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Short Communication

Recovery of degenerate schizostatin production by screening single-basidiospore isolates in *Schizophyllum commune*

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To recover degenerate schizostatin production in a *Schizophyllum commune* isolate, SANK 17785, we examined schizostatin production of 30 single-basidiospore isolates obtained from a basidiocarp of SANK 17785. One of the isolates showed high productivity (136–154 μg/ml) and maintained its high productivity for 26 mo. To explore other isolates with high schizostatin production, 76 single-basidiospore isolates from 7 wild basidiocarps were obtained and their schizostatin production was quantified. Four of the isolates produced more schizostatin than SANK 17785 did originally.

Key Words——basidiocarp formation; genetic recombination; *Schizophyllum commune*; schizostatin; single-basidiospore isolates.

After isolation of a certain novel bioactive metabolite from a fungus, improvement and maintenance of the fungus to ensure a constant supply of the metabolite is important for industry. Schizostatin is a novel inhibitor of squalene synthase produced by a dikaryotic isolate of *Schizophyllum commune* Fr.: Fr., SANK 17785 (Tanimoto et al., 1995; Tanimoto et al., 1996). Although SANK 17785 produced 153 μg/ml of schizostatin originally, its production decreased to 5 μg/ml in a year. SANK 17785 had been maintained in potato dextrose agar (PDA, Nissui, Tokyo) slants at 5°C and preserved as frozen agar plugs in glycerol (10%, v/v) at −100°C. In both cases, SANK 17785 showed only low productivity. To obtain more schizostatin for further studies, recovery of schizostatin productivity in SANK 17785 was required.

Genetic recombination in the sexual cycle has been used to improve certain properties of fungi, e.g., the productivity of commercial mushrooms, and the fermentability of *Saccharomyces cerevisiae* Meyen ex E. C. Hansen (Moore-Landecker, 1996). In the life cycle of basidiomycetes, genetic recombination occurs in basidia within the basidiocarps of the parental dikaryon. Single-basidiospore progeny are monokaryotic and genetically recombined. Basidiocarps of *S. commune* are easily produced in the laboratory (Alexopoulos et al., 1996). Therefore, basidiocarp formation was attempted to obtain single-basidiospore progeny from parental SANK 17785.

Another way of obtaining schizostatin is to find other high-producing isolates from wild basidiocarps. Therefore, other high-producing isolates from wild basidiocarps were also explored, elucidating the distribution of schizostatin-producing isolates in nature.

The isolate SANK 17785 was inoculated onto a PDA plate and cultivated for 5 wk at 23°C to obtain basidiocarps. Part of a gill from a basidiocarp was attached on the inside of the lid of the plate. Basidiospores were allowed to be discharged onto the PDA plate. Single-basidiospore isolation was carried out using Skerman's micromanipulator (Skerman, 1968). The isolates were maintained on PDA slants at 5°C.

Wild basidiocarps of *S. commune* were collected from various sites in Japan (Table 1). Single-basidiospore isolates were obtained using Skerman's micromanipulator. The isolates were maintained on PDA slants at 5°C.

Thirty ml of the seed medium composed of 5% glycerol, 0.5% malt extract (Difco), 0.5% yeast extract (Difco) and 5% fresh mashed potato in a 100-ml Erlenmeyer flask was inoculated with the mycelia of the slant culture, and incubated for 4 d at 26°C on a rotary shaker operating at 210 rpm. Three ml of the seed culture was then transferred to 500-ml Erlenmeyer flasks containing 80 ml of the same medium and incubated for 5 d at 26°C on a rotary shaker operating at 210 rpm, because maximum schizostatin production was detected in the above condition in the previous study of SANK 17785 (Tanimoto et al., 1995).

Schizostatin production in the culture broth was quantified using high-performance liquid chromatography (HPLC). Fifteen ml of the culture broth was extracted with the same volume of acetone. After shaking, the extract was filtered to remove mycelia and chromatographed on a Nova-pak C_{18} column (Waters, 3.9 mm × 150 mm) using 0.3% triethylamine phosphoric acid buffer (pH 3.2): acetonitrile (45 : 55) as the isocratic mobile phase at 40°C. The flow rate was 1.0 ml/minute. Schizostatin showed a single peak monitored by UV at 210 nm with a diode array detector and had a retention time of about 7.8 min (Fig. 1). Standard schizostatin was prepared from SANK 17785 (Tanimoto et al., 1995). Schizostatin production is shown as the amount (μg) per ml culture broth.

To confirm reproductivity in a high-producing isolate, the high-producing isolate was re-fermented and re-examined three times for schizostatin quantification by the same procedure immediately after first quantification and 26 mo later. The high-producing isolates were maintained on PDA slants at 5°C.

From a basidiocarp of SANK 17785, 30 isolates were obtained. All isolates grew well in the liquid medium, and their schizostatin production varied from 2 μg/ml to 172 μg/ml (average 19, standard deviation 32, median 10 μg/ml) (Fig. 2). One isolate (SANK 13394) produced more schizostatin than SANK 17785 did originally.

The amount of schizostatin produced in the first re-examination of SANK 13394 was 136, 144, and 154 μg /ml. The high productivity was maintained (149, 174 and 220 μg/ml, respectively) 26 months later. The large variation (136–220 μg/ml) in production of schizostatin by SANK 13394 may be attributable to experimental error or flask-to-flask variation. Large variation in the production of metabolites by the same isolate of non-basidiomycetes species has been reported (Horn et al., 1996).

Schizostatin production varied from 0 to 228 μg/ml among the 76 isolates from 7 wild basidiocarps collected (Table 1). Four isolates produced more schizostatin than SANK 17785 did originally. Schizostatin production was confirmed in 40 of the 76 isolates. All isolates from SANK 17785 and ba-2 produced schizostatin, while 36 isolates from ba-1 and ba-3-7 produced no schizostatin. The median values of production (Table 1) indicated that most isolates produced small amounts of schizostatin irrespective of the collection site.

Schizophyllum commune was confirmed to have the potential for producing schizostatin. However, the distribution of schizostatin production by single-spore progeny showed that few isolates were high-producing. Our results suggest that numerous single-basidiospore isolates should be examined to enhance the productivity of an bioactive metabolite producer.

Variation in cultural characteristics, such as colony color and growth rate, among single-basidiospore pro-

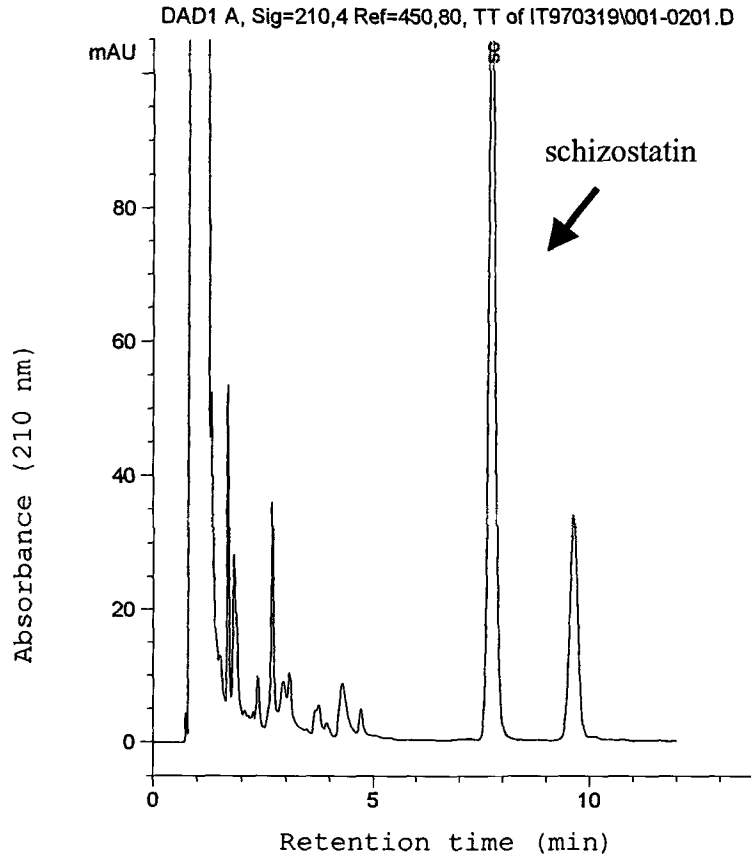

Fig. 1. HPLC profile of an extract of *S. commune* SANK 13394.

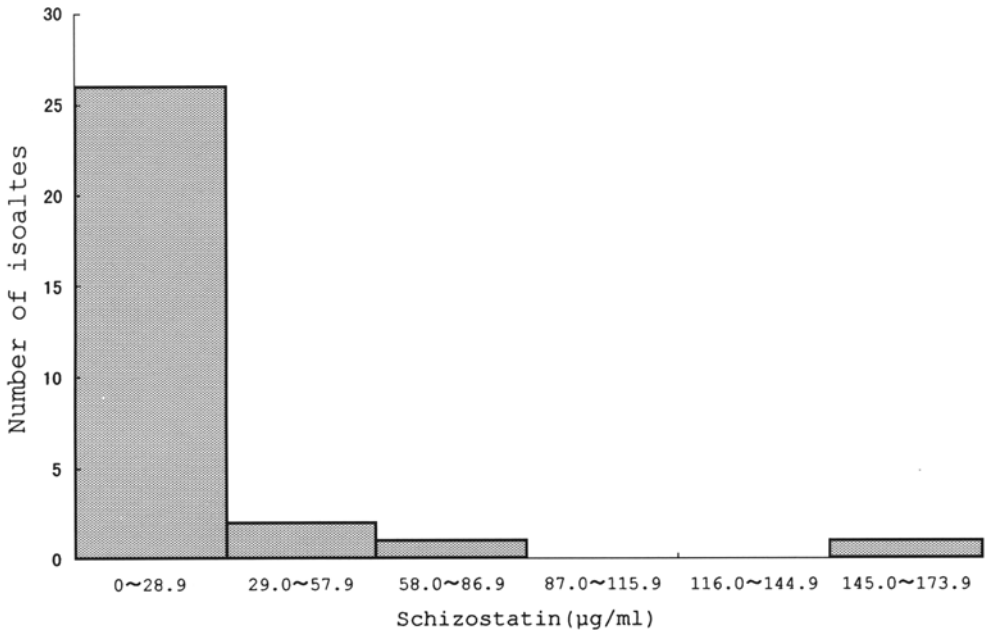

Fig. 2. Distribution of schizostatin production among 30 single-basidiospore isolates obtained from a basidiocarp of SANK 17785. One isolate, which produced maximum schizostatin (172 μg/ml), was assigned as SANK 13394. Minimum production of schizostatin was 2 μg/ml.

Table 1. Number of single-basidiospore isolates obtained from each basidiocarp collected in Japan and schizostatin production by the isolates.

Ba-[a]	Collection site	Number of isolates	NI[b]	Schizostatin production (μg/ml)				
				Mean	SD	Maximum value	Median	Minimum value
1	Morioka-shi, Iwate Pref.	10	3	27	59	190	5	0
2	Utsunomiya-shi, Tochigi Pref.	9	0	30	36	98	11	5
3	Gozenyama-cho, Ibaraki Pref.	9	6	5	11	33	0	0
4	Makabe-cho, Ibaraki Pref.	21	14	44	76	228	0	0
5	Tsukuba-shi, Ibaraki Pref.	9	7	1	2	4	0	0
6	Tsukuba-shi, Ibaraki Pref.	9	2	28	68	208	2	0
7	Minamimaki-mura, Nagano Pref.	9	4	10	17	40	1	0

[a] Suffix for number assigned to the basidiocarps used for isolation
[b] Number of isolates that produced no schizostatin.

geny has been reported (Butler and Pearce, 1999; Noble et al., 1995). This is the first report on variation in bioactive metabolite production among single-basidiospore isolates in basidiomycetes.

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