth media.

Author contributions

Mona W.A. and Mutaman A.K., methodology, software, validation, writing original draft, and formal analysis, Yassir M.A., supervision, investigation, and resources, Mutaman A.K., writing review and editing. All authors have read and agreed to the published version of the manuscript.

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