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Potential Chemopreventive Properties of Anthocyanin-Rich Aqueous Extracts from In Vitro Produced Tissue of Sweetpotato (*Ipomoea batatas* L.)

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Anthocyanin-rich aqueous extracts from cell suspension cultures of a high anthocyanin-producing sweetpotato PL (purple line) cell line grown under two different media conditions, MM (multiplication medium) and APM (high anthocyanin-producing medium) and from the cell line's donor tissue, field-grown storage root (SR) of sweetpotato, cv. Ayamurasaki, were evaluated for antioxidative (DPPH test), antimutagenic (*Salmonella*/reversion assay; mutagen, Trp-P-1), and antiproliferative (human promyelocytic leukaemia cells HL-60) activities. Both cell line extracts MM and APM exhibited higher radical scavenging activities (RSA), 3.8- and 1.4-fold, respectively, than the SR extract. The antimutagenic activity of all extracts was found to be dose-dependent. At a dose of 1 mg/plate, the highest activity exhibited APM (73% inhibition of Trp-P-1-induced reverse mutation of *Salmonella typhimurium* TA98), followed by MM (54% inhibition) and SR (36% inhibition). The MM extract was the strongest inhibitor of the proliferation of human promyelocytic leukemia cells. At a concentration of 1.6 mg/mL medium during 24 h, it suppressed the growth of 47% of HL-60 cells. A significantly lower growth suppression effect displayed APM and SR extracts (21 and 25%, respectively). Total anthocyanin levels and anthocyanin composition in evaluated samples seem to be related to their activities. The MM extract, which exhibited the highest RSA and antiproliferation activities, contained the highest level of anthocyanins. Among them, nonacylated cyanidin 3-sophoroside-5-glucoside dominated. It is speculated that the presence of this anthocyanin contributed toward enhanced activities of MM extract.

KEYWORDS: Sweetpotato; *Ipomoea batatas*; physiological activity; functional foods; polyphenolics; anthocyanins; callus culture

INTRODUCTION

Functional food products are aimed at introducing human dietary ingredients that aid specific bodily functions in addition to being nutritious. Sweetpotato (*Ipomoea batatas* L.)—a root crop cultivated for centuries in various geographic regions and today the world's sixth largest food crop—has been recently rediscovered as a highly nutritious food, rich with vitamins and minerals in favorable ratios. The extract from a purple-fleshed sweetpotato exhibited strong radical scavenging (*1*) and antimutagenic activities (*2*, *3*) and significantly reduced high blood pressure and carbon tetrachloride-induced liver injury (*4*). Other physiological functions of sweetpotato components include antiinflammatory, antimicrobial, and antihypertensive activities and ultraviolet protection effects (*5*). A sweetpotato plant accumulates high levels of polyphenols, among them anthocyanins (*6*) and phenolic acids such as caffeic, monocaffeoylquinic (chlorogenic), dicaffeoylquinic, and tricaffeoylquinic acids (*7*). These components were identified as contributors of strong physiological activities of purple-fleshed sweetpotato plants (*1*, *3*, *5*, *8*). Recently, diacylated anthocyanin isolated from the SR of purple-fleshed sweetpotato cv. Ayamurasaki was identified to possess a postprandial antihyperglycemic (antidiabetic) effect in rats through retardation of maltase activity. On the basis of this quality, the development of antihyperglycemic food from purple-fleshed sweetpotato has been discussed (*9*). Anthocyanins (*10*) and phenolic acids (*11*, *12*) have also been

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reported to possess potential cancer chemopreventive effects. Because of the possible protective effect against chronic diseases, the design of "anthocyanin-tailored" functional food, where a food matrix would serve as a carrier and stabilizer of ingredients with special health effects without reducing their bioavailability, has been undertaken (*13*).

Plant tissue culture offers an opportunity to produce large quantities of tissue in a factory setting independent of environmental conditions. A high anthocyanin-accumulating cell line (PL) has been generated from the SR of sweetpotato cv. Ayamurasaki (*14*). The PL cell line, due to its outstanding ability to accumulate anthocyanin pigment in the dark, is considered to be a suitable source of natural food colorant produced by the means of plant cell cultures. Through changes in the composition of culture medium as well as environmental factors, the quality of in vitro accumulated anthocyanins can be regulated toward biosynthesis of highly acylated or nonacylated components (*15*). These changes in pigment composition may reflect on the color characteristics of pigment extract, its stability, and its nutraceutical properties. In the crude extract of the PL cell line tissue, beside anthocyanins, CHA and CA have been detected (*16*). Their presence may contribute toward chemopreventive attributes of the extract.

Food colorants produced from natural sources represent anthocyanin-rich concentrates, obtained from vegetables or edible fruits by extraction with water, methanol, or ethanol (*17*). These concentrates, beside visible pigment, contain other water soluble compounds such as sugars, phenolics, or vitamins, which are incorporated into food products and may reflect their physiological activities. In a similar manner, a crude anthocyanin-rich extract prepared from the PL cell line tissue produced in vitro may serve as a potential food additive. Therefore, it became of interest to us to evaluate possible physiological activities of the crude anthocyanin-rich extract from the cell line. Aqueous extracts have been prepared from tissue produced under two different media conditions. As a control, a similar extract has been prepared from the cell line's donor—field-grown Ayamurasaki SR. This paper presents our results on the evaluation of antioxidant, antimutagenic, and antiproliferative activities of these extracts.

MATERIALS AND METHODS

Plant Material. The callus culture of the PL cell line, previously established from the SR of sweetpotato cv. Ayamurasaki (*14*), was used for this study. Suspended cell cultures were initiated by transferring about 1.0 g fresh weight (FW) of callus to 50 mL of liquid medium in 250 mL Erlenmeyer flasks. Basal Murashige and Skoog (MS) medium (*18*) supplemented with 1.0 mg/L 2,4-dichlorophenoxyacetic acid and 3% sucrose was used as a multiplication medium. Medium pH was adjusted to 5.8 before autoclaving. Subcultures were done at 7 day intervals. The cultures were incubated on a rotary shaker (130 rpm) at 25 °C in the dark.

For this analysis, suspension cultures were produced under two different medium conditions: MM and APM. The APM was a modified MS medium with 9.4 mM KNO_3, without NH_4NO_3, with 5% sucrose and no growth regulators (*15*). Medium pH was adjusted to 5.8 before autoclaving. Suspension cultures were incubated in 250 mL Erlenmeyer flasks. Five hundred milligrams of cell aggregates was placed in each flask containing 50 mL of medium. The cultures were harvested after 7 days of growth on MM medium and after 14 days on APM medium. SRs of sweetpotato cv. Ayamurasaki were obtained from the Sweetpotato Breeding Laboratory of KONARC.

Preparation of Lyophilized Aqueous Extracts. The aggregates were removed from the culture media, rinsed with distilled water, separated from the liquid by vacuum filtration, and weighed. Fresh aggregates were instantly frozen using liquid nitrogen and stored at −70 °C. Subsequently, the tissue was freeze-dried.

Randomly selected fresh SR of sweetpotato, stored at 15 °C for 3 months, were chopped into small cubes (0.5 mm^3), frozen to −70 °C, and freeze-dried. The freeze-dried tissues were ground and used for extract preparation.

The extracts were prepared at room temperature from the lyophilized flours, using ice-cold water (20 mL per 1 g flour) for 1 h. The suspensions were centrifuged at 18 000*g* for 20 min. The supernatants were collected and lyophilized.

The lyophilized aqueous extracts of the PL cell line tissue produced in MM medium and APM medium were named MM and APM, respectively. The lyophilized aqueous extract of Ayamurasaki SR was named SR.

Quantification of Anthocyanins in Lyophilized Aqueous Extracts. Samples of 20 mg of lyophilized aqueous extracts of MM, APM, and SR (in triplicate) were extracted with 4 mL of 15% acetic acid for 1 h at room temperature. The suspensions were centrifuged at 10 000 rpm for 10 min. The supernatants were collected for identification of anthocyanins and high-performance liquid chromatography (HPLC) analysis.

The supernatants diluted 4-fold with McIlvaine's buffer solution and pH adjusted to 3.0 were used for the measurement of the optical densities at 520 nm with a spectrophotometer UV-1601 (Shimadzu Co., Kyoto, Japan). The color value (CV) of the pigment extract was calculated using the following formula: $CV = 0.1 \times OD_{520} \times D1 \times D2$ (CV/g FW), where OD_{520} is the spectrophotometric reading at 520 nm, and *D*1 and *D*2 are the levels of dilution (*14*).

HPLC Analysis of Aqueous Extracts. For HPLC analysis, the supernatants were filtered through a 0.2 μm filter membrane (DISMIC-13cp, Advantec, Japan). The HPLC system consisted of two LC-10AD Intelligent Pumps, SPD-M10A diode array detector, CTO-10AS column oven, DGV-12A degasser, SIL-10AD autoinjector, and SCL-10A system controller (Shimadzu Co.) equipped with Luna (3μ C18(2), 4.6 mm i.d. × 100 mm) (Phenomenex, U.S.A.) column at 35 °C. The solvents in water with a flow rate of 1 mL/min were used as follows: A, 1.5% phosphoric acid, and B, 1.5% phosphoric acid, 20% acetic acid, and 25% acetonitrile. The elution profile was a linear gradient elution for B 25−85% for 40 min in solvent A. The chromatograms were monitored at 520 nm and recorded. Identification of anthocyanins was carried out by comparing the peaks with YGM-0a (cyanidin 3-sophoroside-5-glucoside) and YGM-0f′ (cyanidin 3-(*p*-coumaroylsophoroside)-5-glucoside) standards isolated from the PL cell line (*19*) and standard peaks of purple-fleshed sweet potato YGM anthocyanins: YGM-1a [cyanidin 3-(caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside], YGM-1b [cyanidin 3-(dicaffeoylsophoroside)-5-glucoside], YGM-3 [cyanidin 3-(caffeoylferuloylsophoroside)-5-glucoside], YGM-4b [peonidin 3-(dicaffeoylsophoroside)-5-glucoside], YGM-5a [peonidin 3-(caffeoyl-*p*-hydroxybenzoylsophoroside)-5-glucoside], and YGM-6 [peonidin 3-(caffeoylferuloylsophoroside)-5-glucoside] (*20*).

Scavenging Activity toward DPPH. Radical scavenging activity (RSA) was determined using a stable radical, DPPH, according to the method reported by Brand-Williams et al. (*21*) with modification as described by Islam et al. (*8*). All reactions were conducted in 96 well microplates with a total volume of 300 μL in each well. The sample solution (75 μL) containing the test compound at concentrations of 0.0, 0.5, 1.0, 5.0, and 7.5 mg in 0.1 M 2-morpholinoethansulfonic acid (MES) buffer (pH 6.0) containing 50% ethanol was added to 150 μL of the same buffer. Furthermore, 75 μL of 0.4 mM DPPH solution in 50% ethanol was added to the mixture, shaken vigorously, and held for 2 min at room temperature. The decrease in absorbance of DPPH at 520 nm was measured with a dual wavelength flying spot scanning densitometer (Shimadzu Co.) with a microplate system. The absorbance of each sample was measured against a blank of ethanol without DPPH. All experiments were carried out in triplicate and repeated twice. DPPH RSA was calculated according to the equation: DPPH RSA (%) = [(absorbance control − absorbance of samples)/absorbance of control] × 100. The RSAs of the aqueous extracts were expressed in terms of IC_{50}—concentration in micromoles of Trolox required for 50% decrease in absorbance of DPPH radicals. The IC_{50} values of purified CA, CHA, and CoA were shown on a molar basis. A plot of absorbance vs concentration was made to establish the standard curve and to calculate IC_{50}.

DPPH, MES, and CA were obtained from Wako Pure Chemical Industries (Osaka, Japan). Trolox was purchased from Aldrich Chemical Co. (Milwaukee, WI), and CHA and CoA were from Sigma Chemical (St. Louis, MO). Other chemicals used were special grades.

Antimutagenicity Assay. Antimutagenicity tests were conducted as described by Yoshimoto et al. (*3*). The antimutagenic potential of aqueous extracts was evaluated for *Salmonella typhimurium* TA98 using mutagen Trp-P-1. Mutagen Trp-P-1 requires a metabolic activation to cause mutations in TA98. For that, it was used (in dose of 0.075 μg/plate) in the presence of S-9 mix. S-9 mix contained 50 μmol of sodium phosphate buffer (pH 7.4), 4 μmol of $MgCl_2$, 16.5 μmol of KCl, 2.5 μmol of glucose-6-phosphate, 2 μmol of NADH, 2 μmol of NADPH, and 50 μL of S-9 fraction in a total volume of 0.5 mL. Mutagen Trp-P-1 was obtained from Wako Pure Chemical Industries. The S-9 fraction, supplied by Oriental Yeast Co. Ltd., was prepared from rat liver treated with phenobarbital and 5,6-benzoflavone and cofactors. All other chemicals were of the highest grade commercially available. All tests were performed in triplicate in two independent experiments. Strain TA98 of *S. typhimurium* was supplied by the Institute of Fermentation (IFO) in Osaka, Japan. The bacteria were cultured in nutrient broth for 20 h at 37 °C on a reciprocal shaker with 100 rpm before the assay was conducted.

Stock solutions of CA, CHA, and CoA were prepared in dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL. From these stock solutions, equivalents of 1.0, 0.5, and 0.1 mg were applied to each plate in triplicate. Similarly, DMSO-dissolved lyophilized extracts were applied at 0.1, 0.5, and 1.0 mg/plate in triplicate.

For the inhibition test, 0.1 mL of mutagen Trp-P-1, 0.1 mL of sample (when the required volume of sample was less than 0.1 mL, the volume was compensated with DMSO), and 0.5 mL of S-9 mix were simultaneously incubated with 0.1 mL of bacterial suspension at 37 °C for 20 min. Subsequently, the incubated culture was mixed with 2 mL of soft agar and poured on minimal glucose agar plates. The plates were incubated for 48 h at 37 °C. The number of bacterial colonies on each plate was counted.

Antiproliferation Assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay (*22*) was used to assess the antiproliferation action of the reconstituted lyophilized powders of aqueous extracts in human promyelocytic leukemia cells (HL-60). MTT was purchased from Sigma Chemical Co. HL-60 cells were obtained from Cell Resource Center for Biomedical Researcher, Tohoku University, Japan, and were cultured in RPMI-1640 medium containing 10% fetal bovine serum at 37 °C in a 5% CO_2/95% air atmosphere. HL-60 cells (5×10^3 cells in 100 μL medium) were plated into each well of the 96 well microtiter plates. After they were incubated for 24 h, the cells were treated with various concentrations of the extracts for 24 h. MTT solution was added to each well and incubated for another 4 h. The resulting MTT formazan product was dissolved by the addition of 100 μL of 0.04 N HCl-2-propanol. The amount of formazan was determined by measuring the absorbance at 595 nm with a microplate reader (Bio-Rad, model 550). The results are expressed as the optical density ratio of the treatment to control. Student's *t*-test was used to determine the difference between the treated and the control. A probability of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Anthocyanins in Aqueous Extracts. The PL cell line, generated from the SR of sweetpotato cv. Ayamurasaki, accumulates high levels of anthocyanin pigments and has the potential to be used for commercial production of natural food colorants by means of plant tissue culture. An anthocyanin-rich extract from in vitro produced tissue could serve as a food additive. Therefore, it became of interest to us to evaluate possible physiological activities of the crude cell line extract and to compare them with the activities of extract from the cell line's donor tissue—field-grown SR of Ayamurasaki. Aqueous extracts have been prepared from cell line tissue produced under two different media conditions: MM and APM. The choice of these media treatments was dictated by our previous finding of distinguished differences in the composition of anthocyanin pigments accumulated at various levels of NH_4^+ in culture medium (*15*). As a control, a similar extract has been prepared from the cell line's donor—field-grown Ayamurasaki SR. The extracts were analyzed for the presence of anthocyanin pigments.

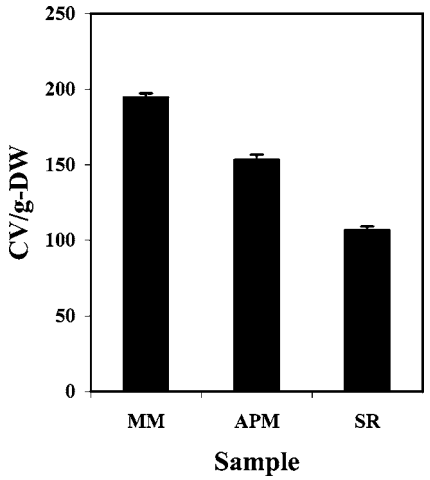
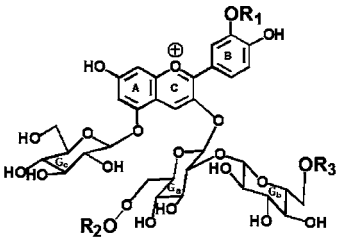


Figure 1. CVs of aqueous extracts prepared from the PL cell line tissue generated from the SR of sweetpotato (*I. batatas* L.) cv. Ayamurasaki grown on MM and APM and from the field-grown Ayamurasaki SR. Each value represents the mean ± SD, $n = 3$.

Anthocyanins, being water soluble, were present in aqueous extracts of the PL cell line and the SR (**Figure 1**). The highest level of total anthocyanins, expressed as a CV/g dry weight (DW) lyophilized powder, was detected in the MM extract. It contained 25% more anthocyanins than APM and 82% more than SR.

Major anthocyanin pigments accumulated by the field grown Ayamurasaki SR and the PL cell line have already been identified (**Figure 2**) (*14*, *19*, *20*, *23*, *24*). All of these pigments are derivatives of cyanidin 3-sophoroside-5-glucoside (YGM-0a) modified by acylation and/or methylation. Because some of them were first isolated from the purple-fleshed sweetpotato cultivar Yamagawa Murasaki, which was Ayamurasaki's ancestor, they were named YGM-anthocyanins (*25*). Some anthocyanins (YGM-0a, -0b, -0d, -0g, -0i, -3, -4b, -5b, and -6) accumulated by the PL cell line in vitro are overlapped with these accumulated by the cell line donor tissue, field-grown SR, but others (YGM-0f′, -0g′, -3′, -7a, and -7e) are cell line specific (*14*). It has been found that YGM-0f′, -0g′, and -3′ are monoacylated, and YGM-7a and -7e are diacylated with CoA, respectively (**Figure 2**). Whereas anthocyanins detected in the SR are acylated with caffeic, *p*-hydroxybenzoic, and ferulic acids (*20*, *23*, *24*).

The HPLC analysis of anthocyanins present in MM, APM, and SR extracts revealed differences in their compositions (**Figure 3**). The major anthocyanins of MM sample were YGM-0a and YGM-0b, previously reported as cyanidin 3-sophoroside-5-glucoside (*19*) and peonidin 3-sophoroside-5-glucoside (*16*), respectively. The YGM-0a constituted 51.2% and YGM-0b constituted 27.1% of total anthocyanin pigments calculated according to the peak area. The major acylated component present in this sample was YGM-0f′ (cyanidin 3-*p*-coumaroyl-sophoroside-5-glucoside (*19*) with a relative concentration of 9.3%. The ratio of cyanidin-based to peonidin-based anthocyanins in this extract was 2:1. The composition of anthocyanins in APM extract was different. Here, the dominant peaks were YGM-0f′, -0g, -0g′, -0i, -7a, and -7e. They constituted, respectively, 20.5, 7.5, 21.3, 9.3, 11.5, and 7.4% of total



YGM Anthocyanin	R_1	R_2	R_3	Cell line *		Storage root*
				MM*	APM*	SR*
0a	H	H	H	51.2	2.7	0.5
0b	CH_3	H	H	27.1	6.7	1.2
0d	H	H	C	1.5	1.0	T
0f'	H	H	*p*C	9.3	20.5	ND
** **0g**	H	H	F	1.5	7.5	T
** **0g'**	CH_3	H	*p*C	3.0	21.5	ND
** **0i**	CH_3	H	F	1.0	9.5	T
1a	H	C	B	ND	T	5.5
1b	H	C	C	ND	ND	3.0
2	H	C	H	ND	ND	4.5
3	H	C	F	ND	T	7.0
3'	H	C	*p*C	ND	3.0	ND
4b	CH_3	C	C	ND	T	20.9
5a	CH_3	C	B	ND	ND	12.4
5b	CH_3	C	H	ND	1.0	14.3
6	CH_3	C	F	ND	3.0	26.0
7a	H	*p*C	*p*C	ND	11.5	ND
7e	CH_3	*p*C	*p*C	ND	7.5	ND

Figure 2. Molecular structures of anthocyanin pigments accumulated in the PL cell line tissue generated from the SR of sweetpotato (*I. batatas* L.) cv. Ayamurasaki and in the field-grown Ayamurasaki SR. The YGM anthocyanins are listed as previously reported (*14*, *19*, *20*, *23*, *24*). *, Relative concentration of individual anthocyanins in MM, APM, and SR aqueous extracts calculated from the peak areas of HPLC chromatograms. T, peaks with area percentage <1.0 (with the exception of YGM-0a in SR); ND, peak not detected; B, *p*-hydroxybenzoic acid; C, caffeic acid; pC, *p*-coumaric acid; F, ferulic acid. **Structures of YGM-0g, -0g′, and -0i are only estimated.

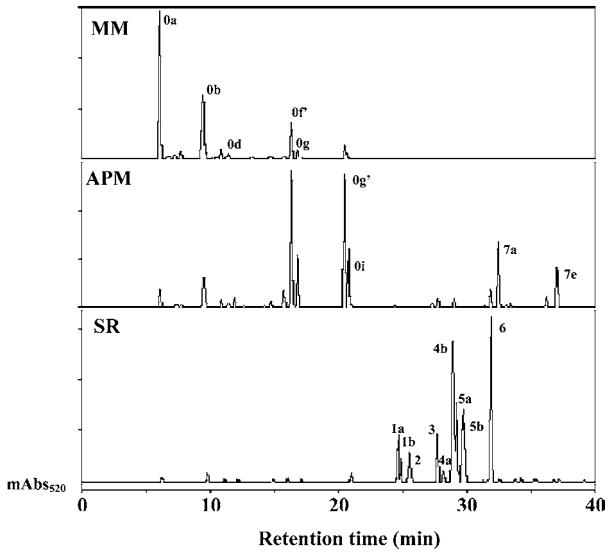


Figure 3. HPLC chromatograms of the PL cell line lyophilized aqueous extracts (MM and APM) and of lyophilized powder of the SR of sweetpotato (*I. batatas* L.) cv. Ayamurasaki (SR). The characters in the chromatograms are the YGM numbers of sweetpotato anthocyanins (*14*, *19*, *20*, *23*, *24*).

anthocyanins. All of these peaks appeared on the HPLC chromatogram with later retention times than YGM-0a and -0b and are suspected to have more complicated molecular structures. Recently, the peak YGM-7a has been identified as cyanidin 3-(di-*p*-coumaroylsophoroside)-5-glucoside and YGM-7e as peonidin 3-(di-*p*-coumaroylsophoroside)-5-glucoside (**Figure 2**). The peaks YGM-0g, -0g′, and -0i are partially

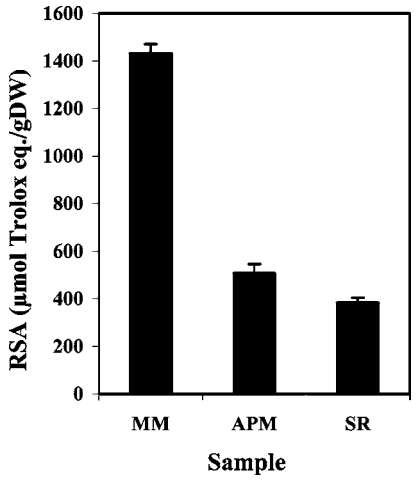


Figure 4. Radical scavenging activities of aqueous extracts prepared from the PL cell line tissue grown on MM and APM media and from the SR of sweetpotato (*I. batatas* L.) cv. Ayamurasaki (SR). Values are means ± SD, $n = 6$.

determined. At present, we estimate that the ratio of cyanidin-based to peonidin-based anthocyanins in this sample is 1:1. The relative concentrations of nonacylated YGM-0a and -0b in APM extract were 2.7 and 6.6%, respectively. Contrary to the MM, where nonacylated anthocyanins dominated, APM consisted predominantly of acylated derivatives of YGM-0a and -0b. The composition of anthocyanin pigments in SR was different to those in MM and APM. The major anthocyanins detected were YGM-4b, -5a, -5b, and -6. These anthocyanins were previously identified as peonidin 3-sophoroside-5-glucosides acylated with *p*-hydroxybenzoic and/or caffeic and/or ferulic acid (*20*, *23*, *24*). In the SR extract, these diacylated anthocyanins made up 73.5% of the total anthocyanins as calculated by peak area. The second group was cyanidin-based YGM-1a, -1b, -2, and -3 (cyanidin 3-sophoroside-5-glucosides with the same acylation pattern) (*20*). These pigments made up 19.9% of total anthocyanins. The relative concentrations of nonacylated YGM-0a and -0b were 0.4 and 1.2%, respectively. With the ratio of cyanidin-based to peonidin-based anthocyanins being 1:3.7 in the SR extract, peonidin type pigments dominated.

RSA. The antioxidant activities of aqueous extracts have been determined using the free radical DPPH. Both tissue culture produced extracts, MM and APM, exhibited higher scavenging activities than the SR extract of field-grown SR (**Figure 4**). Significant differences were obtained between cell line extracts produced under different medium conditions. The scavenging activity of MM was 2.8-fold higher than the activity of APM and 3.8-fold higher than the activity of SR.

Aqueous extracts used for this study are rich in anthocyanins. A strong antioxidative potential of flavonoids and phenolic acids (*26*), among them anthocyanin-based natural food colorants (*27*) as well as purified anthocyanins in various systems such as the DPPH assay, β-carotene bleaching method, linoleic acid emulsion system (*28*), human low-density lipoprotein, and lecitin−liposome systems (*29*) have been reported. Cevallos-Casals and Cisneros-Zevallos (*30*) reported that anthocyanin-accumulating red-fleshed sweetpotato exhibited higher antioxidant capacity and antiradical kinetics in the DPPH test than blueberries. Phenolics from red sweetpotato also reacted faster to stabilize DPPH radicals than Trolox. Higher total phenolic content and larger numbers of active hydroxyl groups as compared to blueberries were indicated to contribute toward these activities (*30*).

The MM extract that displayed the highest RSA consists predominantly of cyanidin-based pigments. Among them, nonacylated YGM-0a dominated. Fukumoto and Mazza (*28*) have compared radical scavenging activities of cyanidin, delphinidin, malvidin, and peonidin and their 3-glucosides and 3,5-diglucosides in various experimental systems. They have reported approximately 2-fold stronger DPPH RSA activity of cyanidin and delphinidin than malvidin, pelargonidin, and peonidin. The authors concluded that an increase in the number of hydroxyl groups in anthocyanin molecules led to higher antioxidant activity. Rice-Evans et al. (*26*) described three criteria for effective radical scavenging by polyphenols, with the first one being the presence of an *ortho*-dihydroxy structure in the B-ring. We have calculated the ratio of *ortho*-dihydroxylated to monohydroxylated anthocyanins in our extracts. In the MM extract, which displayed the highest RSA, this ratio was 2:1. In the APM extract, this ratio was estimated to be 1:1, while in the least active SR extract the ratio was 1:3.7. Dominance of anthocyanins with *ortho*-dihydroxylated structure (cyanidin-based) in MM sample could contribute toward enhanced scavenging ability.

Stacking structures in anthocyanins with highly evolved molecules may hinder the free radical scavenging ability. Addition of a sugar moiety decreases the activity of aglycon, and the addition of a second moiety decreases the activity further (*26*, *28*). All YGM anthocyanins possess sophoroside at position 3 and a single molecule of glucose at position 5 but differ in their acylation pattern (**Figure 2**). We have calculated the ratio of nonacylated to acylated anthocyanins in aqueous extracts. In MM extract, with a ratio 5.3:1, nonacylated anthocyanins dominated. On the contrary, in SR extract, only traces of nonacylated anthocyanins were found (ratio 1:58). In APM extract, this ratio is estimated to be 1:8.7. In our experimental system, extracts with an increased proportion of acylated anthocyanins exhibited lower RSA.

The main anthocyanin in MM extract is nonacylated cyanidin YGM-0a. According to the literature cited above, it possess higher RSA than nonacylated peonidin YGM-0b and the mono- and diacylated cyanidin-based and peonidin-based pigments, which dominate in the APM and SR extracts. On the basis of this information, it can be assumed that the presence of YGM-0a contributes toward superior RSA of the MM extract. This assumption is a subject of our further studies with purified cell line produced anthocyanins.

Beside anthocyanins, the aqueous extracts may contain various water soluble components such as amino acids, vitamins, and polyphenolics. All of these compounds may contribute toward antioxidative capacities. Previously, we have reported the presence of CHA and CA in the PL cell line crude pigment extract (*16*). In MM medium, only trace amounts of these acids were accumulated. Within 4 days after tissue transfer from MM into APM medium, the level of phenolic compounds increased about 3-fold. At day 14 on the APM medium (at this day the APM samples used for the physiological studies were collected), the concentration of CHA reached about 6.0 mg/100 g FW and was approximately 7-fold higher than that on MM medium. In the same time, the concentration of CA doubled and reached 8.9 mg/100 g FW (*16*).

The DPPH RSA activities of the phenolic acids present in the cell line extracts CHA and CA have been examined. CoA was also included in this analysis, as this acid is present in the PL cell line anthocyanins as an acylation group (**Figure 2**). The strongest scavenging activity against DPPH radicals exhibited CHA (IC_{50} = 21.35 nmol), followed by CA (IC_{50} = 11.17 nmol)

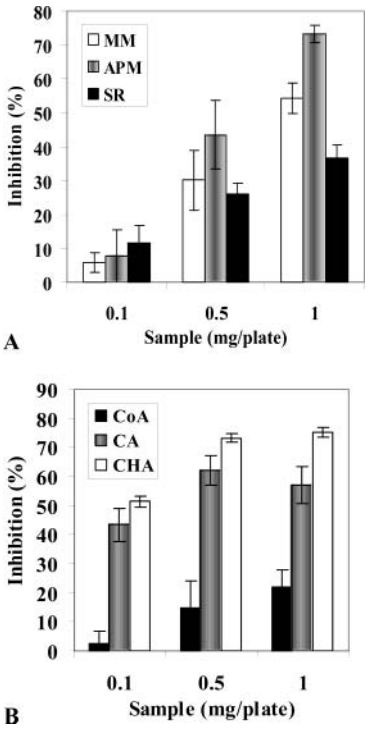


Figure 5. Effect of aqueous extracts of the PL cell line tissue grown on MM and APM and from the field-grown SR of Ayamurasaki (**A**) and CoA, CA, and CHA (**B**) on the inhibition of reverse mutation in *S. typhimurium* TA98 induced by Trp-P-1. Each value represents the mean ± SD, n = 6. Try-P-1 was added at a dose of 0.075 μg/plate. The mutagenicity was tested with S-9 mix. The His^+ revertant values of the controls for aqueous extracts were 574 ± 9/plate and for organic acids 698 ± 40/plate. The values shown have had the spontaneous mutation frequency subtracted.

and CoA (IC_{50} = 2.26 μmol). The amount of Trolox required for scavenging 50% of the DPPH free radicals was 27.00 nmol. These data confirm results reported elsewhere (*8*, *26*, *28*). The RSA of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule (*26*, *28*). CHA possesses five OH− groups, and according to our assay, it displayed the strongest antioxidative potential. CA possesses two OH− groups while CoA only displayed one and displayed the weakest activity. Accumulation of CHA and CA in the PL cell line tissue produced in APM medium was higher than their accumulation in the tissue produced in MM medium (*16*). However, the antioxidant potential of the MM extract was significantly stronger. Although CHA and CA contribute toward RSA of evaluated extracts, it is assumed that the presence of high levels of cyanidin-based anthocyanins, with dominance of nonacylated YGM-0a, exerts strong influence on the scavenging ability of MM.

Antimutagenic Activity. The antimutagenic effect of the lyophilized aqueous extracts of the PL cell line and the field-grown SR of sweetpotato cv. Ayamurasaki is presented in **Figure 5A**. The extracts, used at doses of 0.1, 0.5, and 1.0 mg/plate, were examined against the mutagenicity of mutagen Trp-P-1 on *S. typhimurium* TA98. The addition of lyophilized extracts resulted in an inhibition of the reversed mutation of *Salmonella* in a dose-dependent manner. The effect of the PL cell line produced extracts was superior to that of the field-grown SR. The highest antimutagenic activity was displayed by the APM extract. Applied at the dose of 1.0 mg/plate, it inhibited the reverse mutation of *S. typhimurium* in 73% and was significantly stronger than MM and SR extracts with inhibitory effects of 54.2 and 36.5%, respectively.

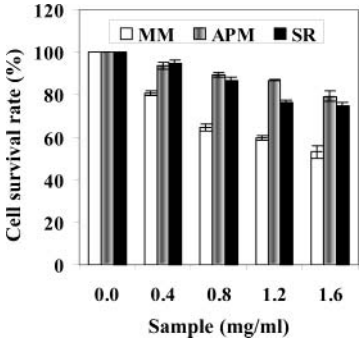


Figure 6. Effects of aqueous extracts of the PL cell line tissue grown on MM and APM and from the field-grown SR of sweetpotato (*I. batatas* L.) cv. Ayamurasaki (SR) on human promyelocytic leukemia cells growth. Values are means ± SD, $n = 6$.

Yoshimoto et al. (*2*) have compared antimutagenic activities of aqueous extracts obtained from SRs of various sweetpotato cultivars, including cv. Ayamurasaki, and found that anthocyanins inhibited the reverse mutation of *S. typhimurium* by direct reaction with activated mutagen. In the following study, they reported that purified nonacylated cyanidin exhibited a stronger effect than nonacylated peonidin. The presence of the catechol structure in the molecule of cyanidin was identified to play an important role in antimutagenicity (*3*). Cyanidin was also identified to inhibit mutagenic activity of benzo[*a*]pyrene and 2-amino fluorene in the *S. typhimurium* test (*31*) in a dose-dependent manner. The domination of cyanidin type pigments in the PL cell line extracts together with an increased amount of total anthocyanin/g DW could contribute to the superior physiological activities of PL cell line extract over the extract of the field-grown SR of sweetpotato cv. Ayamurasaki.

The effects of CA and CHA, previously detected in the PL cell line tissue (*16*) as well as CoA on the reverse mutation of *S. typhimurium* induced by Try-P-1, have been examined. Applied at three different concentrations, 0.1, 0.5, and 1.0 mg/plate, both acids CHA and CA displayed strong antimutagenic activities (**Figure 5B**). At the lowest dose of 0.1 mg/plate, they inhibited the mutagenicity of Trp-P-1 against *S. typhimurium* by about 50%. Significantly less active was CoA. Yamada and Tomita (*32*) reported that a water extract of roasted coffee beans inhibited mutagenicity of cooked food mutagens such as Try-P-1, Glu-P-2, and crude extract of grilled beef in the *S. typhimurium* TA98 system. Furthermore, they found that the CA and CHA identified in the extract effectively inhibited mutagenicity of Try-P-1 and Glu-P-2 in a dose-dependent manner. It can be expected that the inhibitory effect of CA and CHA on cooked food mutagens may have contributed toward increased antimutagenic activity of APM extract.

Antiproliferation Activity. The reconstituted lyophilized powders of aqueous extracts MM, APM, and SR have been applied in the growth inhibition assay of HL-60 cells assayed at concentrations of 0.0, 0.4, 0.8, 1.2, and 1.6 mg/mL culture medium. All extracts inhibited the proliferation of HL-60 cells within 24 h of treatment. At each concentration applied, the maximum inhibition was consistently observed with the MM extract (**Figure 6**). Anticancer effects of anthocyanin such as effective suppression of growth of human malignant cells by anthocyanin from petals of higher plant (*33*) or suppression of the growth of human colon carcinoma cells HCT-15 by hydrolyzed anthocyanins from grape rinds and red rice (*34*) have been reported. Our samples differed in the total amount of anthocyanin pigment with the richest being MM extract. A high concentration of anthocyanin may have contributed toward the pronounced antiproliferation activity. The pigment profile of anthocyanin-rich extracts has been suggested to impact biological activity: purified cyanidin and delphinidin exhibited superior anticancer activity, including the inhibition on TPA-induced cell transformation of mouse epidermal JB6 cells (*10*) and apoptosis induction of HL-60 human leukemia cells and HCT-116 human colon carcinoma cells in vitro (*35*), to other types of anthocyanins such as peonidin. The dominance of cyanidin-based pigments in the MM extract with the major component being nonacylated YGM-0a could contribute toward the highest antiproliferation activity exhibited by the MM.

Beside anthocyanins, other phenolic compounds such as caffeic, ellagic, chlorogenic, and ferulic acids were identified to inhibit tongue carcinogenesis induced by 4-nitroquinoline-1-oxide (*12*). CHA effectively inhibited N-nitrosation of 2,3-diamino-naphthalene due to its ability to scavenge the nitrosating agent, nitrogen sesquioxide, and therefore was suggested to be effective in inhibiting potentially mutagenic and carcinogenic reactions in vivo (*11*). The presence of these acids in the PL cell line extract (*16*) may also have contributed toward the suppression of proliferation of HL-60 cells.

Development of a sweetpotato cultivar, which would accumulate predominantly cyanidin type pigments as a source of physiologically functional food, has been suggested (*3*). On the basis of the information obtained to date, we estimate that the PL cell line developed from the SR of sweetpotato cv. Ayamuraski generates predominantly cyanidin type pigments. Besides anthocyanins, phenolic acids such as CA or CHA may contribute toward physiological activities of cell line extracts. The aqueous extracts of the PL cell line exhibited higher antioxidative, antimutagenic, and antiproliferation activities than extract produced from field-grown SR. Although further research is required to prove physiological activities of purified cell line produced compounds, our results to date suggest that the PL cell line may serve as an alternative source of natural food colorants and physiologically active valuable components of functional food products.

ABBREVIATIONS USED

PL, purple line; MM, multiplication medium; APM, high anthocyanin-producing medium; SR, storage root; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; CA, caffeic acid; CHA, chlorogenic acid; CoA, *p*-coumaric acid; Try-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido-(4,3-*b*)indol.

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