A PROTEASE INHIBITOR ISOLATED FROM *PLANOMONOSPORA PARONTOSPORA*

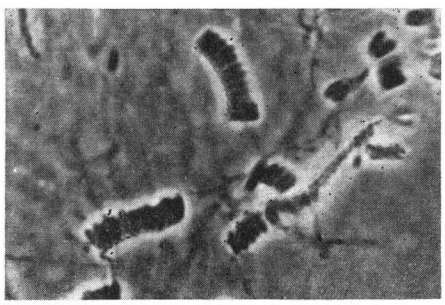
Sir:

As reported by UMEZAWA and co-workers[1~3] all known protease inhibitors of microbial origin (antipain, chymostatin, elastatinal, leupeptin, pepstatin, phosphoramidon) were isolated from the family *Streptomycetaceae*. We would like to report the isolation and characterization of a protease inhibitor from the culture filtrate of *Planomonospora parontospora*, a species belonging to the family *Actinoplanaceae*.

The strain was isolated from a soil sample collected in Arizona, U.S.A., by using the pinus pollen baiting technique and grown in pure culture on modified CZAPEK-agar. On the white to light pink coloured aerial mycelium, there are groups of sporangia with single spores which are arranged in parallel lines in a typical formation (Fig. 1). Furthermore, the mainly light red to red orange colour of the substrate mycelium corresponds to the described characteristics of the strain *Planomonospora parontospora*.

These properties allow us to classify the organism as *Planomonospora parontospora* described by THIEMANN *et al.*[4] The inhibitory activity was determined by the assay method agreed upon by the "Federation International Pharmaceutique", commission for the standardization of pharmaceutical enzymes (FIP-test)[5]. One inhibitory unit is defined as that amount of inhibitor which completely inhibits one trypsin enzyme unit (measured as the rate of hydrolysis of one micromole BAEE per minute).

Fig. 1. *Planomonospora parontospora*, strain SE 294
Sporangia on soil-glucose-yeast-agar magnification ×1,250, lactophenol-cottonblue staining



In a medium containing 1% glucose, 3.2% peptone (Siemsglüss, Hamburg, R.G.F.), 0.5% yeast extract (Difco), 0.1% K_2HPO_4, 0.3% NaCl, and 0.1% $MgSO_4 \cdot 7H_2O$ in tap water, pH 7.2, the maximum amount of inhibitor production (65 FIP-units/ml) was attained after 4~5 days at 29°C in a shake culture. The inhibitor was adsorbed to CM-cellulose in a batch procedure from 30 liters of culture broth and desorbed with 0.05 N HCl. The inhibitor solution was then decolourized with ion-exchange resin (Dowex® 1X2 Cl^-). The supernate was desalted by adsorption to a macroporous polystyrol resin (Lewapol® Ca 9221) and desorbed with 50% ethanol. The inhibitory active fraction was concentrated and lyophilized. The specific activity of the isolated protease inhibitor was 400 FIP-units/mg and the total yield was 50 mg.

The inhibitor was characterized by electrophoresis and amino acid analysis and the data so obtained were compared to antipain*. The inhibitor (SAKAGUCHI positive) showed the same movement towards cathode in acetic acid - pyridine - water (4 : 100 : 900) under 300 V for 90 minutes as antipain. The amino acid analysis (hydrolysis 72 hours, 6 N HCl) yielded the same amino acid molar ratios (valine 1.0, arginine 0.3, phenylalanine 0.3) as antipain[6]. The inhibition spectrum towards papain and trypsin corresponded to that reported by UMEZAWA for antipain.

Therefore, we have shown that antipain is produced not only by the family *Streptomycetaceae* but also can be found in the family *Actinoplanaceae*. Both families are representatives of the order Actinomycetales.

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