Zagazig J. Agric. Res., Vol. 37 No. (4) 2010 889-906

SCREENING OF SOME MICROORGANISMS FOR RED PIGMENTS PRODUCTION IN DIFFERENT MEDIA, AND IDENTIFICATION OF THE MOST PROMISING CULTURE

Maha M.N. Ismail*, E.M. Gewaily, V.S. Bedrous and G.M. Mohamed

Dept. of Agric. Microbiology, Faculty of Agric., Zagazig Univ., Egypt

ABSTRACT

The potential for red pigments production by some microorganisms isolated from different sources was evaluated. A total of twelve isolates were able to produce red pigments on 7 different isolation media, however, the three isolates; ZL_1, ZL_2 and ZL_3 showed more potential in pigments production. Based on phenotypic and electron microscopic investigations, the isolate ZL_1 was characterized as *Monascus* spp. The fungal isolates *Monascus* spp. and the reference (authentic) strain *Monascus purpureus* 4066 resulted highest values of absorbance of extracellular red pigments (2.470 U and 1.929 U) when measured by Spectrophotometer at 500 nm after 10 days of growth on two fermentation media; yeast extract-malt extract (YM) and first fermentation (F1) broth media, respectively. Therefore, the high production yield of red pigments by these fungal isolates demonstrates a possibility of commercial production of such natural colourant that might be used in manufacturing processes.

Keywords: Filamentous fungus, *Monascus*, red pigments, natural colourants, fermentation media.

* **Corresponding author:** Maha M.N. Ismail Tel.: +20105643751

E-mail address: maha_nader@hotmail.com

INTRODUCTION

Colour is an important factor in the acceptability of a food product. Today, food industry used colourants to enhance or restore the original appearance of foods. Synthetic colourants have found their place rapidly in the food industry due to their stability, imparting bright colours and easily recoverable properties. Questioning of safety of synthetic colourants, recent prohibition of some of them and increased consumer consciousness towards the natural products resulted in an increased interest for colourants derived form natural sources (Jackmean and Smith, 1996). For this purpose, pigments extracts from edible sources as well as concentrated juices are commercially available and used in food industry.

There is a wide interest in the production of pigments from natural sources due to a serious safety problem associated with many artificial synthetic colorants which have widely been used in food stuff, cosmetic and pharmaceutical manufacturing process (Kim *et al*., 1995). Many kinds of synthetic dyestuffs have been found to be hazardous to human health and only limited kinds of such dyestuffs are permitted to be used in food in many countries. Therefore, there is a need to develop alternative sources of natural food colournats.

There are a number of natural colorants but only a few available in sufficient quantity for industrial use because they are directly extracted from plant flowers, fruits leaves and roots (Lauro, 1991). The ever- increasing demand for food containing only natural ingredients is responsible for the market trend towards the use of natural rather synthetic pigments (Jûzlová *et al*., 1996)

Although the existing authorized natural food colourants are of either plant or animal origin, fungi have been explored as possible food colourants (Sameer *et al*., 2006). There are a number of microorganism(s) posses an ability to produce pigments in high yields, including species of *Monascus, Paecilomyces, Cardyceps, Penicillium herquei, P. atroventum, Serratia and Sterptomyces* (Yoshimura *et al*., 1975; Wong and Koehler, 1983 and Gunasekaran and Poorniammal, 2008). Amongst them, many species of fungus have attracted special attention because of their capabilities to produce different coloured pigments with high chemical stability (Hajjaj *et*

al., 2000). For industrial application of microbial pigments, higher production of pigments yield, chemical and light stability are essential features. Isolation of new strain for red biopigments production is still of particular interest because of necessity to obtain microorganisms with suitable characteristics for submerged cultivation (Rasheva *et al.*, 1998). *Monascus*, a fungal strain produces a red pigments colorant which can be applied in the food and cosmetic industries (Sardaryan *et al.*, 2004), and have been traditionally used for colouring and securing a number of fermented foods (Lin, 1973).

The purpose of this work was to evaluate the potential for red pigments production among isolated microorganisms, characterize the most potential isolate and define the optimal fermentation media for pigments production.

MATERIALS AND METHODS

Sampling

Soil samples were collected form different orchards (graps and dates) and different fields (rice and sorghum) at each location samples were randomly collected from five sites in sterile bags and thoroughly mixed together to form one representative sample. Also, yoghurt samples were collected from supermarket at Zagazig city.

Isolation Media

a) For fungal isolation, three different media were used. Yeast extract-malt extract-agar (YM-agar, Kreger van Rij, 1984), potato dextrose- agar (PDA, BBL, 11550) and Czapek's-agar (Cz-agar, Sullina and Shantharam, 1998).

b) For bacterial isolation, two different media were used. Plate count-agar (PC-agar, Oxoid. CM 325) and nutrient agar (N-agar, Difco, 1985).

c) For yeast isolation, three different media were used. YM-agar, Malt agar (M-agar, Harrigan and McCance, 1976) and yeast extract-glucose-chloramphenicol-bromophenol blue-agar(YGCB-agar, Perkoppová, 1984).

All cultures were grown on their specific media for 2-10 days at 25°C for fungi and yeast and 30°C for bacteria. Also, a periodical check up was taken place to assure the purity of different microorganism under study, and stored at 4°C.

Isolation of Local Microorganisms for Red Pigments Production

Ten gram of soil was separately transferred to a 250 ml Erlenmeyer flask containing 90 ml of distilled and sterilized 1.0% peptone water and mixed well, then a serial dilution up to 10^{-6} were prepared. One tenth ml of each dilution was transferred on the surface of plates of the three media, PD-agar, YM-agar and Cz-agar media, then incubated at 25°C for 7 to 10 days for fungi isolation. For bacterial isolation two different media were used N-agar and PC-agar media, then incubated at 28 ± 2°C for 48h (Hausler, 1972). Yeasts were grown on three different media YM- agar, M- agar and YGCB-agar. Petri dishes were incubated at 28 ± 2°C for 48h. Colonies of red pigments from fungi, bacteria and yeast were picked up, streaked on slants of individually YM-agar and N-agar test tubes, and then kept at 4°C for screening and identification.

Isolate Selection

The cell dry weight and red pigments intensity were the main criteria used for the selection of isolates. Therefore among the 12 isolates chosen for preliminary investigation, only 3 isolates were selected. All melanoid pigments were ignored due to the interference with any pigments formation.

Screening of Microorganisms for Red Pigments Production

Different microbial cultures were screened for red pigments production:

a) **Local isolates:** Three isolates isolated from rice field soils, rice seeds and yoghurt representing fungi, bacteria and yeast, were screened for red pigments production (Table 1).

b) **Authentic cultures:** Three standard strains (one fungus, one bacteria and one yeast) were obtained from Assiut Univ. Mycological Center, Assiut, Egypt. (Table 1).

Characterization Media

Different media (Czapek's-yeast extract-agar, malt extract-agar and 25% glycerol nitrate-agar-N25G-agar) were used for characterization of fungi as mentioned by Hawksworth and Pitt (1983).

Fermentation Media

Different fermentation media, YM-broth (Kreger-van Rij, 1984), F_1-broth (Lin, 1991), F_2-broth (Wong *et al.*, 1981), F_3-broth (Kim

Table 1. Microorganisms used for red pigments production

Microorganism	Strain or isolate No.	Sources
a• Local isolates:		
Monascus spp.	ZL_1*	Rice field soils
Pseudomonas spp.	ZL_2*	Rice seeds
Rhodotorula spp.	ZL_3*	Yoghurt from Zagazig city
b• Authentic cultures:		
Monascus purpureus	4066	Assiut Univ. Mycological Center (AUMC)
Serratia marcescens	B-55	Assiut Univ. Mycological Center (AUMC)
Rhodotorula mucilaginosa	3877	Assiut Univ. Mycological Center (AUMC)

*ZL_1, ZL_2 and ZL_3 a fungal, bacterial and yeast cultures were isolated at Dept. Agric. Microbiology, Fac. of Agriculture, Zagazig Univ., Egypt.

et al., 2002), N-broth (Difco, 1985) and Tryptone-yeast extract-Dextrose broth (TYD-broth, Oxoid) were used as screening media for red pigments production.

Inoculum Preparation

The stock cultures of fungi, yeast and bacteria were individually grown on YM-agar and N- agar media in a refrigerator, respectively. The agar was washed off with sterile distilled water and the inoculated seed cultures were grown in 200 ml YM-broth and N-broth with shaking for 250 rpm for 4 days at 25°C and 30°C, respectively. When the population density reached $3.6x10^3$, $3.9x10^3$ fpu/ml; $2.1x10^5$, $2.1x10^6$; $2.5x10^3$ and $3.1x10^3$ cfu/ml for *M. purpureus* AUMC 4066, *Monascus* spp. (ZL_1), *S. marcescens*, *Pseudomonas* spp. (ZL_2), *Rhodotorula mucilaginosa* and *Rhodotorula* spp. (ZL_3), respectively, they transferred to fermentation media.

Only 2 ml (5% v/v) of the seed culture was individually inoculated into 40 ml of different fermentation media in 250 ml conical flasks and grown at 25°C and 30°C on a rotary shaker at 250 rpm for 6 and 10 days.

Extracellular Red Pigments

Red pigments production was indirectly evaluated by measuring the absorbance maxima (λ_{max}) of the culture filtrate at 500 nm in a Colourmeter (Sequoia- Turner model 340) and a Spectrophotometer (Jenway 6305 UV/VIS), Lin *et al.* (1992) and Chiu and Poon (1993).

Dry Cell Weight

The broth culture was filtered through filter paper (Whatman No. 4) to separate the fungal mycelium. The biomass yield was estimated by washing with deionized water and drying at 80°C for 24h (Kim *et al.*, 2002).

Identification of Fungal Cultures

A concurrent tests were performed using the local fungus isolate (ZL_I) which gave a high specific red pigments and the authentic fungus *M. purpureus* AUMC 4066. Morphological characteristics (colonies shape and diameter, mycelium colour, soluble pigments production and ascospores diameter) and biochemical tests (enzyme activity test was carried out by using the APIZYM system at Micological center, Assiut University) were used for classifying and identifying new species of *Monascus* according to Howksworth and Pitt, 1983 and Bridge and Howksworth, 1985. The isolated fungus was also examined using scanning electron microscopy (SEM) according to Wong *et al.* (1998).

RESULTS AND DISCUSSION

Screening of Different Microorganisms and the Best Media Used for Growth and Red Pigments Production

In the present study, the purpose was to select the most promising microorganisms and fermentation medium contributing to red biopigments production under shaking flask cultures.

To select the best medium for biopigments production by microorganisms, 6 different media were evaluated. The effect of the media and microorganisms on dry cell weight and extracellular red pigments production were estimated using Colourmeter and Spectrophotometer as represented in Tables 2 and 3.

The maximum extracellular red pigments, dry cell weight, specific production and specific productivity of extracellular red

pigments using Colourmeter at 6 days cultivation by *M. purpureus* AUMC 4066 in YM-broth were 1.420 U, 15.2 g/L, 0.093 u.L/g and 0.00986 u/h respectively, then increased in general to 1.829 U, 16.5 g/L, 0.111 u.L/g and 0.00762 u/h at 10 days, respectively (Table, 2). The maximum extracellular red pigments, dry cell weight, specific production and specific productivity of extracellular red pigments using Spectrophotometer during 6 days cultivation by *M. purpureus* AUMC 4066 in YM-broth were 0.978 U, 15.2g/L, 0.064u.L/g and 0.00679u/h, respectively, then the values increased in general after 10 days of cultivation to 2.470 U, 16.5 g/L, 0.150 u.L/g and 0.0102 u/h.

Data in Table 2 also shows the maximum extracellular red pigments, dry cell weight, specific production and specific productivity of extracellular red pigments using Colurmeter in F_1-broth by *M. purpureus* AUMC 4066 were, 0.595 U, 14.3 g/L, 0.042 u.L/g and 0.00413 u/h respectively at 6 days cultivation, then increased during cultivation at 10 days to 1.876 U, 15.7g/L, 0.119u.L/g and 0.00782 u/h respectively. The absorbance maxima of extracellular red pigments, dry cell weight, specific production and specific productivity using Spectrophotometer in F_1-broth by *M. purpureus* AUMC 4066 during 6 days were 0.760 U, 14.3g/L, 0.053 u.L/g and 0.00528 u/h respectively, then increased during 10 days cultivation to 1.960 U, 15.7g/L, 0.125 u.L/g and 0.00817u/h.

The maximum extracellular red pigments, dry cell weight, specific production and specific productivity of extracellular red pigments using Colourmeter in F_1-broth by *Monascus* spp. (ZL_1) were 1.424 U, 16.7g/L, 0.085 u.L/g and 0.00989 u/h, respectively at 6 days, then increased in general to be 1.538 U, 17.0g/L, 0.090u.L/g and 0.00641 u/h at 10 days. The absorbance maxima, dry cell weight, specific production and specific productivity of extracellular red pigments using Spectrophotometer were 0.990 U, 16.7g/L, 0.059 u.L/g and 0.00687 u/h respectively at 6 days, then increased to be 1.929 U, 17.0g/L, 0.113 u.L/g and 0.00804 u/h at 10 days cultivation.

The results revealed that the YM and F_1-broth media, could be a good fermentation media for red pigments production by *Monascus* spp. These results were in agreement with those found by Lin and Demain (1991);Lee *et al.* (2001);

Table 2. Absorbance of extracellular red pigments at 500nm (Colourmeter), specific production, specific productivity of extracellular red pigments and dry cell weight of different microorganisms during cultivation in different media for 6 and 10 days

Organism	Media	Absorbance maxima									
		6 days					10 days				
		Abs. control	X_{max} (g/L)	$Abs._{maxextra}$ (U)	Pe_{extra} (ul/g)	P_{extra} (U/h)	Abs. control	X_{max} (g/L)	$Abs._{maxextra}$ (U)	Pe_{extra} (ul/g)	P_{extra} (U/h)
***M .purpureus* AUMC 4066**	YM-broth	0.178	15.2	1.420	0.093	0.00986	0.178	16.5	1.829	0.111	0.00762
	F_1-broth	0.030	14.3	0.595	0.042	0.00413	0.030	15.7	1.876	0.119	0.00782
	F_2-broth	0.135	13.1	0.797	0.061	0.00553	0.135	14.5	1.082	0.075	0.00451
	F_3-broth	0.043	15.0	0.489	0.033	0.00339	0.043	17.2	1.016	0.059	0.00423
***Monoscus* spp. (ZL_1)**	YM-broth	0.178	10.2	0.984	0.096	0.00683	0.178	11.7	0.985	0.084	0.00410
	F_1-broth	0.030	16.7	1.424	0.085	0.00989	0.030	17.0	1.538	0.090	0.00641
	F_2-broth	0.135	13.5	0.777	0.058	0.00540	0.135	17.0	1.000	0.059	0.00417
	F_3-broth	0.043	15.0	0.318	0.021	0.00221	0.043	16.2	0.575	0.035	0.00240
***R. mucilaginosa* 3877**	YM-broth	0.178	3.50	0.303	0.086	0.00210	0.178	4.50	0.375	0.0833	0.00156
	F_1-broth	0.030	6.00	0.106	0.0176	0.00073	0.030	7.45	0.757	0.105	0.00315
	F_2-broth	0.135	5.00	0.693	0.077	0.00481	0.135	9.50	0.686	0.124	0.00286
	F_3-broth	0.043	2.50	0.098	0.0392	0.00068	0.043	4.50	0.235	0.094	0.00097
***Rhodotorula* spp. (ZL_3)**	YM-broth	0.178	3.70	0.246	0.0665	0.00171	0.178	4.55	0.270	0.0593	0.00112
	F_1-broth	0.030	5.80	0.148	0.0255	0.00103	0.030	6.45	0.190	0.0294	0.00079
	F_2-broth	0.135	7.00	0.531	0.0758	0.00368	0.135	7.50	0.643	0.0857	0.00268
	F_3-broth	0.043	3.10	0.120	0.0387	0.00083	0.043	4.00	0.154	0.0385	0.00064
***S. marcescens* B-55**	N-broth	0.053	2.50	0.102	0.0408	0.00070	0.053	3.45	0.102	0.0295	0.00042
	TYD-broth	0.146	4.50	0.392	0.087	0.00272	0.146	5.25	0.392	0.0746	0.00163
***Pseudomonas* spp. (ZL_2)**	N-broth	0.053	3.75	0.108	0.0288	0.00075	0.053	5.25	0.317	0.0603	0.00132
	TYD-broth	0.146	2.50	0.299	0.119	0.00207	0.146	6.00	0.349	0.0585	0.00145

M.: *Monascus* S.: *Serratia* R.: *Rhodotorula*

X_{max}: Maximum cell concentration (g/L) $Abs._{max\ extra}$: Maximum extracellular red pigments (U).

Pe_{extra}: Specific production of extracelluar red pigments on cells, obtained at the maximum absorbance for extracellular red pigments (ul/g)

P_{extra}: Specific productivity of extracellular red pigments obtained at the maximum absorbance (u/h).

Table 3. Absorbance of extracellular red pigments at 500nm (Spectrophotometer), specific production, specific productivity of extracellular red pigments and dry cell weight of different microorganisms during cultivation in different media for 6 and 10 days

Organism	Media	Absorbance maxima									
		6 days					10 days				
		Abs. control	X_{max} (g/L)	Abs.$_{max\ extra}$ (U)	Pe_{extra} (ul/g)	P_{extra} (U/h)	Abs. control	X_{max} (g/L)	Abs.$_{maxextra}$ (U)	Pe_{extra} (ul/g)	P_{extra} (U/h)
M. purpureus AUMC 4066	YM-broth	0.400	15.2	0.978	0.064	0.00679	0.400	16.5	2.470	0.150	0.0102
	F_1-broth	0.486	14.3	0.760	0.053	0.00528	0.486	15.7	1.960	0.125	0.00817
	F_2-broth	0.528	13.1	0.796	0.057	0.00518	0.528	14.5	1.067	0.074	0.00445
	F_3-broth	0.463	15.0	0.597	0.040	0.00415	0.463	17.2	0.805	0.047	0.00335
Monoscus spp. (ZL_1)	YM-broth	0.400	10.2	0.791	0.078	0.00549	0.400	11.7	0.967	0.083	0.00403
	F_1-broth	0.486	16.7	0.990	0.059	0.00687	0.486	17.0	1.929	0.113	0.00804
	F_2-broth	0.528	13.5	0.633	0.047	0.00439	0.528	17.0	0.957	0.056	0.00399
	F_3-broth	0.463	15.0	0.550	0.037	0.00382	0.463	16.2	0.629	0.039	0.00262
R. mucilaginosa 3877	YM-broth	0.400	3.50	0.564	0.161	0.00392	0.400	4.50	0.579	0.129	0.00241
	F_1-broth	0.486	6.00	0.140	0.0233	0.00097	0.486	7.45	0.353	0.047	0.00147
	F_2-broth	0.528	5.00	0.820	0.0911	0.00569	0.528	6.50	0.926	0.169	0.00386
	F_3-broth	0.463	2.50	0.493	0.197	0.00342	0.463	8.50	0.496	0.058	0.00206
Rhodotorula spp. (ZL_3)	YM-broth	0.400	3.70	0.531	0.143	0.00368	0.400	4.55	0.580	0.127	0.00242
	F_1-broth	0.486	5.80	0.490	0.0845	0.00340	0.486	6.45	0.522	0.080	0.00217
	F_2-broth	0.528	7.00	0.748	0.106	0.00519	0.528	7.50	0.812	0.108	0.00338
	F_3-broth	0.463	3.10	0.492	0.158	0.00342	0.463	4.00	0.523	0.130	0.00218
S. marcescems B-55	N-broth	0.471	2.50	0.578	0.231	0.00401	0.471	3.45	0.625	0.181	0.00260
	TYD-broth	0.598	4.50	0.655	0.145	0.00455	0.598	5.25	0.746	0.142	0.00311
Pseudomonas spp. (ZL_2)	N-broth	0.471	3.75	0.712	0.189	0.00494	0.471	5.25	0.742	0.141	0.00309
	TYD-broth	0.598	2.50	1.620	0.648	0.0113	0.598	6.00	0.645	0.108	0.00269

Hamano *et al.* (2005); Kim *et al.* (2006); Carvalho *et al.* (2007); Pattanagul *et al.* (2007); Xijun *et al.* (2007); Sandra-Orozco and Kilikian (2008) and Ahmed *et al.* (2009).

On some media (N-broth and TYD-broth media) *S. marcescens* B-55 produce a water soluble pink pigments (Khanafari *et al.*, 2006). The maximum extracellular red pigments using Colourmeter in TYD-broth was 0.392 U at 6 and 10 days cultivation, however, the values as estimated by Spectrophotometer was 0.665 U at 6 days, then increased to be 0.764 U at 10 days (Table 2 and 3). These results could be supported by those found by Trias *et al.* (1988) and Khanafari *et al.* (2006).

The maximum extracellular pigments using Colourmeter by *R. mucilaginosa* 3877 in F_2- broth medium was 0.820 U at 6 days, and then increased to 0.926 U at 10 days while it was 0.693 U at 6 days using Spectrophotometer then decreased to be 0.686 U at 10 days cultivation (Table 2 and 3).

Tables 2 and 3 shows the maximum extracellular red pigments by *Pseudomonas* spp. (ZL_2) and *Rhodotourla* spp. (ZL_3) isolates in TYD-broth and F_2-broth media. The absorbance maxima using Colormeter for *Pseudomonas* spp. (ZL_2) was 0.299 U at 6 days, then increased to be 0.349 U at 10 days, but the absorbance maxima using Spectrophotometer was 1.620 U at 6 days, then decreased to be 0.645 U at 10 days. The maximum extracellular red pigments by *Rhodotorula* spp. (ZL_3) isolate using Colourmeter in F_2-broth was 0.531 U at 6 days, then decreased to be 0.643 U at 10 days. But by using Spectrophotometer, it was 0.748 U at 6 days and 0.812 U at 10 days cultivation.

It is clear from the presented data that both growth and extracellular red pigments production differ among microorganisms.

However, the highest extracellular red pigments production was produced by fungal strains *M. purpureus* AUMC 4066 in YM-broth and F_1-broth, and *Monascus* spp. (ZL_1) in F_1-broth medium at 25°C.

Consequently, the values of dry cell weight were the highest by both *M. purpureus* AUMC 4066 and *Monascus* spp (ZL_1).

Therefore, these organisms were chosen for extracellular red biopigments production in further

studies. Also, Spectrophotometer was preferable for measuring the absorbance maxima (λ_{max}) compared with Colourmeter.

Taxonomy of Fungal Cultures

There are a complicated systems of fungal classification, but for the purpose of this work, the morphological and biochemical characteristics were performed, along with scanning electron microscopy observations.

For the identification of the fungi, Cz-agar, M- agar and N25G-agar were used. The morphological characters such as colonies not exceeding 28 mm in diameter on Malt extract agar (MEA) after incubation for 7 days at 25°C, the mycelium is white in the early stage with abundant crystalline encrustations. These moulds grow in the form of microscopic filaments call hyphae. These hyphae normally contains regular cross- walls called septa, which allow movement of the cytoplasm.

The sexual spores are formed by simple internal divisions or external modifications (conidia) of an individual hyphae. During sexual reproduction, spherical sac-like appendages at the end of each hyphae are formed as a result from a mating between two different hyphae. These structures are called "ascii" and their function is to form and hold the sexually produced spores namely ascospores, which is spherical in shape and has a dimention of (5.5-) 6.0-7.0x (4-) 4.5-5.0 µm. (Figs. 1 and 2).

M. purpureus produce soluble pigments, but have ascomata, conidia which remain colourless and grew well on N25G agar. These results agreed in some aspects with those found by Hawksworth and Pitt (1983), Bridge and Howksworth (1985) and Cannon *et al.* (1995).

From the studies on biochemical characters, enzyme activity (APIZYM-system) *M. purpureus* characterize by the lack of leucin arylamidase and cysteine arlyamidase enzymes (Cannon *et al.*, 1995).

According to these studies *M. purpureus* fungi are eukaryotic organisms of the class Ascomycetes, subdivision Ascomycotina of the family Monoscaceae (Jûzlová, *et al.*, 1996). Summing up the forementioned results we could classify the isolated fungus as *M. purpureus* Went van Tieghem 5705.

These results could be supported by those found by Jones (1995); Read and Beckett, (1996); Wang, *et al.* (1999) and Ho *et al.* (1999). Also, justified by Assiut Mycological Center, Assiut University, Egypt.

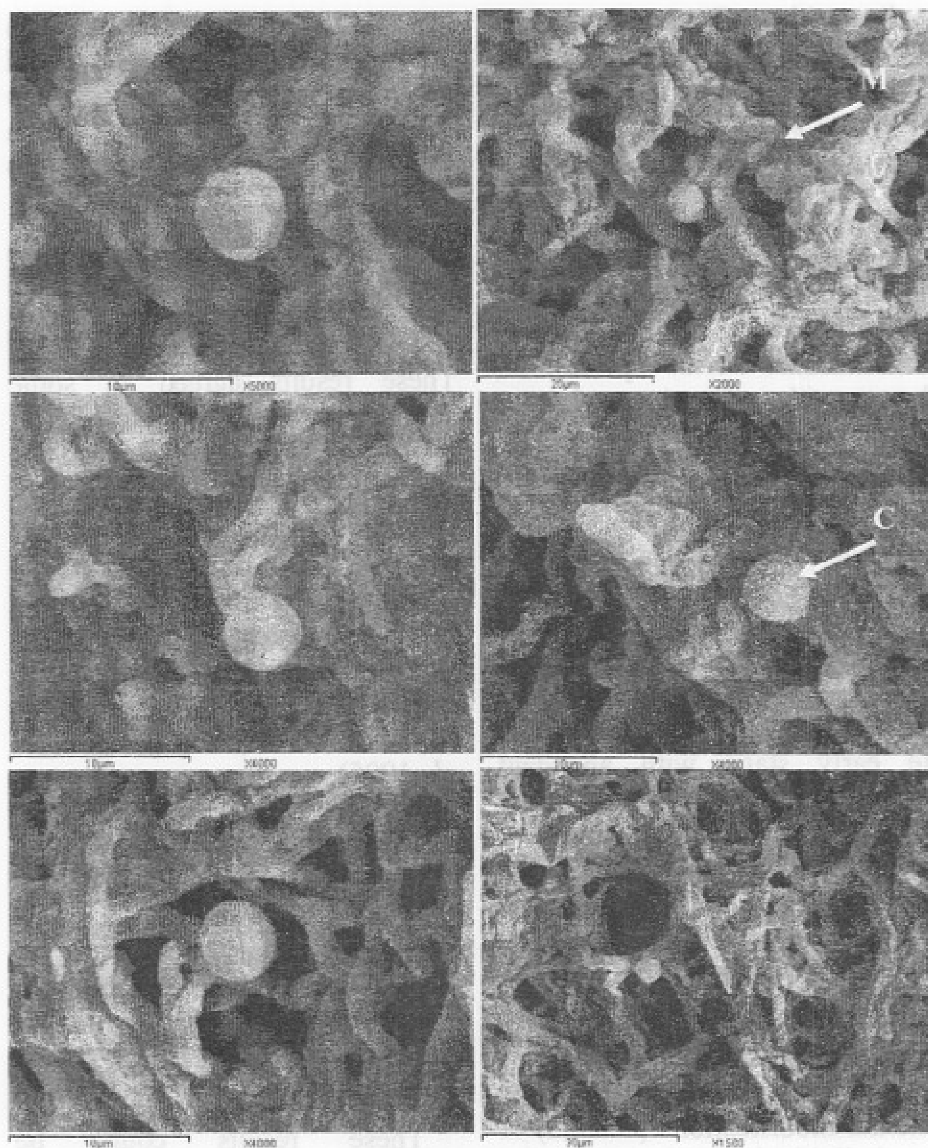

Fig. 1. Scanning electron micrographs of *M. purpureus* AUMC 4066

M = Mycelium C= Conidiospore

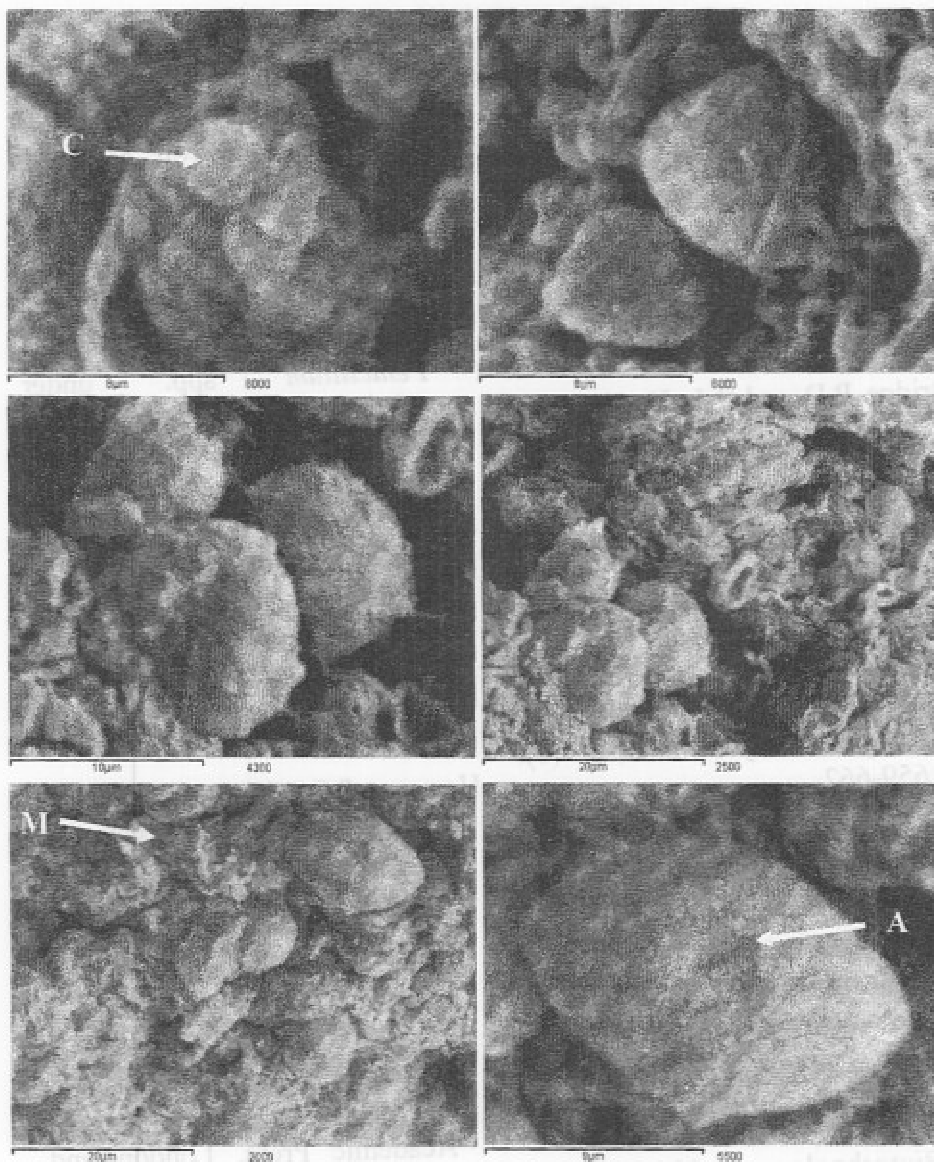

Fig. 2. Scanning electron micrographs of *M.purpureus* Went 5705

M = Mycelium A = Ascospore C= Conidiospore

REFERENCES

Ahmed, M.K, M.S. Nomani and B.P. Panda. 2009. Screening of nutrient parameters for red pigments product by *Monascas purpureus* MTCC369 under submerged fermentation using placket-barman design. Chiang Mai. J. Sci., 36 (1): 104-109.

Bridge, P.D. and D.L. Hawksworth. 1985. Biochemical tests as an aid to the identification of *Manascus* species. Lett. in Appl. Microbiol.,1:25-29.

Cannon, P.F., S.K. Abdullah and B.A. Abbas. 1995. Two new species of *Monassus* from Iraq, with a key to known species of the genus. Mycol. Res., 66 (6): 659-662.

Carvalho, J.C., B.O. Oishi, A.L. Woiciechowsk, A. Pandey, S. Babitha and C.R. Soccol. 2007. Effect of substrates on the production of *Monascus* biopigments by solid-state fermentation and pigments extraction using different solvents. Indian. J. Biotechnology, 6 : 194-199.

Chiu, S.W. and Y.K. Poon. 1993. Submerged production of *Monascus* pigments. Mycologia, 85(2): 214-218.

Difco Manual. 1985. Dehydrated culture media and reagents for microbiology. 10th (ed) Difco Laboratories Detroit Michigan 48232 USA.,pp.487-623.

Gunasekaram, S. and R. Poorniammal. 2008. Optimization of fermentation conditions for red pigments production from *Penicillium* spp. under submerged cultivation. African J. Biotech., 7(12): 1894-1898.

Hajjaj, H., P. Blanc, E. Grbussac, J.L. Uribelarrea, G. Goma and P. Loubiere. 2000. Kenetic analysis of red pigments and citrinin by *Monascus ruber* as a function of organic acid accumulation. Enzyme Microb. Technol., 27: 619-625.

Hamano, P.S., S.F.B. Orozco and B.V. Ilikan. 2005. Concentration and determination of extracallular and intracellular red pigments produced by *Monascus* pp. Barzilian Arch. of Bio. and Technol., 48: 43-49.

Harrigan, W.F. and E. McCance-Margaret. 1976. Laboratory Methods in Microbiology. Academic Press, London and New York.

Hausler, W.J. 1972. Standard Methods for Examination of Dairy Products 13th ed. Washington, D.C.: American Public Health Association.

Hawksworth, D.L. and J.J. Pitt. 1983. A new taxonomy for *Monoscus* species based on cultural and microscopical characters. Australian J. Botany, 31: 51-61.

Ho, W.H., V. Male Ranghoo, K. D. Hycle and I.J. Hodgkiss. 1999. Ascal ultrastructural study in *Annulatascus hongkongensis*, sp. nov., a freshwater ascomycete. Mycologia, 91 (5): 885-892.

Jackman, R.L. and J.L. Smith. 1996. Anthocyanins and betalains. In G.A.F. Hendry and J.D. Houghton (Eds), Natural Food Colourants (seconded.). UK: Chapman and Hall.

Jones, E.B.F. 1995. Ultrastructure and toxonomy of the aquatic ascomycetous order Halosphaeriales. Can. J. Bot., 73.

Jûzlová, P., L. Martinková and V. Kren. 1996. Secondary metabolites of the fungus *Monascus*: a Review. J. Indust. Microbiology, 16: 163- 170.

Khanafari, A., M.M. Assadi and F.A. Fakhr. 2006. Review of prodigiosin pigmentsation in *Serratia marcescens*. Online J. Biological Sci., 6(1): 1-13.

Kim, J.J., J.H. Kim, H.S. Oh and C.S. Shin. 2002. Morphology control of *Monascus* cells and scale- up of pigments fermentation process. Biochemistry, 38: 649-655.

Kim, J.K., S.M. Park and S.J. Lee. 1995. Novel antimutagenic pigments produced by *Bacillus licheniformis* SSA3. J. Microbiol. Bioctechnol., 5:48-50.

Kim. C., H. Jung, I.H. Kim and C.S. Chin. 2006. Effect of *Monascus* pigments derivatives on the electrophoretic mobility of bacteria and the cell adsorption and antibacterial activities of pigments. Colloids and Surfaces B. Biointefaces, 47: 153-159.

Kreger-van Rij. N.J.W. 1984. The yeasts, a taxonomy study, 3. Aufl. Groningen, The Netherlands. Elsevier Sciences Publishers, B.V. Amesterdam.

Lauro, G.J. 1991. A primer on natural colours. Cereal Food World, 36: 949-953.

Lee, B.K., N.H. Park, H.Y. Piac and W.J. Chung. 2001. Production of red pigments by *Monascus purpureus* in submerged culture. Biotechnol. Bioprocess Eng., 6 : 341-346.

Lin, C.F. 1973. Isolation and culture condition of *Manascus* sp. for the production of pigments in submerged culture. J. Ferment. Technol., 51:407 414.

Lin, T.F. 1991. Studies on the formation of *Monascus* red pigments. Ph.D. Thesis, Massachusetts Institue of Technology, Cambridge, Mass, USA.

Lin, T.F. and A.L. Demain. 1991. Effect of nutrition of *Monascus* spp. on formation of red pigments. Appl. Microbial. Bioteehnol., 36 (1): 70-75.

Lin, T.F., K. Yakaashijin, G.H. Buchi and A.L. Demain. 1992. Formation of water-soluble *Monascus* red pigments by biological and semi-synthetic process. J. Ind. Microbiol., 9: 173-179.

Pattanagul, P., R. Pinthong, A. Phianmongkhol and N. Leksawasdi. 2007. Review of angkak production (*Monascus purpureus*). Chiang. Mai. J. Sci., 34 (3): 319-328.

Perkoppová, J. 1984. Comparison of nutrient media for determination of yeasts and moulds in cheese. Bulletin potervinarskeka, 23(4): 253-561.

Rasheva, T., J.N. Hollet and A. Kujumdzieva. 1998. Taxonimic investigation of *Monascus purpureus* strain. J. Cult. Collect., 2: 51-59.

Read, N.D. and A. Beckett. 1996. Ascus and ascospore morphogenesis. Mycol. Res., 100: 1281-1314.

Sameer, A.M., S.M. Anne and T. Uif. 2006. Colorimetric characterization for comparative analysis of fungal pigments and natural food colourants. J. Agric. Food Chem., 54: 7027-7035.

Sandra, F., B. Orozco and B.V. Kilikian. 2008. Effect of pH on citrinin and red pigments production by *Manascus purpureus*. World J. Microbial. Biotechonl., 24: 263- 268.

Sardaryan, E., H. Zihlova, R. Strand and Z. Cermakova. 2004. Arpink red meet a new natural red food colourant of microbial origin. In. Pigments in Food, more than colours., L. Dufosé (Ed), Université de Bretagne Occidenrtate Publ., Quimper, Frances pp. 207- 208.

Sullina, S.B. and S. Shantharam. 1998. General Microbiology. Science Puplishers., Inc., USA.

Trias, J., M. Vinas, J. Guinea and J.G. Loren. 1988. Induction of yellow pigmentsation in *Serratia marcescens*. Appl. Environ. Microbiol., 54: 3138-3141.

Wang, S.L., T.C. Yieh, I.L. Shih. 1999. Purification and characterization of a new antifungal compound produced by *Pseudomonas aeruginosa* K-187 in a shrimp and crab shell powder medium. Enzyme Microb. Technol., 25: 439-446.

Wong, H.C. and P.E. Koehler. 1983. Production of red water-soluble *Monascus* pigments. J. Food. Sci., 48: 1200-1203.

Wong, H.C., Y.C. Lin and P.E. Koehler. 1981. Regulation of growth and pigmentsation of *Monascus purpureus* by carbon and nitrogen concentrations. Mycologia, 73: 649-654.

Wong, S.W., K.D. Hyde and E.B.G. Jones. 1998. Annulatonascaceae, a new ascomycete family from the tropics. Syst. Asscomyc., 16: 17-25.

Xijun, L., C. Wang and K. Guo. 2007. Identification of new red pigments producted by *Monascus ruber*. Dyes and Pigments. 73: 121- 125.

Yoshimura, M., S. Yamanaka; K. Mitsugi and Y. Hirose. 1975. Production of *Monascus* pigments in a submerged cultures. Agr. Biol. Chem., 39: 1789-1795.

إنتقاء بعض الميكروبات المنتجة للصبغات الحمراء في بيئات مختلفة وتعريف أهم المزارع الواعدة

مها محمد نادر إسماعيل – عصام الدين محمود جويلي

فيكتور صموئيل بدروس – جمال الدين مصطفي محمد

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق– مصر

فى هذه الدراسة تم تقييم إمكانية إنتاج الصبغات الحمراء بواسطة بعض الميكروبات المعزولة من مصادر مختلفة. وجد أن إثنتا عشرة عزلة من مختلف الميكروبات لها القدرة على إنتاج صبغات حمراء على سبعة بيئات عزل مختلفة، وقد اظهرت ثلاثة عزلات منها (ZL_3, ZL_2, ZL_1) قدرة اكبر على إنتاج هذه الصبغات. وبناءاً على الصفات المظهرية والميكروسكوب الإلكتروني وبعض الإختبارات البيوكيميائية تم تعريف العزلة المحلية ZL_1 على انها فطر *Monascus spp.*

وقد أعطت كلتا عزلتى الفطر: *Monascus purpureus* AUMC4066 وهى سلاله تم الحصول عليها من مركز الفطريات جامعة أسيوط والعزلة الثانية المعزولة محلياً ZL_1 وهي *Monascus* Went 5705 والتي تم تعريفها في مركز الفطريات – جامعة أسيوط أقصى كمية من الصبغات الحمراء المفرزة خارج الخلايا (U 2.47 و U 1.929) على التوالى (لمدة ١٠ أيام من التحضين) وذلك عند قياسها بطريقة لونية بإستخدام جهاز الاسبكتروفوتوميتر عند طول موجى ٥٠٠ نانوميتر وذلك عند التنمية على بيئتى تخمرهما: بيئة مرق مستخلص الخميرة والمولت (YM) وبيئة التخمر الأولى (F_1) على التوالى.

وعلى هذا فإن الإنتاج العالى من الصبغات الحمراء المنتج بواسطة هذه الفطريات يوضح إمكانية الإنتاج التجارى لهذه الصبغات واستخدامها كألوان طبيعية فى العمليات التصنيعية المختلفة.