PERIODICO di MINERALOGIA Volume 89, No. 3, 2020

Correlative effect of solid media on yellow pigmentogenesis at an *Epicoccum* sp. strain

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Abstract

This study is intended to determine the optimum solid media composition able to stimulate yellow pigmentogenesis at a new fungal Epicoccum sp. selected strain. Experiments show the possibility to increase pigment production by 4.8 times through substitution of corn embryos (shown as the best medium by previous research) in proportion of 86.66% with corn flour. By using corn flour in combination with corn embryos or soy flour pigment production can be increased by 2.5 times or by 1.67 times respectively.

Keywords: *Epicoccum* sp., yellow pigments biosynthesis, solid-state fermentation.

Introduction

The colour of a food product is a major quality component. This determines its acceptability while enhancing its attractiveness. An unsuitable colour may be associated with an improper processing, fake or alteration. Loss of colour due to processing calls for adding colorants. In this case, reproducibility of a certain colour is another significant aspect.

Pigments of chemical synthesis have been used for many years and are still in use. Theoretically, they are inert physiologically but contain harmful by-products. It is therefore imperative the use of natural non-toxic and physiologically accessible colorants [7]. More often than not, natural colorants are extracted from vegetal tissues and the most widely used are:

- annatto (bixine) extracted from *Bixa orleana;*
- paprika (capsantine) obtained from *Capsicum annuum;*
- saffron (crocetine, crocine) obtained from *Crocus sativus.*

Using vegetal pigments is advantageous from the following points of view:

- structural diversity that determines solubility in aqueous and/or fatty media;
- high tincture potential and a wide colour-spectrum;
- increased inocuity.

Carotenoids are most widely distributed class of pigments in nature displaying yellow, orange and red colour [8].

Industrially carotenoid pigments such as β-carotene and astaxanthin are utilised as food or feed supplements. They are also precursors of vitamin A. Several carotenoids have recently attracted much attention due to their beneficial effect on health, e.g. the functions of lycopene and astaxanthin include strong quenchers of siglet oxygen, an involvement in cancer prevention and enhancers of immune responses [9].

Carotenoids comprise a major class of pigment molecules, with a broad natural distribution. All photosynthetic organisms from bacteria to higher plants and nonphotsynthetic organisms including certain bacteria, fungi and red yeasts synthesize carotenoids.

Nowadays, however, the use of vegetal pigments is limited by the seasonal nature of the raw materials, by the extensive conditions necessary to obtain it (duration, large areas), low productivity, and dependence on the geographical and agro-technical conditions as well as the instability of pure pigments.

Given these circumstances, obtaining natural pigments from microbial sources (bacteria, fungi and algae) is the most rewarding perspective [5].

The numerous advantages of this approach are those of every microbial biotechnology: cheap and easy-to-get raw materials, short cultivation time, high productivities by a strictly controlled process (automated), as well as the possibility to increase production by a proper selection of producers and improving their fermentative performance by mutations and genetic engineering.

The microbial pigments are secondary metabolites being synthesized in idiophase (an exception are the photosynthesis pigments) [6]. Micro-organisms produce a large variety of pigments, which have been identified and structurally classified as: carotenoides, quinons, piranes, flavones etc. Specialists focus on identification and selection of new strains, optimisation of fermentative conditions, extraction, purification and characterization of microbial pigments, along with their toxicity potential and behaviour *in vivo* or in foods [4].

Pigmentogenesis varies according to culture conditions, namely nitrogen, sugar, oxygen or temperature variations.

Fungus *Epicoccum nigrum* is known to synthesize secondary metabolites including isoprenoids. *E. nigrum* also produces pigments including flavonoids and carotenoids as β-carotene, γ-carotene, rhodoxanthin and torularodin [10, 11, 12].

The MIUG Collection of Industrial Microbiology Laboratory of the "*Dunãrea de Jos*" University Galaţi, has selected *Epicoccum* sp. strains which have been investigated for their potential of yellow pigment biosynthesis, all of them located inside cells [13].

For such strains, the optimum fermentative and high pigmentogenesis conditions have been studied. The best results were achieved by solid state cultivation on a medium containing natural ingredients, wheat and corn embryos, as solid base, moistened at 1:2.5 ratio with a nutritive broth based on molasses, glycerol and mineral salts [1, 2].

Starting from these results, the current study intended to identify the qualitative and quantitative effect of the nutritive solid base substrate on the pigment biosynthesis in order to optimise the fermentative medium composition and make it more efficient for the yellow pigmentogenesis at a new highly productive strain.

Material and Methods

Micro-organism. An *Epicoccum* sp. (coded MIUG 2.15) strain displaying a yellow pigments biosynthesis potential has been obtained by our own isolation and selection studies.

Culture conditions. Cultivation has been made by surface culture on semisolid media where the composition of the solid base has been varied (C and N sources) while keeping constant the composition of both moisturising medium and the solid/liquid ratio (1/2.5). The moisturising broth made of molasses (7.5%), glycerol (1%), urea (2%) and mineral salts containing Ca^{2*}, Mg^{2+}, K^{+}, P [1,2].

Pigmentogenesis potential evaluation. After cultivation, during 10 days, at 25ºC, in the absence of light, the fermentative media have been dried (40-45ºC) and then finely grounded; from the powder thus obtained yellow pigments were extracted for 1 hour at 50ºC using a mixture of acetone: methanol (7:2) as solvent.

The biosynthesis potential was evaluated by quantitative estimation of the yellow pigments in the extract against a reference curve of pure β-carotene dissolved in the same solvent. The maximum absorbance has been identified by spectrum analysis at λ =455 nm (identical to the pure commercial reference unit).

Results and discussions

Previous studies have pointed out the stimulating effect of the corn embryos on the pigmentogenesis and consequently suitable recipes have been elaborated, in which they represent the solid base of the solid state fermentation exclusively [1].

Since corn embryos are hard to find in our country nowadays, the new raw material has been identified and estimated in terms of quality and quantity in order to partially or totally replace them. Thus, by keeping constant the composition of the liquid fraction and the solid/liquid ratio, new variants of media have been conceived by making use of corn embryos and vegetal flours (corn, rice, soy and barley).

The results obtained are illustrated in **Figure 1**. Using the corn embryos medium as reference, research has shown that an almost similar pigment yield can be obtained by totally replacing the corn embryos with rice flour and even spectacular achievements are reached (pigment production increase by 76-144%) when using only corn flour or mixtures of corn flour with other vegetal flours.

The above data suggested further research by making new variants of fermentative media based on ingredients which provided the best results, i.e. corn and rice flour. The studies also attempted an assessment of the qualitative and quantitative effect provided by the solid substrate. The results are illustrated in **Figure 2**.

From the data presented in **Figure 2** the stimulating effect of the corn and rice flours on the yellow pigment production is confirmed.

The soy flour acts as inhibitor when combined with corn flour (1: 6.5 ratio) and stimulator in a similar combination of rice flour.

The flour of citric peels used for their content of pigment precursors (such as terpenes; some authors recommend including citric molasses) has a different qualitative effect depending on other vegetal flours.

It should be noted that, when replacing the corn embryos by corn flour, the yield coefficient increases by 1.6 times and 1.4 times respectively when using the mixture 6.5:1 rice flour and soy flour.

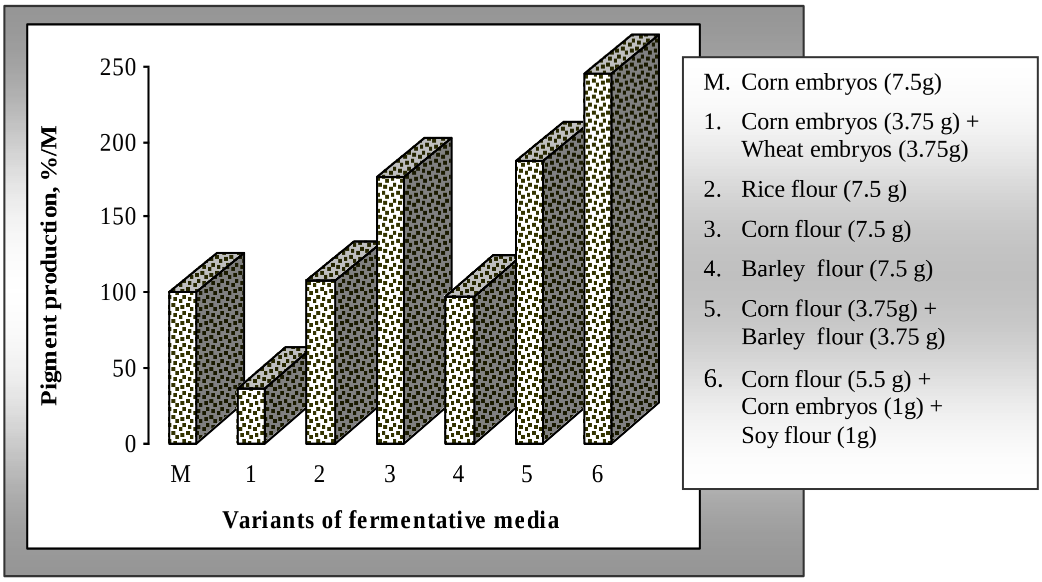

Figure 1. Qualitative and quantitative effect of solid substrate upon pigment production

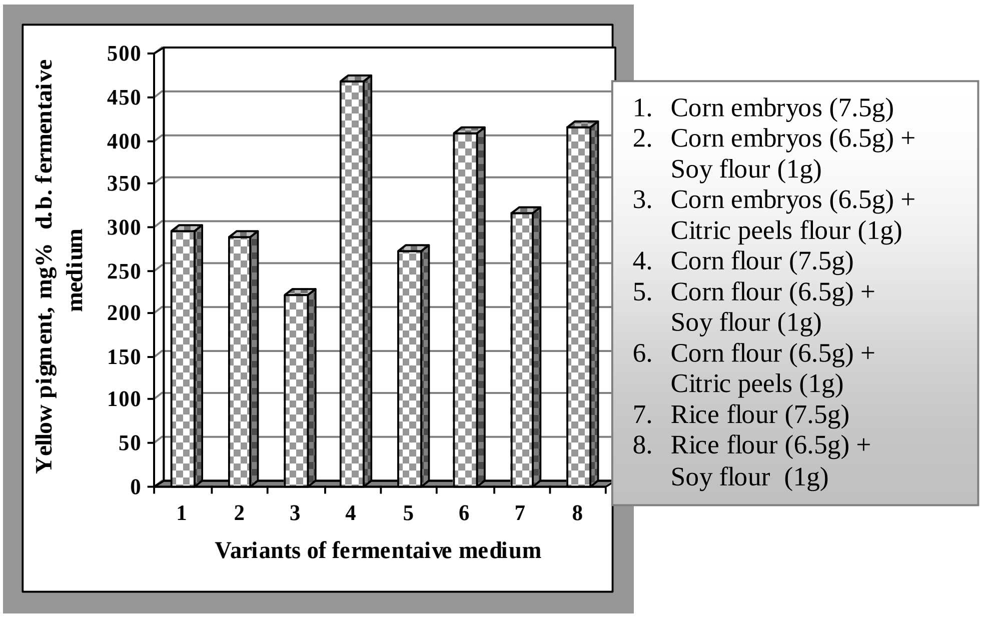

Figure 2. Effect of the corn embryos and some vegetable flours upon yellow pigment production

In order to certify the previous results a new experiment has been performed to see the qualitative and quantitative effect of he corn embryos, soy flour and citric peels flour used in mixture with the corn and rice flour.

The data in **Figure 3** assess the corn stimulating effect that is synergetic in combinations of 6.5:1 with corn embryos and soy flour. If the three ingredients are used in combinations of 5.5:1:1 a slightly inhibiting effect is reported.

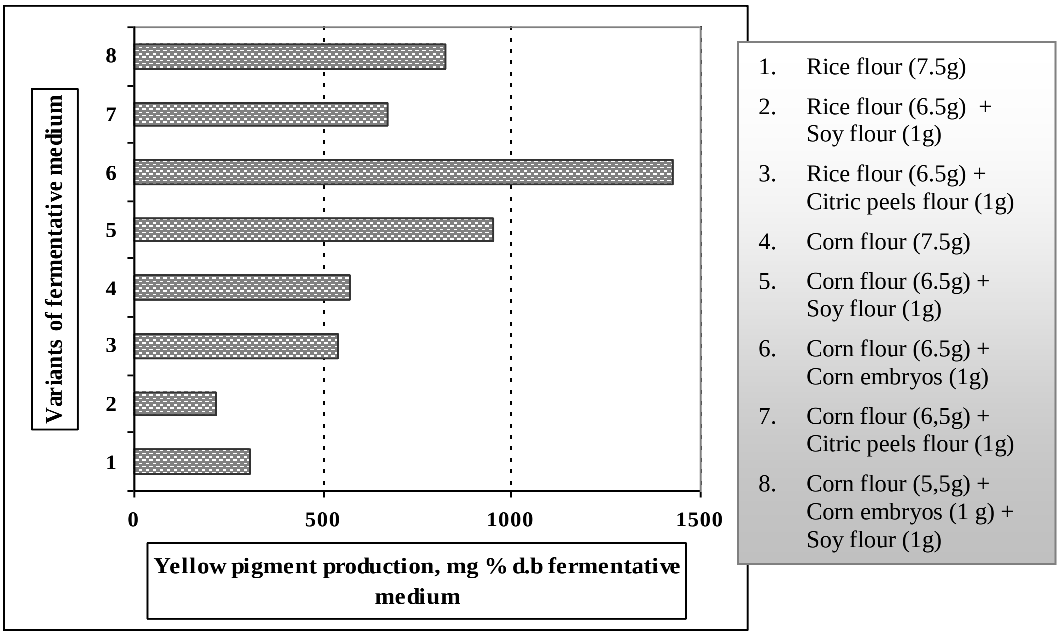

Figure 3. Pigment production on corn and rice flour based media

Conclusion

A new fungal strain *Epicoccum* sp. has been identified and selected as it features a high potential of yellow pigment biosynthesis.

Quantitative studies were carried out to observe the effect of some natural ingredients used as solid base in solid-state fermentations upon the pigment production in order to achieve high yield coefficients of yellow pigments.

Studies have shown the possibility of increasing the biosynthesis 4.8 times yield by replacing the corn embryos with 86.66% corn flour.

Growth and pigment production for *Epicoccum* MIUG 2.15 are susceptible to be increased by managing culture conditions in solid-state fermentation system.

The mould is very sensitive to medium composition variations. For this reason next investigations will be focused on fermentative conditions optimisation, in order to obtain a high yield of biomass and increasing yellow pigments production.

Acknowledgements

This work was supported by CNCSIS Grant 450/2004

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