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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: The pigment produced by *Monascus purpureus* is used in Asia as a food colouring and for medicinal purposes. The diametric growth was evaluated at the tenth day in culture media based on quinoa flour enriched with carbon, nitrogen and salt supplements, measured with a digital vernier where 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% sodium chloride at pH 6.

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

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1. Introduction

Monascus purpureus also known as "red rice yeast", "red rice koji", "ang kak", "akakoji", "anka" is consumed in Asia, since 800 A.C. [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 meat preservation [2]. Thus, the pigments produced by *Monascus* have high economic value worldwide, as a coloring agent, have many advantages, such as easy production on inexpensive substrates, good solubility in water and ethanol, numerous bioactive metabolites. Researchers are trying to replace synethetic food coloring with natural *Monascus* pigments, as they improve sensory characteristics in food. In pharmacology and medicine, *Monascus* pigments have wide uses in the prevention and treatment of numerous human diseases as antioxidant, antihypertensive, anti-inflammatory, neuroprotective, antihyperlipidemic, antitumor, antibiosis, etc [3].

On the other hand, quinoa (*Chenopodium quinoa* Willd) is a herbaceous plant belonging to the Chenopodiaceae family, it was cultivated and consumed since 5000 years ago in the populations of the Andean indigenous region [4]. In recent years, quinoa has been recognized as an alternative crop to cereals due to its excellent nutritional value. It is currently grown mainly in 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, becoming largely promoted by the Food and Agriculture

Organization of the United Nations, which dedicated 2013 to this plant [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. Microorganism

We worked with the filamentous fungus *Monascus purpureus* CECT 2955, from the Spanish Type Culture Collection (CECT). The instructions for resuspension of lyophilized cultures of CECT were followed. After activation, the microorganism was seeded in Petri dishes with PDA medium (HiMEDIA), where they were incubated at 30°C for 7 days, then preserved at 4°C for later use.

2.2. Inoculum Production

The strain was inoculated again in a Petri dish containing quinoa flour agar (QFA). It was incubated at 30°C for 7 days, and a roast of the previous plate was taken and striated by depletion with the help of a sterile swab. It was kept at 30°C for 7 days. After this time, the medium invaded by the fungus was liquefied with sterile water for 15 seconds. The homogeneous inoculum was used for the investigation.

2.3. Growth Experiment

2.3.1. Preparation of Culture Media

QFA was used as the base culture medium, in the ratio 1:20 g/ml; agar agar 1.5 %. The seven-culture media proposed for this research were based on quinoa flour agar supplemented at 0.5 and 1 % with: glucose, fructose, molasses, fermented fish, fish hydrolysate, monosodium glutamate and sodium chloride. The pH of the seven media was adjusted to 5, 6 and 7.

2.3.2. Inoculation

Culture medium was poured per Petri dish (20 ml). After solidification, a well (hole / pit) was made in the center of the culture medium with the help of a 0.5 mm diameter punch where the homogeneous strain was inoculated with 40 μL per hole. The petri dishes were incubated at 30°C for 10 days, and all treatmente were carried out in triplicate (three replicates).

2.3.2. Growth rate

To evaluate the diametricgrowth, 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 measurments per plate. The average of these 2 measuremnts per plate was taken into account for one repetition. The diametral growth rate was calculated by linear regression of the mean diameter as a function of time in mm per day.

2.4. Statistical Analysis

The results obtained for diametric growth (mm) of *M. purpureus* were analysed by ANOVA using Statgraphics 19 software (Statpoint Technologies Inc, USA). Means were analysed 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 7x2x3 factorial arrangement with three replications

3. Results and Discussions

Table 1 shows the results of the analysis of variance for 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 was 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 (α = 0.05), significant differences were obtained only in 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. 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

Table 2 shows that no differences ($P > 0.05$) were found among the supplements: fish hydrolysate, fermented fish, fructose and molasses. While the sodium chloride supplement showed the highest morphological growth evaluated by the diameter in vitro (Figure 1). The lowest growth was obtained with the monosodium glutamate supplemented.

No significant differences were found at the three pH levels (5, 6, 7), nor at the concentrations of 0.5, 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.

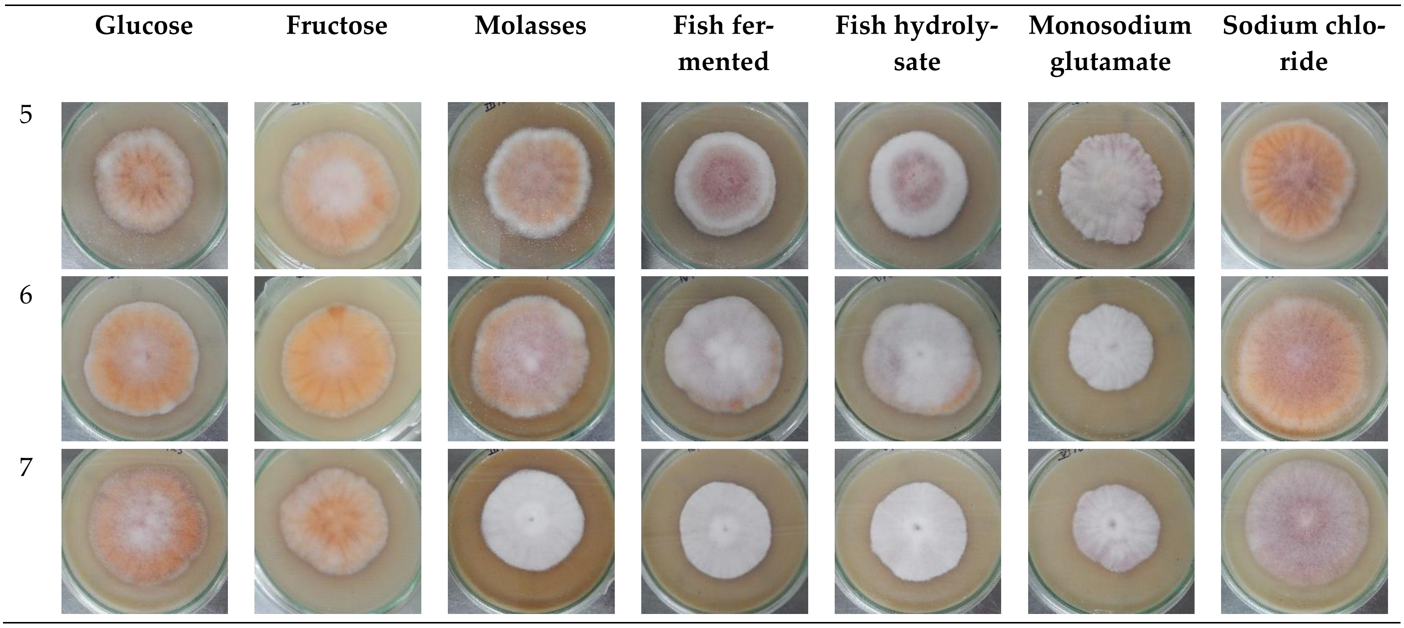
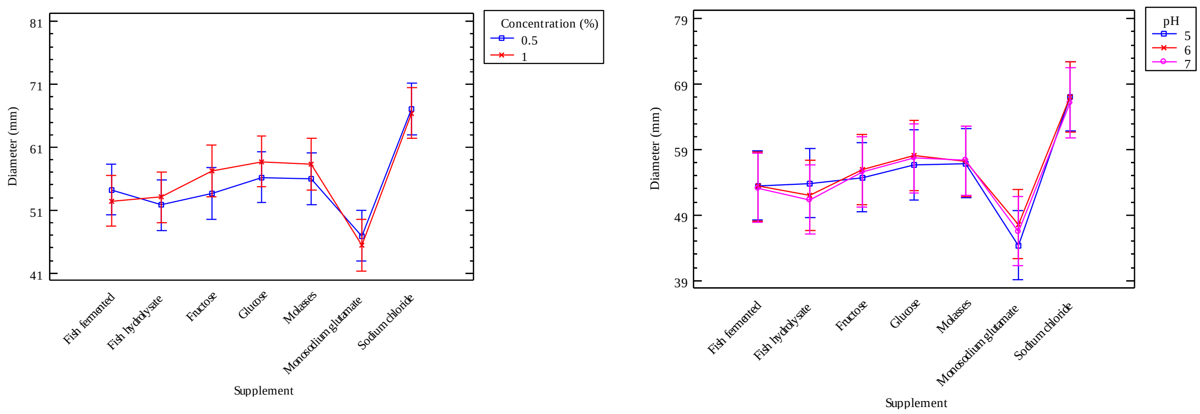


Figure 1. Mycelial development of *M. purpureus* in culture media based on quinoa flour supplemented with different sources of carbon, nitrogen and salts at 0.5% (*w*/*v*), at 3 pH levels (5, 6 and 7) after 10th days of incubation.

The highest diametral growth of the fungus was obtained with sodium chloride, independently of the concentration used, being the lowest growth obtained with monosodium glutamate. Considering the other supplements tested, the mycelia showed similar growth without being affected too much by the supplement concentration (Figure 2a). On the other hand, the pH does not affected the *Monascus purpureus* diametral growth, being again the highest growth obtained with sodium chloride and the lowest with monosodium glutamate (Figure 2b).



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

Figure 2. The figure shows, (**a**) the interactions of supplement and substrate concentration in percentages; (**b**) the interaction of supplement and pH on the diametric growth of the fungus in vitro.

4. Conclusion

From this research, it was deduced that different supplement sources have effects on the development of the *M. purpureus*, and factors such as pH and concentration can also make changes in the morphology of the colonies affecting their growth rate.

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F.T.-H; resources, M.S.-J.; data curation, E.Q.-R.; writing—original draft preparation, E.Q.-R.; writing—review and editing, U.G.-B.; visualization, E.Q.-R. and J.J.M..; supervision, M.S.-J., V.C., U.G.-B, and J.J.M.; project administration, M.S.-J.; funding acquisition, M.S.-J. All authors have read and agreed to the published version of the manuscript.

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