ORIGINAL ARTICLE

A C G publications

Bioorg. Med. Chem. Rep. 1:1 (2018) 16-21

Bioorganic & Medicinal Chemistry Reports

Citric acid synthesis efficiency of *Aspergillus niger* in carob molasses based media

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(Received December 01, 2018; Revised December 25, 2018; Accepted December 31, 2018)

Abstract: Citric acid is an inactive ingredient that is widely used in pharmaceutical, cosmetic and food industries. It is an organic acid that is produced during the Krebs cycle of the cell metabolism therefore it is present in the living organisms. Natural resource of the citric acid is citrus fruits and it is well known for its preservative action. Synthetically by utilizing from biotechnological tools, it can be produced by *Aspergillus niger* in a media that can supply the sµgar source. In this study we compared the efficiencies of carob molasses and potato based broths to grow *A. niger* for citric acid production. Carob molasses are widely found in the region and their utilization as media in biotechnological production of the citric acid would be advantageous. Our results support that this media can be used as an efficient source for the citric acid producing fungus *A. niger* in the bioreactors.

Keywords: Microbiology; citric acid; carob; pharmaceutical industry; cosmetics; food science.

1. Introduction

Citric acid has been widely used in pharmaceutical, cosmetic and food industries as an inactive ingredient. [1-15] Citric acid can be produced by microbial fermentation and *Aspergillus niger* (*A.niger*) strains have been the major fungal species that has been in use due to its high efficiency [1-15]. *A. niger* can produce citric acid even at low pH values 2.5 to 3.5 and the yield increases by increasing the sµgar content of the growth media[6,7,12].

Althoµgh the usage of *A. niger* for the biotechnological production of the citric acid has been vital for the industrial purposes, there has always been a room for improvement in the efficiency[1-15]. Especially usage of alternative sµgar sources from the agricultural waste products as a growth medium for the fungus is crucial for economical purposes[1-15].

Citric acid is used as an inactive ingredient of many drµgs, cosmetics as well as food products[1-15]. Therefore, there will always be a high demand for its production. In order to satisfy the high demand more efficient and economical solutions should be created for the production of this important biotechnological product.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/bioorganic-medicinal-chemistry-reports © January-December 2018
DOI: http://doi.org/10.25135/bmcr.15.18.12.1124

Our study aimed to decipher the citric acid production efficiency by *A. niger* from a common agricultural waste product carob molasses. Carob production and processing have been a common practice in the Mersin province of Turkey due to its unique and optimal climate conditions. Usage of carob molasses a waste product from the processing of carob, would enable great economic advantages to the region for the biotechnological production of the citric acid.

In our study we tested the citric acid production efficiencies of *A. niger* in carob molasses and potato based (as a control) growth media. Previous studies sµggest high citric acid production levels at 30 ºC after 192 hours of incubation period of *A.niger*[1-15]. Studies also sµggest the advantage of lower pH values for pure and higher citric acid production levels compared to the high pH values and in our study we are presenting results that delineate the effect of pH on the citric acid production by *A. niger* in potato based and carob molasses based growth media [1-15]. Our results support that carob molasses based media would be an economical alternative for the conventional potato based broth for the production of citric acid by *A. niger.*

2. Experimental

2.1. Materials and apparatus

All starting materials and reagents were purchased from commercial suppliers and carob molasses was produced by the carobs grown and collected in the region. *Aspergillus niger* 1688 and *Escherichia coli* 25922 were purchased from ATCC.

2.2. Fungal and bacterial growth for the production of citric acid.

Basic potato based broth was used for the microbial growth. *A. Niger* and *E.coli* were grown at 30 ºC in a shaker with aseptic conditions in 50ml of final volume. pH of the carob molasses and potato based media were 3.5, 5.5, 7.5, 9.5 and unmanipulated (5.1 for carob molasses and potato based broth 5.6). Carob molasses media was prepared by 1:4 dilution of its malt in distilled water. These pH values were chosen based on the previous studies and citric acid production was measured after 192 hours of incubation at 30 ºC. 192 hours time point was also chosen based on the findings of the previous studies which sµggest the highest yield after 192 hours incubation of *A.niger* at 30 ºC[1-15].

2.3. Measurement of the citric acid production

Concentration of the citric acid was determined by forming the citric acid crystals from the growth media of *A. niger* and *E.coli* after 192 hours of incubation at 30 ºC. The weights of the citric acid crystals were divided into the total volume of 50ml in each sample to determine the concentrations. Experiments were conducted as triplicates and statistical analysis was done for three independent data sets.

2.3. Statistical analysis

Graphpad prism version 5 was used for the statistical analysis. Unpaired t test was applied for each data set and each set consisted of at least 3 biologically independent data points.

3. Results and discussion

Different ranges of pH values were used for the growth media. This range spanned pH of 3.5 and 5.5 as acidic values to pH of 7.5 as the neutral value and pH of 9.5 as the basic one. In the control groups we used natural pH values of the media and for the carob molasses media it was pH of 5.1 and for the potato broth media it was pH of 5.6. Other than the normal pH of the carob molasses media there was no production of citric acid at different ranges of pH's after 8 days of incubation. This emphasizes

that the pH of the media was the major factor contributing the citric acid production. We observed the growth of the fungus under all conditions but we were unable to obtain citric acid crystals from carob molasses based media whose pH values were manipulated. Moreover, natural pH and pH 5.5 of potato based media had similar levels of citric acid production. While carob molasses media lacked the citric acid content after 8 days of incubation under all conditions except the natural pH conditions, potato based media had the highest yield at the neutral (22 µg/mL) and basic (30µg/mL) pH values. pH3.5 was also a good condition for the potato based media for the citric acid production by *A. niger* since it yield in average 17µg/mL of citric acid crystals (Figure 1-5).

Carob molasses based media lacked the high efficiencies in differing ranges of pH values for the citric acid production compared to those of potato based media but at its normal pH value it lead to a substantial production of citric acid. It doubled the citric acid production compared to that of potato based medium. Therefore, it has potential to be used for the production of citric acid without pH manipulation which would also increase the costs for the production. Moreover, since it is an agricultural side product that is usually considered as waste; its usage would be more economical than other types of media. In the future studies, our group will be testing the citric acid production efficiency of *A. niger* in different media types that could be produced simply by using the agricultural wastes and side products with substantially low costs.

4. Conclusion

In conclusion, the novel carob molasses based media enabled the production of citric acid from *Aspergillus niger* as efficiently as potato based commercially purchased media after 192 hours of incubation at 30 ºC . Citric acid production was the most efficient at the normal pH of the carob molasses based media which further saves the producers from the costs of the pH adjustments. Our results support that carob molasses based media can be used efficiently in the bioreactors to produce citric acid for the pharmaceutical, cosmetic and food industries.

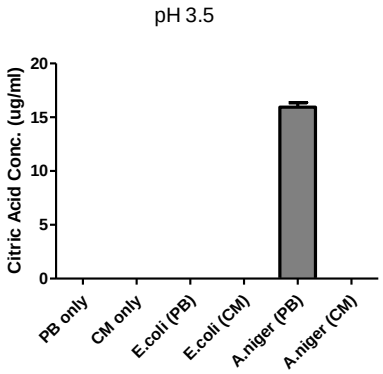

Figure 1. Citric acid concentration (µg/mL) in the 50 mL media of each group after 192 hours incubation at 30 ºC. *E.coli* was used as a control bacteria that would not produce citric acid. Both *E.coli* and *A.niger* were grown in potato based broth (PB) or carob molasses based broth (CM) at the pH of 3.5. Unpaired two tailed t test was conducted for statistical analysis, $p<0.0001$ N=3.

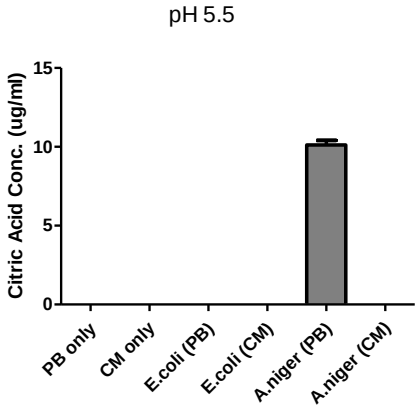

Figure 2. Citric acid concentration (µg/mL) in the 50 mL media of each group after 192 hours incubation at 30 °C. *E.coli* was used as a control bacteria that would not produce citric acid. Both *E.coli* and *A.niger* were grown in potato based broth (PB) or carob molasses based broth (CM) at the pH of 5.5. Unpaired two tailed t test was conducted for statistical analysis, $p<0.0001$ N=3.

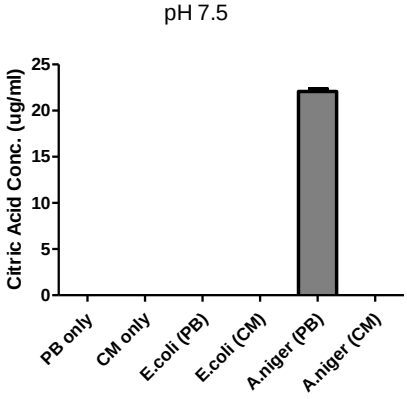

Figure 3. Citric acid concentration (µg/mL) in the 50 mL media of each group after 192 hours incubation at 30 °C. *E.coli* was used as a control bacteria that would not produce citric acid. Both *E.coli* and *A.niger* were grown in potato based broth (PB) or carob molasses based broth (CM) at the pH of 7.5. Unpaired two tailed t test was conducted for statistical analysis, $p<0.0001$ N=3.

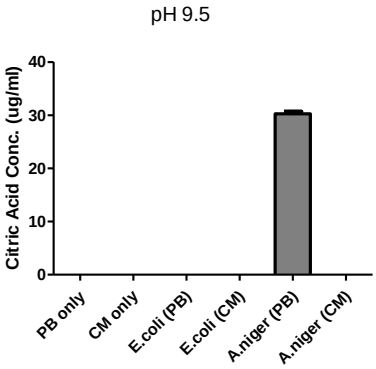

Figure 4. Citric acid concentration (µg/mL) in the 50 mL media of each group after 192 hours incubation at 30 °C. *E.coli* was used as a control bacteria that would not produce citric acid. Both *E.coli* and *A.niger* were grown in potato based broth (PB) or carob molasses based broth (CM) at the pH of 9.5. Unpaired two tailed t test was conducted for statistical analysis, $p<0.0001$ N=3.

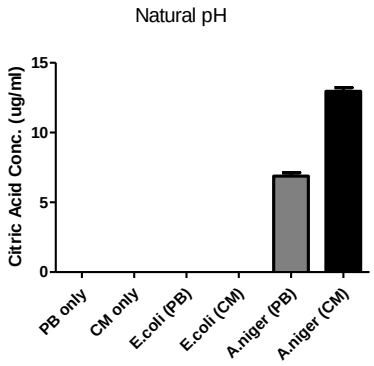

Figure 5. Citric acid concentration (µg/mL) in the 50 mL media of each group after 192 hours incubation at 30 ºC. *E.coli* was used as a control bacteria that would not produce citric acid. Both *E.coli* and *A.niger* were grown in potato based broth (PB) or carob molasses based broth (CM) at the natural pH of each media. Unpaired two tailed t test was conducted for statistical analysis, $p<0.0001$ N=3.

Acknowledgements

This article is a part of the master thesis of Busra Olcel Dur. We gratefully acknowledge financial support from the Mersin University BAP department with the project number of 2018-1-TP2-2820.

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