

Tf-26Vx, AN ANTIBIOTIC PRODUCED
BY A THERMOPHILIC FUNGUS

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Thermophilic fungi are commonly found in self-heated habitats, such as composts, and help to decompose various plant materials. However, there have been few reports describing production of biologically active substances by these fungi. During a screening programme for new antibiotics produced by thermophilic microorganisms, a substance with activity against Gram-positive and strictly anaerobic Gram-negative bacteria was isolated from the mycelium of a thermophilic fungus. The present paper deals with the production, isolation, identification and biological activity of the antibiotic, Tf-26Vx.

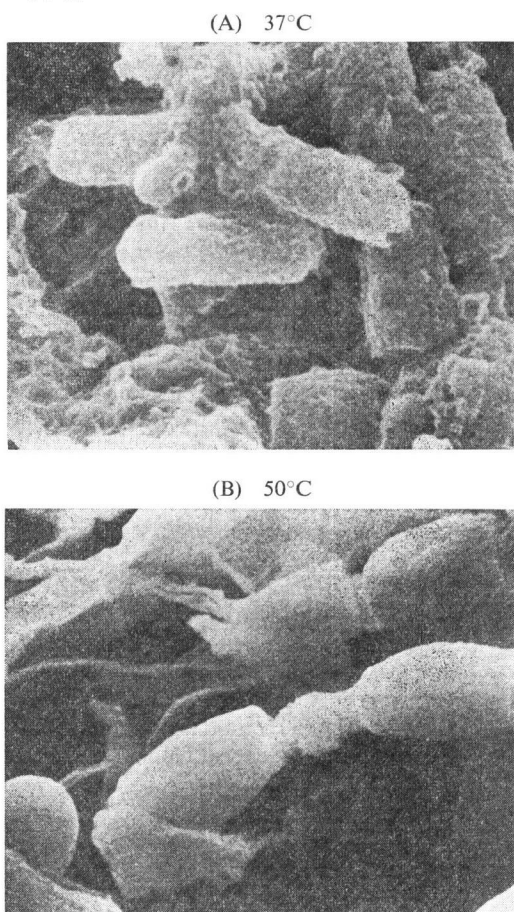
The fungus, strain No. 26, was isolated from compost and was identified as *Malbranchea pulchella* SACCARDO and PENZIG var. *sulfurea* (MIEHE) COONEY and EMERSON, a member of the thermophilic Fungi Imperfecti which has been reported to produce several hydrolases and penicillin¹⁾. On yeast-glucose agar, strain No. 26 grows at temperatures ranging from 28° to 55°C.

The optimum is between 45 and 50°C and growth was very slow at the lower limit. Marked changes were observed in the surface of arthrospores grown at different temperatures.

Viewed under the scanning electron microscope, those grown at 50°C were cylindrical or oblong and their surface was smooth. Arthrospores grown at 37°C were cylindrical with distinctly rough walls (Fig. 1).

For the production of Tf-26Vx, *Malbranchea pulchella* var. *sulfurea* strain No. 26 was cultured at 45°C with aeration and agitation in the following medium: glucose 3.0%, yeast extract 0.5%, malt extract 0.5%, NaNO₃ 0.3%, KCl 0.05%, K₂HPO₄ 0.1%, MgSO₄·7H₂O 0.05%,

Fig. 1. Difference of the surface of arthrospores of *Malbranchea pulchella* var. *sulfurea* strain No. 26 grown on yeast-malt extract agar at 37°C and 50°C.

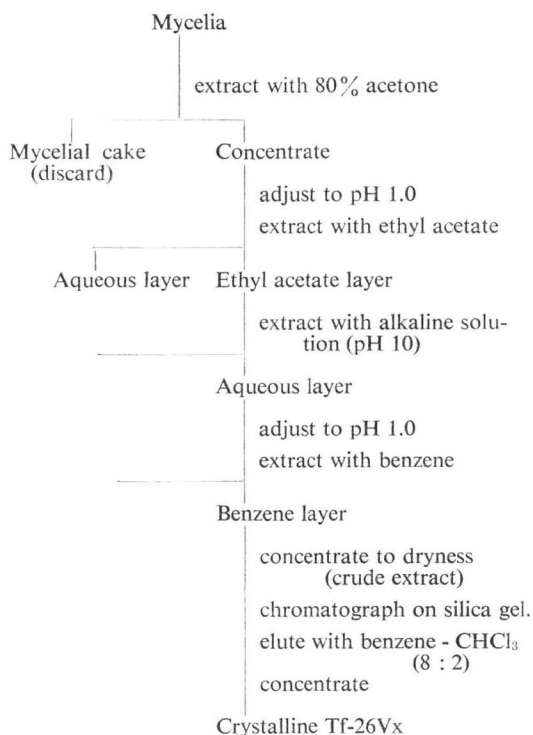


FeSO₄·4H₂O 0.001%, Adecanol (Asahi Denka Co., non-ionic detergent) 0.01% (pH 6.5 before sterilization). Tf-26Vx was produced mainly in the mycelium and was extracted with 80% acetone.

The extraction and purification procedure is summarized in Fig. 2. Tf-26Vx is pale yellow, crystalline, and melts at 332°C with decomposition. It shows $[\alpha]_D^{25} + 3.46^\circ$ (c 0.95, CHCl₃). A molecular formula of C₃₀H₂₆O₁₀ was established by elemental analysis and by mass spectrometric determination of the molecular weight (M.W. 546). Tf-26Vx is soluble in chloroform or dimethylformamide, and insoluble in water, methanol or ethyl acetate. The UV spectrum in chloroform has absorption maxima at 273 and 385 nm. The IR spectrum (KBr) shows charac-

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Fig. 2. Isolation procedure for Tf-26Vx.



teristic bands at 2975 and 2935 (alkyl group), 1635 (carbonyl group), 1580, 1505, 1498, 1462 and 1413 (aromatic group), 1382 (methyl group), and 1250 cm^{-1} (methoxy group).

The NMR spectrum in deuteriochloroform, using tetramethylsilane as an external reference,

shows a $\text{CH}_3\text{-CH-CH}_2$ group [δ 1.54 (CH_3 , doublet), 3.00 (2H, doublet), 4.71 (1H, multiplet)], two aromatic protons at 6.72, 6.98, a methoxy group at 3.84 and two exchangeable protons at 9.72 and 13.86 ppm.

From the physicochemical properties described above, it was concluded that Tf-26Vx has the structure shown in Fig. 3, which is the same as that proposed for vioxanthin, a metabolite of *Trichophyton violaceum*²³. The only significant difference between Tf-26Vx and vioxanthin is that the melting point of the latter is reported as 190~195°C with decomposition. Vioxanthin has not so far been found in thermophilic fungi and has not been known to have biological activity.

Tables 1 and 2 show the antimicrobial spectrum of Tf-26Vx in the agar-plate diffusion test. The

Fig. 3. Structure of Tf-26Vx.

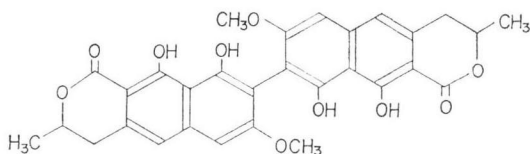


Table 1. Antibacterial activity of Tf-26Vx against aerobic and facultative anaerobic bacteria.

Test organism	MIC (mcg/ml)*
<i>Bacillus subtilis</i> PCI-219	≤0.045
<i>Bacillus cereus</i> Ch-1	≤0.045
<i>Bacillus thuringiensis</i> Val-II	0.095
<i>Staphylococcus aureus</i> 209P	0.39
<i>Staphylococcus aureus</i> B-5	1.56
<i>Staphylococcus aureus</i> 222	0.19
<i>Staphylococcus epidermidis</i> A-4	0.095
<i>Staphylococcus epidermidis</i> TO-3	0.095
<i>Staphylococcus epidermidis</i> TO-5	≤0.045
<i>Sarcina lutea</i> A	≤0.045
<i>Sarcina lutea</i> B	0.19
<i>Escherichia coli</i> NIHJ	>100
<i>Escherichia coli</i> No. 9	>100
<i>Escherichia coli</i> No. 49	>100
<i>Klebsiella</i> sp. 3K25	>100
<i>Klebsiella</i> sp. 15C	>100
<i>Shigella flexneri</i> 2b	>100
<i>Shigella sonnei</i>	>100
<i>Salmonella typhi</i> Tanaka	>100
<i>Salmonella paratyphi</i> A	>100
<i>Salmonella paratyphi</i> B	>100
<i>Salmonella enteritidis</i> M-11	>100
<i>Salmonella enteritidis</i> WT-1	>100
<i>Proteus mirabilis</i> 9'	>100
<i>Pseudomonas aeruginosa</i> NC-5	>100

* Agar dilution method; Nutrient agar (Eiken), 37°C, 18 hours, 10⁸ cells/ml.

compound is highly active against Gram-positive and strictly anaerobic Gram-negative bacteria, whereas facultative anaerobic Gram-negative bacteria are not affected at concentrations up to 100 $\mu\text{g/ml}$. Recently, SC-30532, an antibiotic with the same aromatic hydrocarbon skeleton as Tf-26Vx, was obtained from the culture broth of *Spicaria divaricata* grown in the presence of sitosterol²³. However, its antimicro-

Table 2. Antibacterial activity of Tf-26Vx against strictly anaerobic Gram-negative bacteria.

Test organism	MIC (mcg/ml)
<i>Bacteroides fragilis</i> V-6	< 0.39
<i>Bacteroides fragilis</i> V-7	< 0.39
<i>Bacteroides fragilis</i> V-8	< 0.39
<i>Fusobacterium glutinosum</i> 1006	> 200
<i>Fusobacterium necroforum</i> s-45	12.5 ~ 25.0

* Agar dilution method; Gam agar (Nissui). Plates were incubated anaerobically in GasPak Jar (BBL) at 37°C for 48 hours. Inoculum size: 10⁸ cells/ml.

bial activity seemed to be weaker against both Gram-positive and strictly anaerobic bacteria.

Besides antimicrobial properties Tf-26Vx was active in other biological systems. These activi-

ties will be reported elsewhere.

Acknowledgement

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