Short Communication

Anthranilic Acid, as a Fruiting
Body Inducing Substance
in *Favolus arcularius*,
from a Strain TA 7
of Actinomycetes

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The formation of fruiting bodies in Basidiomycetes is the most dramatic expression of differentiation and morphogenesis that can be found among the fungi. In general this formation process requires appropriate environmental conditions such as adequate illumination, suitable temperature and an adequate supply of essential nutrients.¹⁾ Cyclic AMP has been reported to be effective in inducing fruiting bodies in monokaryotic mycelia of the *fis*⁺ strain of *Coprinus macrorhizus*.²⁾ Cerebrosides have also been reported to stimulate the fruiting body formation of *Schizophyllum commune*.³⁾ Fruiting body inducing substances, if any can be obtained, are useful to clarify the mechanism of fruiting. Moreover, such substances would economize the commercial production of mushrooms, since such environmental stimuli as temperature reduction and photo-irradiation are no longer necessary. From such standpoints, we performed screening for microbial products that would induce fruiting body formation. For the screening we used a bioassay with *Favolus arcularius* ATCC 24461 in which light is required for the initiation and development of fruiting.⁴⁾ This edible mushroom is named Amisugi-take in Japan. Our screening of soil isolates revealed that the broth of the strain TA 7 induced the formation

of the stipe of the fruiting body even under dark conditions. The strain was considered to be a species belonging to the rare Actinomycetes by its morphological and physiological properties. This paper deals with the isolation and identification of the active constituent, and the biological activity of the formation of the fruiting body.

For the production of the fruiting body inducing substance, the strain was aerobically cultured at 30°C in a 30-liter jar fermentor which contained a 20 liters medium consisting of 2% glucose, 1% meat extract, 1% polypeptone and 0.3% NaCl (pH 7). After a 46 hr cultivation period, the culture broth was centrifuged to remove mycelia.

The filtrate (80 liters) was concentrated and the aqueous extract chromatographed on silicic acid by a stepwise elution from hexane to ethyl acetate. The fruiting body inducing activity was found in a fraction eluted with 25% ethyl acetate in hexane. Further purification was successively carried out by column chromatography on Florisil (hexane-ethyl acetate) and silicic acid (hexane-acetone). Finally, the active constituent (9 mg) was obtained as colorless needles, mp 122~125°C, from hexane-

TABLE I. FRUITING RESPONSE OF
F. arcularius TO ANTHRANILIC
ACID

Dosage (ng/disc)	No. of discs producing the fruiting body ^a
0	0
0.01	0
0.03	0
0.1	3
0.3	4
1	2
3	1
10	0
30	0
100	0

^a Ten discs loaded with the same amount of anthranilic acid were prepared. Five discs loading various amounts of anthranilic acid were placed in the same Erlenmeyer flask and incubated at 25°C for two weeks in continuous darkness.

TABLE II. EFFECTS OF ANTHRANILIC ACID AND SOME RELATED COMPOUNDS ON FRUITING OF *F. arcularius*

Compound	Activity	Dosage range
Anthranilic acid	+	0.1 ~ 3 ng/disc
<i>m</i> -Aminobenzoic acid	—	
<i>p</i> -Aminobenzoic acid	+	1 ~ 3
Methyl anthranilate	—	
<i>N</i> -Methyl anthranilic acid	—	
Tryptophan	—	
Kynurenine	—	
Folic acid	—	

methanol.

The active constituent showed UV absorption maxima at 218 (in MeOH, $\epsilon=20,200$), 248 (7000) and 335 nm (4700), and IR absorption bands (KBr pellet) at 3500, 3400 and 1590 (NH_2), 1678 (COOH), 1490 and 755 cm^{-1} (benzene ring), respectively. The FD-MS gave a M^+ ion peak at m/z 137, indicating a molecular formula of $\text{C}_7\text{H}_7\text{NO}_2$. The ^1H -NMR spectrum in CD_3OD gave signals at δ 6.50 (1H, ddd, $J=9, 7, 2\text{ Hz}$), 6.69 (1H, dd, $J=9, 2\text{ Hz}$), 7.17 (1H, ddd, $J=9, 7, 2\text{ Hz}$) and 7.79 (1H, dd, $J=9, 2\text{ Hz}$). This spectral evidence indicated the active constituent to be *o*-aminobenzoic acid (anthranilic acid) and its identity was confirmed by direct comparison with an authentic specimen.

The fruiting body inducing assay was carried out by the following method. Discs (6 mm in diameter) with various amounts of samples to be tested were placed on a margin of an Erlenmeyer flask (100 ml volume), in which *F. arcularius* ATCC 24461 was spotted on the center of a semi-synthetic agar medium (20 ml).⁴⁾ The flask was incubated at 25°C for two weeks in continuous darkness. Fruiting bodies were formed around the discs with active compounds. When *F. arcularius* was cultured under the inducing condition, i.e., under continuous light, this mushroom exhibited about 80% of fruiting.

The fruiting responses of *F. arcularius* cultured in the dark with the addition of anthranilic acid are shown in Table I. Anthranilic acid induced the stipe of the fruiting body in a

dosage range of 0.1 ~ 3 ng/disc. Further cultivation under illumination by daylight fluorescent tubes led to the pileus formation on the top of the induced stipe, resulting in the formation of a complete fruiting body. Although it remains to be clarified whether anthranilic acid is an endogenous fruiting regulator or an exogenous inducer, this result strongly suggests that at least two factors, namely the inducer of stipe formation and that of the pileus formation, take part in the development of fruiting bodies.

Some related compounds were also tested on this mushroom (Table II). Fruiting was induced by *p*-aminobenzoic acid, while *m*-aminobenzoic acid was not effective in the concentration range tested (0.01 ~ 100 ng/disc). Methyl anthranilate and *N*-methyl anthranilic acid were also ineffective, indicating that both an amino and a carboxyl group play significant roles to promote the activity. In addition, tryptophan and kynurenine were tested because anthranilic acid takes part in a metabolic pathway of tryptophan, but these compounds showed no activity. Folic acid, which contains a *p*-aminobenzoic acid moiety, did not induce fruiting.

Further studies on the structure/activity relationship of anthranilic acid and related compounds are in progress.

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