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Effect of Different Types of Water on Growth and Pigment Production by *Monascus purpureus*

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Summary

While deionized water, distilled water, tap water, rain water, well water, ground water and canal water gave equivalent growth for *Monascus purpureus*, tap water, well water, ground water and canal water promoted pigment production when compared to deionized water, distilled water and rain water.

Introduction

The mold *Monascus purpureus* has been used for centuries in a fermentation to produce red coloring for red rice wine and red soy bean cake in China and Japan, and today its fermentative pigments are well accepted for coloring foodstuffs. Numerous investigators have carried out studies of *M. purpureus*(1-7), the chief interest being the production of food coloring pigments. These studies have shown that many factors affect growth and pigment production in *M. purpureus*. The factors include (i) environmental conditions (ii) nutrients (iii) some unusual substrates, e.g., various oils, and (iv) some heavy metals(8-11). However, these studies have not determined effects of different types of water on *M. purpureus*, and this could be of some importance to pigment factories using different water supplies. Thus, the present study examined seven different types of water for any influence on growth and pigment production by this mold.

Materials and Methods

Isolation of M. purpureus. Red rice obtained from commercial source was spread on sabouraud dextrose agar (SDA; Difco) plates. After incubation at 30°C for 5 days, representative colonies were sampled and restreaked on SDA slants for storage.

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Types of water. Seven different types of water were used in this study. Deionized water and distilled water were those routinely used in the microbiological laboratory, Faculty of Pharmacy, Mahidol University, Bangkok. Tap water, rain water, well water, ground water and canal water were all obtained from the Bangkok area. These different types of water were analysed for the presence of five metals (Cu^{++}, Fe^{+++}, Mg^{++}, Mn^{++} and Zn^{++}) using an atomic absorption spectrophotometer (Techtron, Australia).

Growth and piqment production. Cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of sabouraud dextrose broth (SDB; Difco) prepared using the seven types of water listed above. The initial pH of all media was about 6.3. Inoculum was prepared from two 10-day-old cultures of *M. purpureus*, each grown on 100 ml of SDA in a 250 ml Erlenmeyer flask. Spores were washed off each culture with 50 ml of sterile deionized water and the washings were pooled. Two-ml portions were added to the experimental flasks which were then incubated at 30° C. On the 3^{rd}, 5^{th} and 8^{th} day during growth, the contents of puplicate flasks were filtered through Whatman No. 1 paper. The pigment was extracted by washing the residue mycelium with three 20 ml portions of 95 % ethanol. The mycelium was then dried to constant weight at 90° C. to determined growth. The dissolved pigments in both SDB and ethanol were measured with a spectrophotometer (Bausch & Lomb) at 498 nm with the uninoculated SDB and ethanol as the respective colorimetric bianks. Measurements of pH were made with a digital pH meter (Model pHsar-1, Beckman).

Crude extract of red piqment. The pigment was extracted from red rice by procedure outlined above. About 10 g of red rice were extracted with three 100 ml of ethanol. The combined extract was evaporated to yield crude monascus pigment. Beginning with 0.2 % W/V, 2-fold dilutions of this crude extract were made with ethanol. Such preparations were then measured for absorption spectrum at 498 nm. The curve was plotted between the ethanol extracted pigment concentration and absorbancy. This curve was used as standard for determination of the amounts of the amounts of pigment in samples.

Results and Discussion

After growing of *M. purpureus* in media prepared with different types of water for 3 and 5 days, it was found that the growth of molds was likely to be equal in these media (see Table 1). On 8-day-old cultures, the growth was markedly increased to be 3-6 times more when compared to growth on the 3^{rd} and 5^{th} days. This study demonstrated no effect of different types of water on growth of *M. purpureus*, but pigment production was greatly influenced by the type of water used. On day 3, high productivity was observed in media prepared with well water, ground water and canal water, medium productivity was found in

Table 1. Growth of *M. purpureus* in SDB prepared with different types of water

Types of water	Growth dry weight (g)		
	Day 3	Day 5	Day 8
Deionized	0.030	0.025	0.109
Distilled	0.028	0.027	0.114
Tap	0.030	0.022	0.172
Rain	0.032	0.033	0.188
Well	0.040	0.027	0.120
Ground	0.033	0.035	0.177
Canal	0.036	0.039	0.146

Tadle 2. Pigment production in *M. purpureus* grown in SDB prepared with different types of water

Types of water	Day 3			Day 5			Day 8		
	Pigment (mg)		Total (mg)	Pigment (mg)		Total (mg)	Pigment (mg)		Total (mg)
	Media	EtOH		Media	EtOH		Media	EtOH	
Deionized	0.29	1.02	1.32	5.35	3.66	9.01	9.80	8.55	18.35
Distilled	0.34	0.71	1.05	8.15	2.43	10.58	15.25	6.03	21.28
Tap	2.05	0.99	3.04	16.80	6.78	23.58	28.20	19.92	48.12
Rain	0.85	0.92	1.77	6.30	2.22	8.52	23.65	11.10	34.75
Well	3.50	2.46	5.96	9.85	6.93	16.78	17.80	10.14	27.94
Ground	2.95	1.56	4.51	12.70	5.25	17.95	33.20	24.48	57.68
Canal	4.25	2.58	6.83	12.80	8.58	21.38	25.00	18.96	43.96

Tadle3 3. Percentage weight by weight of pigment to growth in *M. purpureus* grown in SDB in different types of water

Types of water	Pigment/growth (% W/W)		
	Day 3	Day 5	Day 8
Deionized	4.37	36.04	16.83
Distilled	3.75	39.19	18.67
Tap	10.13	107.18	27.98
Rain	5.53	25.82	18.48
Well	14.90	62.15	23.28
Ground	13.67	51.29	32.59
Canal	18.97	54.82	30.11

Table 4. pH change in SDB prepared with different types of water in the presence of growth and pigment production in *M. purpureus*

Types of water	pH			
	Initial	Day 3	Day 5	Day 8
Deionized	6.31	5.58	4.86	4.50
Distilled	6.32	5.84	5.27	5.05
Tap	6.35	6.07	5.00	4.85
Rain	6.35	5.72	5.51	5.09
Well	6.31	5.90	5.11	4.52
Ground	6.30	6.02	5.62	5.02
Canal	6.32	5.62	5.00	4.72

Table 5. Presence of Cu^{++}, Mg^{++}, Fe^{++}, Mn^{++} and Zn^{++} in different types of water for preparing os SDB

Types of water	Amounts of elements (μg/liter)				
	Cu^{++}	Fe^{++}	Mg^{++}	Mn^{++}	Zn^{++}
Deionized	10.4	58.2	187.8	4.0	34.7
Distilled	1.0	38.8	11.9	5.0	32.6
Tap	4.0	66.0	1006.0	8.5	119.5
Rain	0.8	54.3	199.8	4.3	1565.0
Well	6.7	112.6	1018.0	5.0	18.6
Ground	3.2	81.5	993.0	98.0	467.0
Canal	3.2	48.5	1000.0	6.0	90.2

tap water, and low productivity was found in deionized water, distilled water and rain water. The data of the experiment were tabulated in Tables 2 and 3. On day 5, the highest productivity of pigment was found in media prepared with tap water. This situation persisted to day 8. Table 2 shows that lengthening incubation resulted in increasing pigment contents both in the media and in the ethanol extract. The increase in pigment in the liquid medium with increasing culture time could have resulted from accumulation of alcohol produced during metabolism. This could enhance the solubilization of pigment from the cells.

Table 4 shows the pH of all the media constantly dropped during growth and pigment formation with *M. purpureus*. This indicates the accumulation of acidic compounds during metabolism. In the case of the media using tap water, the pH was much lower on day 5 than on day 3. This sudden change of pH correlated well with a noticeble increase in pigment production (Tables 2 and 3). A possible explanation for this event is that some slowly pigment promoting substances were present in tap water, and that after day 3, they were actively involved in cell metabolism which increased pigmentation and gave rise to highly acidic compounds.

There are studies reporting that Zn^{++} promotes growth in *M. purpureus*[(9)] and that Fe^{+++} enhances red pigment formation in *Candidapulcherrima*[(12)]. Since pigment production was noticely affected by the type of water used in preparing media, each type was tested for Cu^{++}, Fe^{+++}, Mg^{++}, Mn^{++} and Zn^{++}. The results are shown in Table 5. Among these five heavy metals, only the quantity of Mg^{++} seemed to have some effect on pigment production. As has been previously shown, pigment production in media prepared with tap, well, ground and canal waters was greater than media using deionized, distilled and rain waters. In the detection of heavy metals in these types of water, Mg^{++} was found to be approximately 1,000 μg/liter in the former groups. These amounts of Mg^{++} were about 5, 83 and 5 times higher than those contained in deionized, distilled and rain waters, respectively. Therefore, it was probable that Mg^{++} might be one of the important metals in the biosynthesis of this mold pigment. For other elements, i.e., Cu^{++}, Fe^{++}, Mn^{++} and Zn^{++}, this effect was not seen. The possibility was that their amounts were too more or few from the optimum concentration or they really did not have any roles in the synthesis of the red pigment in *Monascus*. However, other than the metals there might have some water soluble compounds that would influence the effect. This needs further detection.

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Reference

1. Church, M.B. (1920) Laboratory experiments on the manufacture of Chinese Ang-khak in the United States, J, Indus. and Engineer. Chem,, 12, 45-46.
2. Nakazawa, R. and Sato, K. (1930) On the *Monascus* of Taiwan, the red seed of rice. J. Agri. Chem. Soc. Japan, 6, 352-355.
3. Kawabata, S. and Sato, K. (1976) Water-soluble *Monascus* pigment, Kokai (Japawese), 88, 519.
4. Kim, C.S., Rhee, S.H. and Kim, I. (1977) Studies on production and characteristics of edible red color pigment produced by mold (*Monascus* sp.), Hanguk Sikp'um Kwahakhoe chi (Korean), 9, 277-283.
5. Wong, H.C. and Bau, Y.S. (1978) A comparison of conidial and ascospore germination of *Monascus purpureus*, Trans. Br. Mycol. Soc., 70, 277-282.
6. Bau, Y.S. and Wong, H.C. (1979) Zinc effects on growth, pigmentation and antibacterial activity of *Monascus purpureus*, Physiol. Plant, 46, 63-67.

7. Hiroi, T., Shima, T., Suzuki, T., Tsukioka, M. and Ogasawara, N. (1979) Hyperpigment-productive mutant of *Monascus anka* for solid culture, Agri. Biol. Chem., 43, 1975-1976.
8. Sato, K. and Naito, J. (1935) Acids and alcohols as nutrients for *Monascus*, J. Agri. Chem. Soc. Japan, 11, 473.
9. McHan, F. and Johnson, G.T. (1970) Zinc and amino acids: important components of medium promoting growth of *Monascus purpureus*, Mycologia, 62, 1018-1030.
10. Manandhar, K.L. and Apinis, A.E. (1971) Temperature relations in *Monascus*, Trans. Br. Mycol. Soc., 57, 465-472.
11. Yoshimura, M., Yamanaka, S., Mitsugi, K. and Hirose, Y. (1975), Production of *Monascus*- pigment in a submerged culture, Agr. Biol. Chem., 39, 1789-1795.
12. Beijerinck, M.W. (1918) Levures chromogênes. Nouvelle, réaction biologique du fer, Arch. neerl. Physiol., 2, 609-615.