Short research article

Cryptococcus neoformans – New science for discovering melanin modifiers

Abstract

Aim- The present study was taken up to establish the effect of niacinamide on phenoloxidase lead melanogenesis and to prove the reliability of C.neoformans based screening methodology.

Methods

The organism was grown in the Minimal media in presence and absence of L- DOPA and Niacinamide and checked for its pigment producing ability at different time intervals.

Results- Niacinamide did not affect the pigmentation in Cryptococcus neoformans in the absence or presence of L-Dopa.

Conclusion - Cryptococcus neoformans as a biological tool for studying the mechanism of action of various melanin promoters/ inhibitors. The present study highlights the importance and usefulness of Cryptococcus neoformans based screening invention as it is cost effective rapid and 'living cell model'.

Keywords

Vitamin B3, Tyrosinase, Hyperpigmentation, L-DOPA

Introduction

Niacinamide, is otherwise called as Vitamin B3 or Nicotinamide or 3-pyridinecarboxamid. This is a biologically effective form of niacin that is found in root vegetables of many plants and also in certain yeast fungi. Niacinamide functions as a precursor for the co-factors such as Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP). Along with their reduced forms NADH and NADPH, and that would act as antioxidant [1].

Niacinamide has several medicinal applications for skincare including anti-inflammation, prevention of photo-immunosuppression and increased intercellular lipid synthesis. Topical Niacinamide is known to offer anti-aging benefits to the skin, improved barrier function and significant improvement in the appearance of photo aged facial skin such as texture, hyperpigmentation, redness, fine lines and wrinkles. [2, 3, 4 &5]

Additionally, Niacinamide is believed to influence the cutaneous pigmentation by down-regulating the transfer of melanosomes from melanocytes to keratinocytes. Studies done by Hakozaki *et al.* suggest that Niacinamide has no effect on tyrosinase activity, melanin synthesis or melanocyte number in a monolayer culture system. The authors also found that Niacinamide had down-regulated the number of melanosomes transferred from melanocytes to keratinocytes from 35 to 68% in a co-culture model system. The actual process by which Niacinamide down-regulates melanosome transfer yet to be established [6, 7, and 8].

Cryptococcus neoformans (C.neoformans) is yeast like fungus belongs to the class basidimycota and is known to produce melanin like pigment. The pigment production is associated with virulence and drug resistance [9,10]. The mechanism of melanogenesis in C.neoformans is through an enzyme analogue of tyrosinase- Phenoloxidase. It is well known that Niacinamide doesn't affect tyrosinase or melanin synthesis however would abrogate melanin transfer to keratinocytes.

We have already established the usefulness of C.neoformans in rapid screening of actives that may have the pigment modifying property. However the absolute reliability of the C.neoformans based screening approach require testing with a known tyrosinase non-inhibitors. The present study was taken up to establish the effect of niacinamide on phenoloxidase lead melanogenesis and to prove the reliability of C.neoformans based screening methodology. Findings are presented in the paper.

Materials and methods

C. neoformans culture was obtained YRG care, Chennai. C.neoformans was grown in a defined minimal media (15 mM glucose, 10 mM MgSO4, 29.4 mM KH2PO4, and 13 mM glycine, 3 mM thiamine, with and without 1.0 mM L-dopa. The organism was grown in the above media was incubated for 14 days at room temperature. The intensity of pigment produced was observed at different time intervals.

Evaluation of Niacinamide in the melanization of C.neoformans

To the above defined media containing L- DOPA, 1% Niacinamide was incorporated. Media without L- DOPA was used as negative control. All the media plates in triplicate were inoculated with C.neoformans and were incubated for 14 days at room temperature. The intensity of pigment produced by the organism in media plate containing L- DOPA and Niacinamide was observed and the similarity in the observation was compared with control plate which was devoid of L- DOPA

Result

C.neoformans required 14 days to produce melanoid pigmentaion. The C.neoformans grown in media containing L- Dopa (10mM) on day 2, mild pigmentation was observed and which further deepened from day 4 to day 14. Table- 1

When C.neoformans was grown in media containing Niacinamide and L- DOPA, the intensity and extent of pigmentation was similar to that in L- DOPA alone treated media. Niacinamide did not seem to either positively or negatively influence the pigment formation in C.neoformans where phenoloxidase is involved in melanoid pigmentogenesis. Table- 1

Experiments	**Presence of pigment vs days**			
	2	4	7	14
C.neoformans	-	-	-	+++
C.neoformans+ Dopa	+	++	+++	+++
C.neoformans+ Niacinamide	-	-	-	+++
C.neoformans+ Niacinamide+ Dopa	+	++	+++	+++
Dopa alone	-	-	-	-

- = No black pigmentation

78 + = Mild pigmentation

79 ++ = Moderate pigmentation

80 +++ = Deep pigmenttaion

81

82 **Fig -1 4 day old C.neoformans (Control)** **Fig- 2 4 day old C.neoformans treated**
83 **with Niacinamide**

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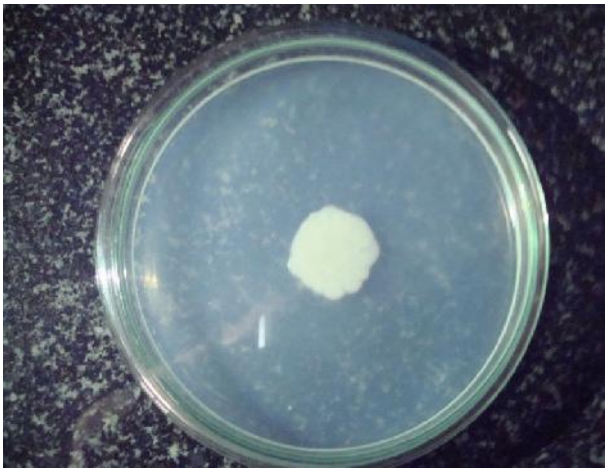

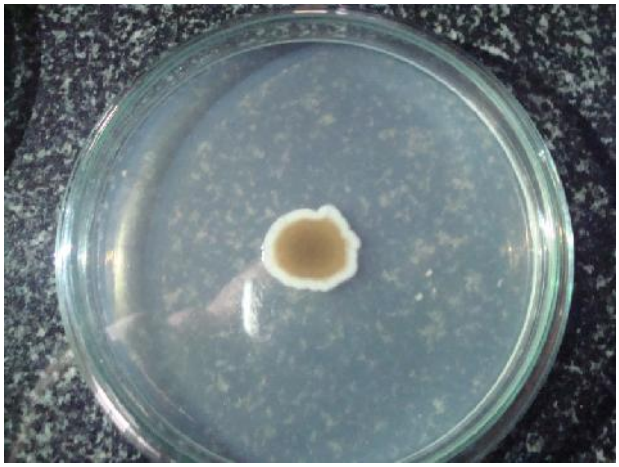

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86 **Discussion**

87 The present study has undoubtedly established the usefulness of *Cryptococcus neoformans* as
88 a biological tool for studying the mechanism of action of various melanin promoters/
89 inhibitors. Further the above tool also has established the mechanism of action of
90 Niacinamide.

91 Addition of Niacinamide did not alter the pigment producing ability of *Cryptococcus*
92 *neoformans* when DOPA was supplemented in the media which suggests Niacinamide does
93 not inhibit the enzymatic pathway in melanogenesis.

94 It's already established that Niacinamide does not affect the process of melanognesis through
95 tyrosinase enzyme pathway. *Cryptococcus neoformans* produce melanin through an alternate
96 mechanism by using tyrosinase analogue-phenoloxidase. However, the effect of Niacinamide
97 on phenoloxidase is not clearly known. The present study has also revealed that Niacinamide

does not affect phenol oxidase lead melanogenesis like that of tyrosinase linked melanogenesis. This proves that *Cryptococcus neoformans* is quite reliable tool for screening ingredients that may have melanin promotion/inhibition property. Tyrosinase based assays as well as the cell culture based assays are followed for the above purpose. However the invitro studies may provide only indicative results whereas *Cryptococcus neoformans* model is perfect living cell biological model and can predict the results more accurately than the invitro studies.

In the present we have used two known positive indicators to predict the usefulness of *Cryptococcus neoformans* based screening method. The first indicator is Niacinamide which does not affect the tyrosinase activity. The second indicator being *Cryptococcus neoformans* which produce melanoid pigmentation in selective media supplemented with L- DOPA. However the pigmentation in *Cryptococcus neoformans* is due to phenol oxidase enzyme which is an analogue of tyrosinase enzyme seen largely among vertebrates.

It is already known that Niacinamide does not affect the enzymatic pathway in melanogenesis however block the melanin transfer from melanocytes to keratinocytes. Since the Niacinamide has not affected the melanoid pigmentation in *Cryptococcus neoformans* which proves phenoloxidase based screening shall go in concordance with the findings obtained through tyrosinase assay. This validates the scientific credence and sanctity of *Cryptococcus neoformans* based screening method for melanin promotors/inhibitors. This method is reliable, rapid, cost effective as well as 'living cell model' than invitro cell culture based assay.

120 **Reference**

121 1. Jody P. Ebanks, R. Randall Wickett, and Raymond E. Boissy. Mechanisms
122 Regulating Skin Pigmentation: The Rise and Fall of Complexion Coloration. Int J
123 Mol Sci. 2009 Sep; 10(9): 4066–4087.

124 2. Bissett D, Miyamoto K, Sun P, Li J, Berge C. Topical niacinamide reduces
125 yellowing, wrinkling, red blotchiness, and hyperpigmented spots in aging facial
126 skin. Int. J. Cosmet. Sci. 2004;26:231–238.
127 3. Bissett D, Oblong J, Berge C. Niacinamide: A B vitamin that improves aging facial
128 skin appearance. Dermatol. Surg. 2005;31:860–865.
129 4. Bissett D. Topical niacinamide and barrier enhancement. Cutis. 2002;70S:8–
130 12. [PubMed]
131 5. Bissett D, Oblong J, Saud A, Berge C, Trejo A, Biedermann K. Topical niacinamide
132 provides skin aging appearance benefits while enhancing barrier function. J. Clin.
133 Dermatol. 2003;32S:9–18

134 6. Hakozaki T, Takiwaki H, Miyamoto K, Sato Y, Arase S. Ultrasound enhanced skin-
135 lightening effect of vitamin C and niacinamide. Skin Res. Technol. 2006;12:105–113

136 7. Greatens A, Hakozaki T, Koshoffer A, Epstein H, Schwemberger S, Babcock G,
137 Bissett D, Takiwaki H, Arase S, Wickett R, Boissy R. Effective inhibition of
138 melanosome transfer to keratinocytes by lectins and niacinamide is reversible. Exp.
139 Dermatol. 2005;14:498–508

140 8. Boissy R. Melanosome transfer to and translocation in the keratinocyte. Exp.
141 Dermatol. 2003;12:5–12.[PubMed]

142 9. D. C. McFADDEN & A. CASADEVAL. Capsule and melanin synthesis in
143 *Cryptococcus neoformans*. Medical Mycology 2001, 39, Supplement 1, 19-30
144 10. Polacheck I. The discovery of melanin production in Crypto- coccus neoformans and its
145 impact on diagnosis and the study of virulence. Zentralbl Bakteriol 1991; 276: 120–123.