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Proceeding Paper

Effect of Carbon, Nitrogen and Salt Sources on the Growth of *Monascus purpureus* in Quinoa (*Chenopodium quinoa*)-Based Culture Media †

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Abstract: *Monascus purpureus* is produced in Asia and used as a food dye and for medicinal purposes. In the present study, the radial growth of quinoa flour-based culture media was evaluated on the tenth day after cultivation with different sources of carbon (glucose, fructose and molasses), nitrogen (monosodium glutamate, fermented fish and fish hydrolysate) and sodium chloride in two percentages (0.5% and 1%) at different pH (5, 6 and 7). The highest value obtained was 72.59 mm with a radial growth rate of 3.629 mm/day, corresponding to the effect of 0.5% (*w/v*) sodium chloride at pH 6, and the lowest value was 42.05 mm with a radial growth rate of 2.10 mm/day, due to the effect of 0.5% (*w/v*) monosodium glutamate at pH 7. From this investigation, it was deduced that different sources of carbon, nitrogen and sodium chloride have effects on the development of *M. purpureus* and that factors such as pH and supplement concentration did not cause changes in the morphology of the colonies.

Keywords: exponential phase; kinetics; supplement; mycelium; radial measurement

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1. Introduction

Monascus purpureus also known as "red rice koji", "ang kak", "akakoji", "anka", and misnamed "red rice yeast", as it is a filamentous fungus, has been consumed in Asia since 800 BC [1] as a traditional food ingredient, for food coloring and medicinal use. *Monascus* has also been used to make fermented foods, red soybean cheese, red wine, medicines and to preserve meat [2]. Therefore, there are numerous pigments produced using *Monascus* with a worldwide economic value as a natural coloring agent, as well as being produced on cheap substrates and being easily extractable with solvents such as water and ethanol. Researchers are trying to replace synthetic food coloring with natural *Monascus* pigments, as they improve the sensory characteristics of food. In pharmacology and medicine, *Monascus* pigments have wide uses in the prevention and treatment of numerous human diseases due to their antioxidant, antihypertensive, anti-inflammatory, neuroprotective, antihyperlipidemic, antitumor, antibiosis properties, etc. [3].

On the other hand, quinoa (*Chenopodium quinoa* Willd) is an herbaceous plant belonging to the Chenopodiaceae family, and was cultivated and consumed 5000 years ago by the

populations of the Andean indigenous regions [4]. In recent years, quinoa has been recognized as an alternative crop to cereals due to its excellent nutritional value. It is currently grown in mainly Peru, Bolivia, Ecuador and Chile, from where it is exported, with Peru being the main producer (59.8%), followed by Bolivia (38.8%). This grain gained increasing attention, being widely promoted in 2013 by the Food and Agriculture Organization of the United Nations [5]. Nitrogen and pH affect the culture conditions in the biosynthesis of pigment production [6].

In this research, we propose to evaluate the growth of *M. purpureus* in quinoa flour-based culture media enriched with different carbon, nitrogen and salt supplements.

2. Materials and Methods

2.1. Inoculum Production

The filamentous fungus *M. purpureus* CECT 2955 from the Spanish Type Culture Collection (CECT) was used. The instructions for the resuspension of lyophilized cultures from the CECT were followed. After activation, the micro-organism was seeded in Petri dishes with PDA medium (HiMEDIA), incubated at 30 °C for 7 days, and stored at 4 °C for later use. The strain was streak inoculated onto a Petri dish containing QFA: quinoa flour agar medium (quinoa 5% *w/v*, 1.5% *w/v* agar, and 100 mL distilled water). It was incubated at 30 °C for 7 days. After this time, the medium invaded by the fungus was liquefied with sterile water at room temperature for 15 s. The homogeneous inoculum was used for the investigation.

2.2. Growth Experiment

2.2.1. Preparation of Culture Media and Inoculation

The culture media proposed for this research were based on quinoa flour agar supplemented at 0.5% and 1% with glucose, fructose, monosodium glutamate and sodium chloride (CDH, India), and molasses, fermented fish and fish hydrolysate (donated by the Environmental Biotechnology Laboratory—Bioremediation UNALM). Each of the two concentrations of each supplement was tested at three pH levels 5, 6 and 7. The culture medium was poured per Petri dish (20 mL). After solidification, a well was made in the center of the culture medium with the help of a 0.5 mm diameter punch where 40 µL of the homogenate strain was inoculated per hole. Petri dishes were incubated at 30 °C for 10 days, and all treatments were performed in triplicate (three replicates).

2.2.2. Growth Rate

To evaluate the radial growth, two perpendicular lines were drawn at the base of each Petri dish. With a digital vernier, the colony diameter was measured after 10 days of incubation, making two measurements per plate. The average of these two measurements per plate was taken into account in each repetition. The radial growth rate was calculated by linear regression of the mean diameter as a function of time in mm per day.

2.3. Statistical Analysis

The results obtained for radial growth (mm) of *M. purpureus* were analyzed using ANOVA and Statgraphics 19 software (Statpoint Technologies Inc., Warrenton, VA, USA). Means were analyzed by Tukey's test, considering a significance level of 5% throughout the study. To compare the data, a completely randomized statistical design was used with a $7 \times 2 \times 3$ of the substrate, percentage of substrate and pH, respectively, in a factorial arrangement with three replicates.

3. Results and Discussion

Table 1 shows the results of the analysis of variance for the diameter (mm) of the *M. purpureus* growth trial. The highest diameter obtained was 72.59 mm, which corresponded to a radial growth rate of 3.629 mm/day, with the treatment of 0.5% (*w/v*) sodium chloride at pH 6. The lowest diameter 42.05 mm, corresponding to a radial growth rate of

2.10 mm/day, was obtained for the treatment with 0.5% (*w/v*) monosodium glutamate at pH 7. Tukey's test was applied, and significant differences ($p < 0.05$) were obtained only for the supplements.

Table 1. Analysis of variance for diameter (mm) obtained in vitro.

Sources of Variation	Sum of Squares	Df	Mean Square	F-Ratio	*p*-Value
Main effects					
A: Supplement	4227.09	6	704.515	28.12	0.0000
B: Concentration (%)	21.10	1	21.099	0.84	0.3611
C: pH	9.07	2	4.533	0.18	0.8348
Interactions					
AB	122.11	6	20.352	0.81	0.5630
AC	59.47	12	4.956	0.20	0.9983
BC	0.59	2	0.293	0.01	0.9884
Residuals	2405.39	96	25.056		
Total (Corrected)	6844.82	125			

Table 2 shows that no differences ($p > 0.05$) were found among the supplements of fish hydrolysate, fermented fish, fructose and molasses. No significant differences were found at the three pH levels (5, 6, 7), nor at the concentrations of 0.5 or 1% (data not shown), so the culture medium at pH 6 was chosen as it is close to the initial pH (5.81) of the culture medium formulation and the concentration of 0.5%, reducing costs and formulation time.

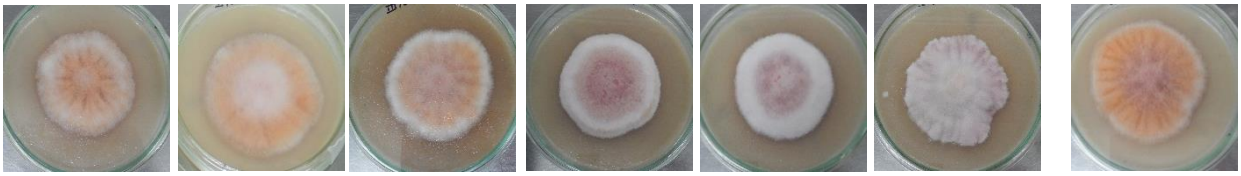
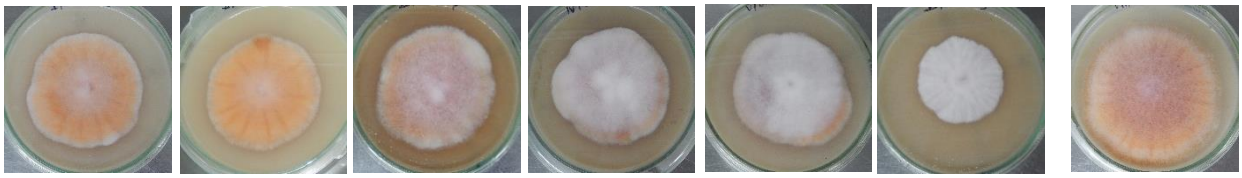
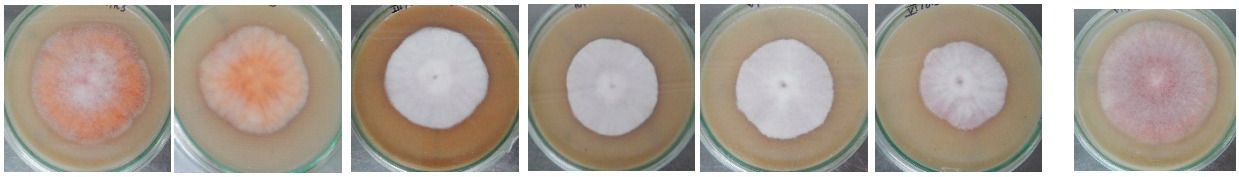
Table 2. Multiple range tests for the diameter (mm) per supplement used in quinoa flour-based culture media.

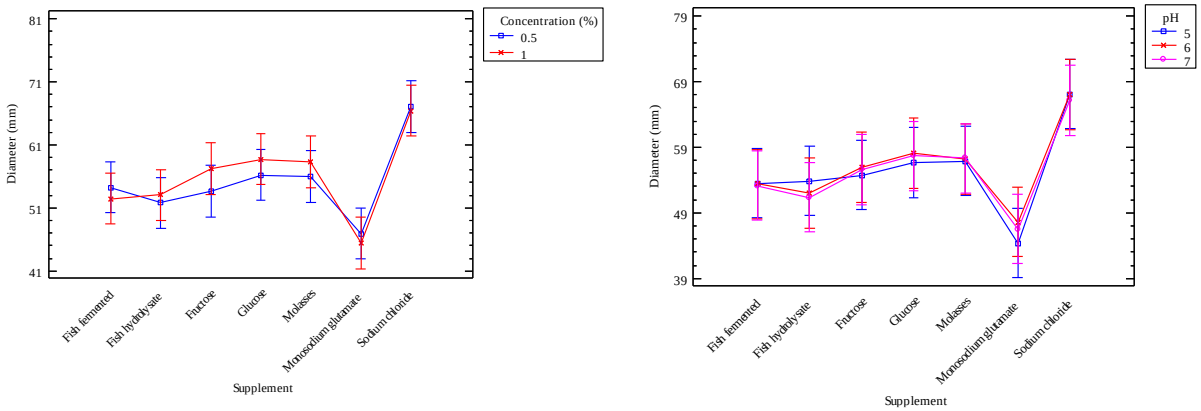
Supplement	Count	LS Mean	LS Sigma	Homogeneous Groups
Monosodium glutamate	18	46.165	1.1798	X
Fish hydrolysate	18	52.421	1.1798	X
Fish fermented	18	53.342	1.1798	X X
Fructose	18	55.444	1.1798	X X
Molasses	18	57.147	1.1798	X X
Glucose	18	57.482	1.1798	X
Sodium chloride	18	66.766	1.1798	X

Count: percentage of substrate (0.5, 1%) × pH (5, 6, 7) × three replicas; LS Mean: least squares mean; LS Sigma: least squares sigma.

Table 3 shows the mycelial development of *M. purpureus* on quinoa flour-based culture media enriched with seven supplements. After 240 h of incubation, the greatest radial growth occurred with the sodium chloride supplement at pH 5, 6 and 7 when compared with the other six supplements at their respective pH levels (Figure 1), as well as showing greater pigmentation both on the front of the plate and on the back (images not shown). On the other hand, it is observed that with the glucose and fructose supplements the hyphae were pigmented, which indicated that a stationary phase had been reached where the secondary metabolites were formed in their majority, among them the pigments, in contrast to the white areas on the mycelium, which would indicate that they were mainly forming biomass, such as the media containing molasses, fermented fish, fish hydrolysate and monosodium glutamate supplements at pH 7; it should be noted that the slowest growth and deformed edges of the mycelium occurred with the latter supplement.

Table 3. Mycelial development of *M. purpureus* in culture media based on quinoa flour supplemented with different sources of carbon, nitrogen and salts at 0.5% (*w/v*), at 3 pH levels (5, 6 and 7) after 10th days of incubation.

	Glucose	Fructose	Molasses	Fermented Fish	Fish Hydrolysate	Monosodium Glutamate	Sodium Chloride
5							
6							
7							



(**a**) (**b**)

Figure 1. The figure shows (**a**) the interactions of supplement and substrate concentration in percentages; and (**b**) the interaction of supplement and pH on the radial growth of the fungus in vitro.

4. Conclusions

This research determined that the growth of *M. purpureus* on quinoa flour agar is affected by the type of supplementation which causes changes in the growth rate and morphology of the fungus. Sodium chloride at a concentration of 0.5% (*w/v*) and pH 6 showed the highest growth of 72.59 mm with a radial growth rate of 3.629 mm/day. Supplements such as glucose and fructose affected the early pigment production observed in the culture plates. Finally, the lowest growth was produced with a concentration of 0.5% monosodium glutamate at pH 7.

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