Characteristics of Red Pigment from Marine Bacterium Utilizing Colloidal Chitin

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Abstracts

Studies on extraction of red pigment was performed to provide the basic information for the utilization of red pigment as s new source of natural food colorant. A bacterium isolated from marine resources were carried out the test for excretion of red pigment. One strain of a marine bacterium, KSR-97 showed a high production of red pigment on the medium of colloidal chitin, peptone-yeast extract with minerals. In physicochemical and sensory properties in aqueous solution of red pigment was investigated at various condition of pH, temperature, concentration of ethanol and stability of storage. Potent antioxidative of red pigment was separated by thin layer chromatograpy, silica gel chromatography and reverse high performance liquid chromatography using ODS hypersil column.

Materials and methods

Microorganism and Media

KSR-97 used in this experiment was isolated from the sea waters. The medium was contained 20% colloidal chitin, 2.5g peptone, 2.5g yeast extract, 1g KH_2PO_4, 0.01g $MgSO_4 \cdot 6H_2O$, 0.01g $ZnSO_4$ and 0.01g $MnSO_4$(per *l*)

Assay for red pigment produced in culture broth

The amount of pigment was determined on a spectrophotometer by measuring its absorbance at 450~550nm of the culture filtrate.

pH, temperature and culture period

The initial pH of the medium was varied from 4 to 8 with 1N-hydrochloric acid or sodium hydrooxide. On the other hand, to find out maximum temperature were incubated at temperature ranges from 15℃ to 40℃

Antimicrobial test

Paper disk susceptibility test: The agar diffusion disk technique is the method commonly used in laboratories for measuring the susceptibility of various bacteria.

HPLC analysis

Reverse phase HPLC analysis was done on a ODS gel column and monitored RI detector. The column was eluted at a rate of 1.0㎖/min with solvent solution(H_2O-MeOH 5:95, v/v).

Results and discussion

Various mono, di and polysaccharide sugar alcohol and organic acids were checked to determine the most suitable carbone sources for red pigment production. L-arabinose, xylose and rhamnose were found to be no effective for red pigment. Polypeptone, tryptone and yeast extract were proved to be the best of the various source tested. The most suitable concentration was 1% polypeptone. Red pigment increased gradually with increasing concentration of sodium chloride. The pigment showed highest level of productivity when 4% sodium chloride added in culture were almost less than 1% and over than 5% in the medium, its inhibited a pigment. The maximum yield of red pigment was obtained when the initial pH of the medium was adjusted to pH 7 for 72 hours days cultivation. The red pigment production increased with rising temperature and reached a maximum at 25℃, but the production of the pigment markedly decreased at temperature of higher than 30℃(Fig. 1). In Sensory tests, red pigment was fairly stable in the ranges of pH 3 to 8(Fig. 2). The effects of storage were stable until 7 days(Fig. 3). Until ethanol concentration of 40% added to the red pigment was shown to be suitable(Fig. 4). Red pigment was extracted well with the non-polar solvents. The ethyl acetate extract showed the highest antimicrobial activities for gram positive and negative strains(Table 1, 2). When ethyl acetate fraction was applied to silica gel column, antioxidant substances were eluted with chloroform:methanol mixture. This active fractions was separated to several spots (Rf=0.81, 0.30, 0.24 and 0.12) on the TLC in chloroform:methanol system(Fig. 5). In this system Rf=0.24 spot indicated high est antioxidant activity. From HPLC analysis on silica gel column, 1 peak was obtained.

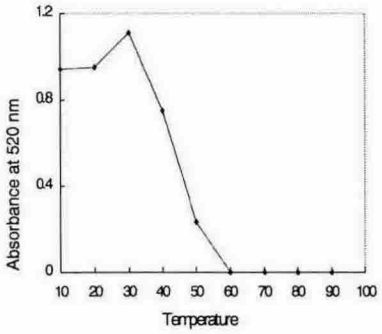

Fig. 1. Effect of temperature on the absorbance of red pigment extract

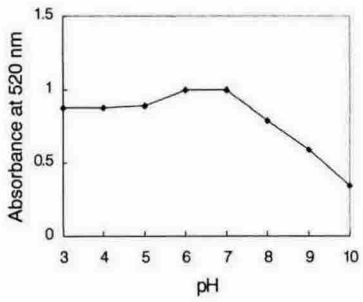

Fig. 2. Effect of pH on the absorbance at 520nm by red-pigment extracts.

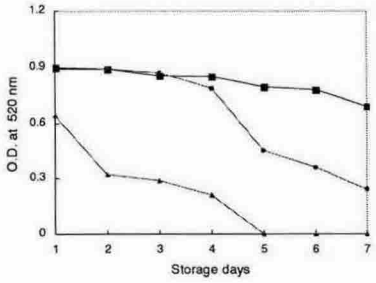

Fig. 3. Comparative absorbance of red pigment extract during storage at 4℃, 25℃ and 37℃.

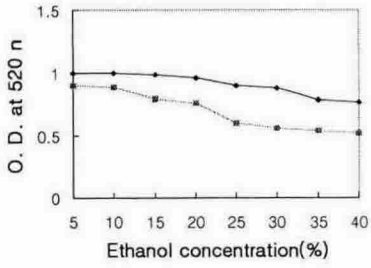

Fig. 4. Effects of ethanol concentration

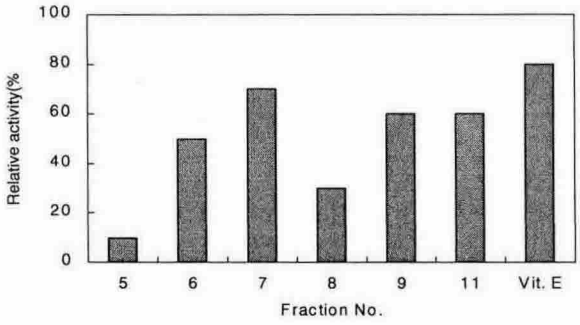

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Fig. 5. Antioxidative activity of the various fractions by silica gel column chromatography with chloroform and methanol ratio.

Table 1. Antimicrobial effect of the extracts against strains by agar diffusion method using paper disc

Strains	Inhibitory zone (ϕ, ㎜)		
	Methanol	Etylacetate	Buthanol
Staphylococcus aureus ACTC 65389 -in Gram(+) bacteria	2	18	8
Escherichia coli -in Gram(-) bacteria	8	15	8
Fusarium oxysorum IFO 5901 -Fungi	6	13	-

Table 2. Antimicrobial effect of the ethyl acetate extract under various conditions

Strains	Treatment(ϕ, ㎜)			
	None	Heat (121℃, 1atm and 20min)	Acid (pH 3.0)	Alkali (pH 11.0)
Staphylococcus aureus ACTC 65389	18	17	17	16
Escherichia coli	15	15	14	14
Fusarium oxysorum IFO 5901	13	13	12	13

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