# A NEW IMMUNOMODULATOR, FR-900494: TAXONOMY, FERMENTATION, ISOLATION, AND PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERISTICS

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FR-900494 is a new type of immunoactive substances produced by an actinomycete named *Kitasatosporia kifunense* sp. nov.

FR-900494 exhibits a competitive action against immunosuppressive factor produced in the serum of tumor bearing mice and has the capacity to restore the depression of lymphocytes.

In our screening program for new type of immunoactive substances from microorganism[1], FR-900494 isolated from the fermentation broth of a new actinomycete *Kitasatosporia kifunense* enhances the activity of the mouse immune system *in vitro*.

The present paper describes the taxonomy of producing strain, isolation, physico-chemical properties and biological activities.

## Materials and Methods

### Taxonomy

#### Bacterial Strain

Strain No. 9482 was isolated from a soil sample obtained at Mt. Kifune, Kyoto Prefecture, Japan. *Kitasatosporia setae* IFO 14216 (ATCC 33774), *Nocardiopsis dassonvillei* IFO 13901, *Streptomyces hygroscopicus* subsp. *hygroscopicus* IFO 13472 (ATCC 27438), and *Actinomadura verrucosospora* IFO 14100 (ATCC 27299) were obtained from the Institute for Fermentation, Osaka (IFO), Japan.

#### Morphological Characterization

The aerial and vegetative mycelium of the organism grown on yeast extract - malt extract agar, oatmeal agar or inorganic salts - starch agar were examined directly under the microscope. To observe fragmentation or vegetative mycelium spores, the surface of the yeast extract - malt extract agar was taken out with a glass knife. This specimen was observed on a glass slide under a light microscope. Electron micrographs of the spores were obtained with electron microscopes (model HV-12 Hitachi and model S-450 Hitachi).

#### Cultural and Physiological Characterizations

For cultural and physiological characterizations, the media recommended by WAKSMAN[2] and endorsed by the ISP[3] were used. Cultures were incubated for 14 days at 30°C. The color names used in this study were based on Color Standard (Nihon Shikisai, Co., Ltd.). The ability to utilize carbohydrates was determined by the method of PRIDHAM and GOTTLIEB[4]. Growth-permissible temperature range and optimum growth temperature were determined on yeast extract - malt extract agar using a model TN-3 temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd.).

#### Cell Analysis

Strains were cultured in GY medium (glucose 1%, yeast extract 0.5%, peptone 0.5%, adjusted

to pH 7.0 by 0.1 N NaOH) or modified GY medium (supplemented 3% peptone in GY medium) incubated on a rotary shaker (300 rpm) at 30°C. The mycelium grown in GY medium was harvested by centrifugation (6,000×*g*, 20 minutes), and washed twice with distilled water. The mycelium was further washed with ethanol, followed by drying at room temperature. The dried mycelium was used for the analysis of whole-cell. The washed mycelium was used for cell wall preparation.

Cell wall preparations were obtained by the method of BECKER *et al.*[5] or YAMAGUCHI[6]. Cell walls were hydrolyzed for the analysis of amino acid or sugars. Amino acid composition was analyzed using an amino acid analyzer (model 835 Hitachi). Whole-cell and cell wall sugars were analyzed by high performance liquid chromatography (HPLC, using Hitachi anion-exchanger 3013-N column) according to the method of HONDA *et al.*[7,8].

Deoxyribonucleic acid was prepared by the method of MARMUR[9]. Guanine-plus-cytosine content was determined as described by TAMAOKA and KOMAGATA[10]. Phospholipid and mycolic acid compositions were determined by the method of LECHEVALIER *et al.*[11,12]. Menaquinones were prepared by the method of COLLINS *et al.*[13], and determined by the method of TAMAOKA *et al.*[14]. The determination of whole-cell and cell wall diaminopimeric acid (DAP) isomers were carried out by HPLC.

Analysis of DAP Isomers

*o*-Phthalaldehyde-DAP adducts (acid hydrolysate of whole-cell or cell wall) were prepared according to the method of LINDROTH and MOPPER[15], or LARSEN and WEST[16], and analysis with HPLC (Lichlosorb RP-18, Merck, 10 $\mu$m, 4×250 mm). The analysis was carried out with a flow rate of 2.0 ml/minute and a column pressure of 130 kg/cm$^2$. The mobile phase consisted of 40% methanol, 5% acetonitrile, 30 mM $NaH_2PO_4$, 9 mM $Na_2HPO_4$ (pH 7.2). LL- and *meso*-type of *o*-phthalaldehyde-DAP adducts, eluted at 6 minutes and 7 minutes, respectively, were determined by fluorometry (excitation 360 nm, emission 440 nm).

Time Course Study in a Submerged Culture

The spores were inoculated into 80 ml of YM medium (in 250 ml flask), and incubated at 30°C on a rotary shaker (300 rpm). Portions of the culture were taken at various times, and whole mycelium was analyzed by HPLC to determine contents of DAP isomers.

Fermentation

The microorganism *Kitasatosporia kifunense* No. 9482 was used in this study.

The seed medium contained corn starch 1%, glucose 0.5%, glycerol 1%, cotton seed meal 1%, dried yeast 0.5% and $CaCO_3$ 0.2%, pH 6.5. The production medium contained soluble starch 2%, dried yeast 0.2% and gluten meal 0.3%.

A loopful of slant culture of *K. kifunense* No. 9482 was inoculated to each of eight 250-ml Erlenmeyer flasks containing 80 ml of the seed medium and incubated at 30°C for 72 hours on a rotary shaker (200 rpm). Six hundreds ml of the culture were inoculated to the production medium (160 liters) in a 200-liter jar fermentor and incubated for 96 hours at 30°C under aeration of 160 liters/minute and agitation of 300 rpm.

FR-900494 was assayed and purified from the cultured broth by monitoring the competitive action against the inhibitory activity of proliferative responses of mouse spleen cells to concanavalin A (Con A) which induced by adding the immunosuppressive factor[1].

Isolation and Purification

The fermentation broth (160 liters) was filtered with the aid of diatomaceous earth (4 kg). The filtrate was passed through a column of activated carbon (45 liters). The column was washed with deionized water (90 liters) and eluted with 80% aq MeOH (100 liters). The active fraction was concd *in vacuo* to a volume of 10 liters. The concentrate was absorbed on a column of DEAE-Sephadex A-25 ($OH^-$) (8 liters), washed with deionized water (12 liters) and eluted with 0.2 N NaOH (15 liters). The eluate was adjusted to pH 7.0 with 6 N HCl and passed through a column of activated carbon. After washing with deionized water (15 liters), the active fractions were eluted with 40% aq MeOH (20 liters) and concd *in vacuo* to a volume of 300 ml. The concentrate was absorbed on a column of

CM-Sephadex C-25 ($H^+$) (10 liters) and developed with deionized water. The active fractions (1.2 liters) were concd *in vacuo* to a volume of 50 ml and absorbed on a column of DEAE-Sephadex A-25 ($OH^-$) (500 ml). After washing with deionized water (1 liter) saturated with carbon dioxide, the column was eluted with 1% ammonia water. The active eluate was passed through a column of activated carbon (50 ml). The column was washed with deionized water (150 ml) and eluted with 80% aq MeOH. The active eluate was concd *in vacuo* to a volume of 10 ml and lyophilized. The purified powder of FR-900494 (160 mg) was obtained.

Biological Activity

The competitive action of FR-900494 against the inhibitory activity of proliferative responses of mouse spleen cells to Con A, which induced by the immunosuppressive factor was measured by the method described previously[1].

The antimicrobial activity of FR-900494 was determined by a serial dilution method in bouillon medium for bacteria and in Sabouraud medium for fungi and yeast. The minimum inhibitory concentration (MIC) was expressed in term of $\mu$g/ml after overnight incubation at 37°C for bacteria and 48~72 hours incubation at 28°C for fungi and yeast.

## Results

### Taxonomy

Morphology

The mature sporophores were moderately long, and formed straight, hooked or spiral spore chains (Fig. 1). The spores were short cylindrical, and 0.6~0.7×1.2~1.3 $\mu$m in size. Spore surfaces were smooth (Figs. 2 and 3). No fragmentation of substrate mycelium was observed in agar medium. The strain incubated with modified GY medium developed into fragmented short mycelium. The strain incubated with GY medium for 3 days at 30°C, formed some of submerged spore like bodies. No synnemata, sporangia, zoospores or vegetative mycelium spores are observed.

Cultural Characteristics

Cultural characteristics of strain No. 9482 studied with various agar media were summarized in Table 1. Aerial mycelium formed on oatmeal agar or yeast extract - malt extract agar was in the gray color series. The vegetative growth of the organism was yellowish brown. No soluble pigments were produced in the media examined.

Fig. 1. Sporophores of strain No. 9482.
Sporophores (A and B) were observed on aerial mycelium (incubated on yeast extract - malt extract agar for 7 days at 30°C). Magnification, ×800.

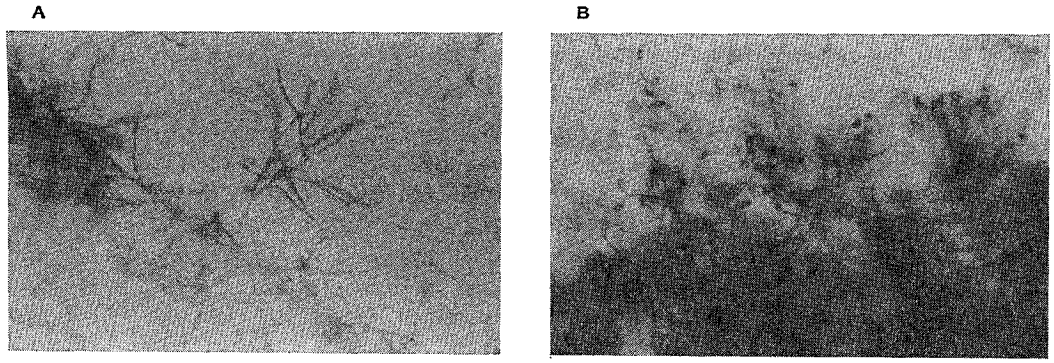


Fig. 2. Electron micrograph of spore chain of strain No. 9482 on yeast extract - malt extract agar (10 days culture).

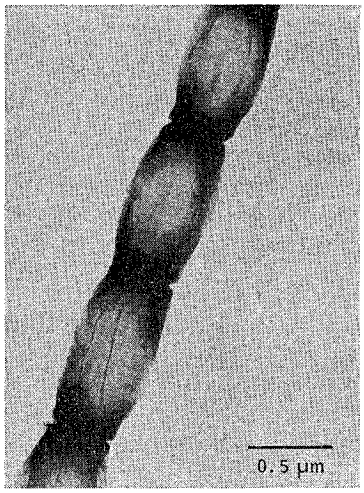



Fig. 3. Scanning electron micrograph of strain No. 9482 on yeast extract - malt extract agar (10 days culture).

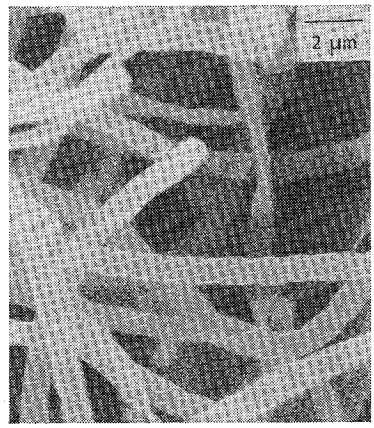



Table 1. Cultural characteristics of strain No. 9482.

| Medium | Growth | Aerial mass color | Reverse side color | Soluble pigment |
|---|---|---|---|---|
| Oatmeal agar | Poor | Grayish white, poor | Colorless | None |
| Yeast extract - malt extract agar | Moderate | Light gray | Yellowish brown | None |
| Inorganic salts - starch agar | Moderate | Light gray | Yellowish brown | None |
| Glucose - asparagine agar | Moderate | Grayish white | Pale yellowish brown | None |
| Glycerol - asparagine agar | Moderate | Grayish white | Pale yellowish brown | None |
| CZAPEK's agar | Poor | None | Colorless | None |
| Nutrient agar | Poor | None | Colorless | None |
| Potato - glucose agar | Abundant | Yellowish gray | Light brown | None |
| Tyrosine agar | Moderate | Grayish white | Dark orange | Brown |
| Peptone - yeast extract - iron agar | Poor | None | Colorless | None |

## Physiological Characteristics

Physiological characteristics of strain No. 9482 are summarized in Table 2. The optimum growth temperature of the strain was 30°C. The growth-permissible temperature range was very narrow (25°C~33°C). Melanoid pigment was produced in tyrosine agar, but not in peptone - yeast extract - iron agar or Tryptone - yeast extract broth. The organism could grow on yeast extract - malt extract agar supplemented with 3% NaCl, but did not grow on the same medium with 5% NaCl. The strain was able to utilize glycerol, D-glucose, mannitol, D-trehalose, L-arabinose or maltose as a carbon source, but D-xylose and other carbohydrates were not utilized (Table 3).

Table 2. Physiological characteristics of strain No. 9482.

| | |
|---|---|
| Temperature range for growth | 25~33°C |
| Optimum temperature | 30°C |
| Nitrate reduction | Negative |
| Starch hydrolysis | Positive |
| Milk coagulation | Negative |
| Milk peptonization | Negative |
| Melanin production | Positive |
| Gelatin liquefaction | Negative |
| $H_2S$ production | Negative |
| NaCl tolerance | >3~<5% |

Table 3. Carbohydrate utilization by strain No. 9482.

| | | | |
|---|---|---|---|
| D-Glucose | + | D-Mannose | ± |
| Sucrose | ± | D-Trehalose | + |
| Glycerol | + | Inositol | − |
| D-Xylose | − | Mannitol | + |
| D-Fructose | − | Inulin | − |
| Lactose | − | Cellulose | − |
| Maltose | + | Salicin | − |
| Rhamnose | − | Chitin | − |
| Raffinose | − | Sodium citrate | ± |
| D-Galactose | + | Sodium succinate | − |
| L-Arabinose | + | Sodium acetate | − |

Symbols: +; Utilization, ±; doubtful utilization, −; no utilization.

Fig. 4. Time course of LL- and *meso*-diaminopimelic acid (DAP) contents in the whole-cell of strain No. 9482 grown in submerged culture with GY medium at 30°C.

○ *meso*-DAP, ● LL-DAP.

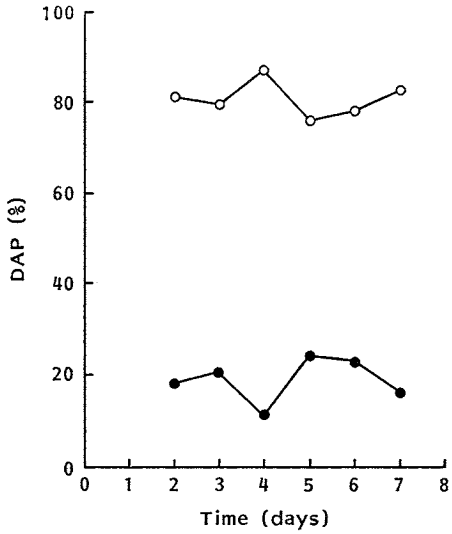
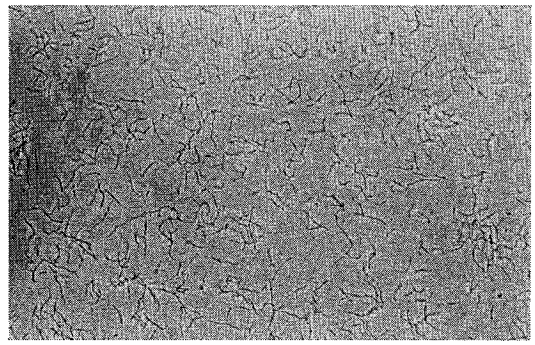



Fig. 5. Photograph of strain No. 9482 in submerged culture with modified GY medium for 3 days at 30°C.

The organism was observed with an optical microscope (×800).



## Cell Analysis

Submerged mycelium of strain No. 9482 contained both LL- and *meso*-DAP, but the major component was the *meso* isomer in all growth phases (Fig. 4). The major amino acids and sugars in the cell wall were *meso*-DAP, glutamic acid, alanine (Tables 4 and 5), mannose, galactose and glucose (Table 6). Mannose in the cell wall was very large amount (1.7% of dry cell wall). LL-DAP and glycine were detected. Strain No. 9482 contained ribose, mannose, galactose, and glucose in the whole-cells; arabinose and rhamnose were not detected. Diagnostically important whole-cell sugars were not detected (type C). Aerial mycelium of strain No. 9482 contained *meso*-DAP (70% of total DAP) and lesser amount of LL-DAP (30% of total), while that of *K. setae* contained far more amount of LL-DAP (80% of total) and lesser amount of *meso*-DAP (20% of total). Whole-cell DAP isomers of strain No. 9482 and *K. setae* (incubated with modified GY medium for 3 days at 30°C) were analyzed. *K. setae* contained LL-DAP (75% of total DAP) and lesser amount of *meso*-DAP (25% of total). On the other hand, strain No. 9482 contained more *meso*-DAP (60% of total) and lesser amount of LL-DAP (40% of total). Strain No. 9482 and *K. setae* contained phospholipids of the type P II pattern. Nocardomycolic acids were not

Table 4. Comparison of diaminopimelic acid (DAP) isomers in the cell wall of strain No. 9482 and related genera.

| | DAP (100%) | |
|---|---|---|
| | LL- | *meso-* |
| Strain No. 9482 | 29.5 | 70.5 |
| *Actinomadura verrucosospora* | 8.1 | 91.9 |
| *Kitasatosporia setae* | 36.5 | 63.5 |
| *Nocardiopsis dassonvillei* | 0 | 100 |
| *Streptomyces hygroscopicus* subsp. *hygroscopicus* | 100 | 0 |

Table 5. Comparison of the amino acids composition (molar ratio) in the cell wall of strain No. 9482 and related genera.

| | DAP | Gly | Glu | Ala |
|---|---|---|---|---|
| Strain No. 9482 | 1 | 0.31 | 0.81 | 1.48 |
| *Actinomadura verrucosospora* | 1 | 0.03 | 0.68 | 1.51 |
| *Kitasatosporia setae* | 1 | 0.38 | 0.71 | 1.58 |
| *Nocardiopsis dassonvillei* | 1 | 0.05 | 0.79 | 1.43 |
| *Streptomyces hygroscopicus* subsp. *hygroscopicus* | 1 | 0.84 | 0.81 | 1.21 |

Abbreviations: DAP; Diaminopimelic acid, Gly; glycine, Glu; glutamic acid, Ala; alanine.

Table 6. Sugar composition of cell wall and whole-cell in strain No. 9482 and *Kitasatosporia setae*.

| | % of sugar in dry weight of | | | |
|---|---|---|---|---|
| | Cell wall | | Whole-cell | |
| | No. 9482 | *K. setae* | No. 9482 | *K. setae* |
| Rhamnose | — | 0.97 | — | 2.21 |
| Ribose | — | — | 0.69 | 0.30 |
| Mannose | 1.68 | 0.03 | 5.41 | 0.95 |
| Arabinose | — | — | — | — |
| Galactose | 0.42 | 1.23 | 2.34 | 2.15 |
| Xylose | — | — | — | — |
| Glucose | 0.13 | 0.18 | 0.27 | 0.17 |
| Madurose | — | — | — | — |

— : Not detected.

Table 7. Cell analysis of strain No. 9482.

| | |
|---|---|
| Cell wall amino acid composition | *meso*-DAP, alanine, glutamic acid, a small amounts of LL-DAP and glycine |
| Cell wall sugar composition | Mannose, galactose, glucose |
| Whole-cell sugar composition | Ribose, mannose, galactose, glucose |
| Phospholipid pattern | P II |
| Nocardomycolic acid | Not detected |
| Menaquinones | MK-9 ($H_6$), MK-9 ($H_8$), trace of MK-8 ($H_8$), MK-9 ($H_2$), MK-9 ($H_4$), MK-10 ($H_6$) and MK-10 ($H_8$) |
| Guanine-plus-cytosine | 71.2 mol% |

detected. The predominant menaquinones present in the cells were MK-9 ($H_6$) and MK-9 ($H_8$), with trace of MK-8 ($H_8$), MK-9 ($H_2$), MK-9 ($H_4$), MK-10 ($H_6$) and MK-10 ($H_8$). The predominant menaquinones of *A. verrucosospora* and *K. setae* were also MK-9 ($H_6$) and MK-9 ($H_8$). The guanine-plus-cytosine content of DNA in the strain was estimated to be 71.2 mol%. Results were summarized in Table 7.

Table 8. Physico-chemical properties of FR-900494.

| | |
|---|---|
| Appearance | White powder |
| Molecular formula | $C_8H_{12}N_2O_6$ |
| Molecular weight | 232 |
| Elementary analysis | |
| Found: | C 38.63, H 5.13, N 10.49. |
| Calcd for $C_8H_{12}N_2O_6 \cdot \frac{1}{2}H_2O$: | C 39.84, H 5.43, N 11.61. |
| SI-MS (*m/z*) | 233 $(M+H)^+$ |
| MP | 120~136°C (dec) |
| $[\alpha]_D^{23}$ (*c* 0.1, $H_2O$) | +33° |
| UV $\lambda_{max}^{H_2O}$ nm ($\varepsilon$) | 226 (230) |
| IR $\nu_{max}^{KBr}$ $cm^{-1}$ | 3400~2800, 1730, 1710, 1450, 1400, 1100, 1010, 810 |
| TLC (Silica gel plate) | |
| Rf[a] | 0.38 |
| Rf[b] | 0.05 |

[a] Solvent system: 2-Propanol - $H_2O$, 9: 1.
[b] Solvent system: Chloroform - MeOH - 28% ammonia water, 5: 3: 1.

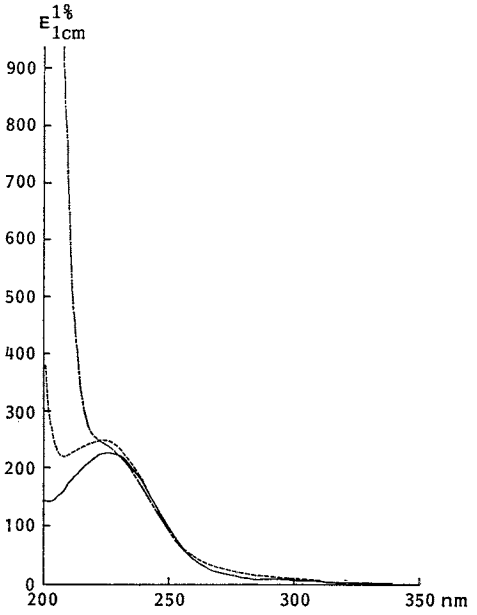
## Physico-chemical Properties

The physico-chemical properties of FR-900494 are summarized in Table 8. The UV, $^1H$ NMR and $^{13}C$ NMR spectra are represented in Figs. 6~8, respectively.

FR-900494 was readily soluble in water, slightly soluble in methanol and ethanol, and insoluble in acetone, ethyl acetate and chloroform. FR-900494 gave positive reactions to iodine reagent, though negative to cerium sulfate, potassium permanganate, Molisch and ninhydrin reactions.

The $^{13}C$ NMR spectrum shows eight carbon signals. This data together with secondary ion mass spectrum (SI-MS) and elemental analysis established the molecular formula of FR-900494 to be $C_8H_{12}N_2O_6$. The study on the structure of FR-900494 is under progress and the results will be published elsewhere.

Fig. 6. UV spectra of FR-900494.
—— $H_2O$, ------ 0.05 N NaOH, ---- 0.05 N HCl.




## Biological Activity

### Competitive Effect of FR-900494 against the Immunosuppressive Factor Obtained from Tumor Bearing Mice Serum

The competitive action of FR-900494 against the inhibitory activity of proliferative responses of mouse spleen cells to Con A, which induced by the immunosuppressive factor was shown in Table 9.

The addition of FR-900494 to the culture containing immunosuppressive factor prevented the suppression. This result suggests that FR-900494 has the capacity to restore the depression of mitogenic responses of mouse spleen cells by immunosuppressive factor.

Fig. 7. $^{1}H$ NMR spectrum of FR-900494 (in $D_2O$+NaOD, pD=11, 270 MHz).

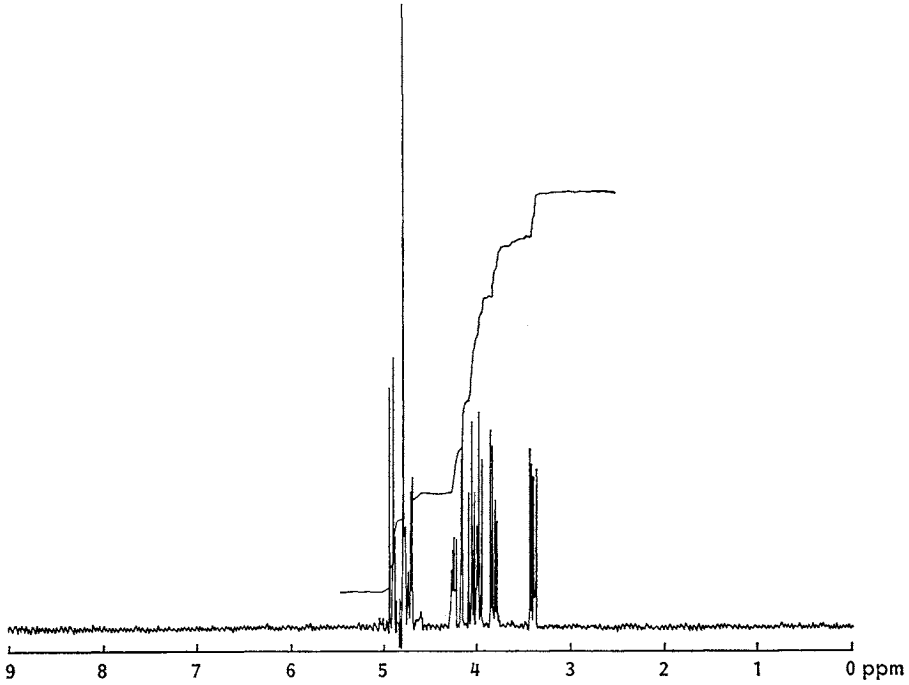
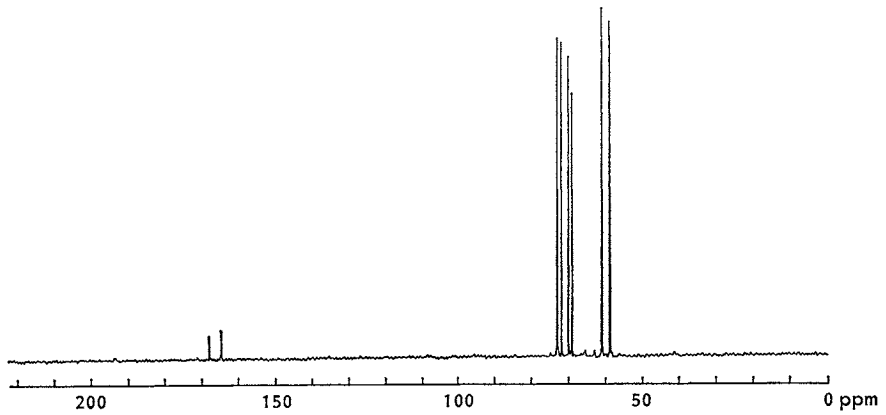



Fig. 8. $^{13}C$ NMR spectrum of FR-900494 (in $D_2O$, pD=7, 270 MHz).




The addition of FR-900494 to the mouse spleen cell culture did not effect mitogenic activity of mouse spleen cells or spleen cell viability.

Antimicrobial Activity

FR-900494 was devoid of antimicrobial activity when tested versus the following organisms at 100 μg/ml: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Aureobasidium pullulans* and *Aspergillus niger*.

## Discussion

### Taxonomy

Strain No. 9482 contained *meso*-DAP and lesser amount of LL-DAP and glycine in the cell wall.

Table 9. Suppression of Con A induced mouse spleen cell prolifiration by immunosuppressive factor and its restoration by FR-900494.

| Treatment of spleen cell added | [$^3$H]Thymidine uptake (cpm)* |
|---|---|
| Non-treated (control) | 965±69 |
| Con A 1 μg/well | 59,540±1,140 |
| Immunosuppressive factor 6 μg/well | 497±14 |
| FR-900494 1.0 μg/well | 1,354±50 |
| 0.1 μg/well | 776±47 |
| Con A 1 μg/well+immunosuppressive factor 6 μg/well | 2,721±152 |
| Con A 1 μg/well | |
| +FR-900494 1.0 μg/well | 46,744±2,980 |
| 0.1 μg/well | 43,560±3,285 |
| Con A 1 μg/well+immunosuppressive factor 6 μg/well | |
| +FR-900494 1.0 μg/well | 34,256±405 |
| 0.1 μg/well | 52,424±4,641 |
| 0.01 μg/well | 39,795±4,037 |
| 0.001 μg/well | 4,570±149 |

* Mean±SE ($n$=4).

Therefore, cell wall analysis of strain No. 9482 was again performed according to the procedure of LECHEVALIER[17]. We obtained a same results after ninhydrin treatment of the paper chromatography. Cell wall composition analysis shows that strain No. 9482 resembles the genus *Kitasatosporia* ŌMURA *et al.* 1982[18~20]. On the other hand, the morphological and cultural characteristics of strain No. 9482 resemble those of the genus *Streptomyces* WAKSMAN and HENRICI 1943 and *Actinomadura* LECHEVALIER and LECHEVALIER 1970[21]. But, strain No. 9482 contained *meso*-DAP as a major constituent of the cell wall. Moreover, diagnostically important whole-cell sugars were not detected (type C). The genus *Streptomyces*, as currently described[22], has LL-DAP (but not *meso*-DAP) in the cell wall (type I). The genus *Actinomadura* has madurose as the characteristic whole-cell sugar (type B). The cell wall type has been one of the most important criteria in the classification of genus in the order *Actinomycetales*[17]. Therefore, strain No. 9482 should not be classified as a member of the genera *Streptomyces* or *Actinomadura*.

The predominant menaquinone composition of strain No. 9482 [MK-9 ($H_6$) and MK-9 ($H_8$)] was in good agreement with those of *A. verrucosospora* and *K. setae*. On the other hand, menaquinones of *N. dassonvillei*[23] [MK-10 ($H_8$), MK-10 ($H_6$) and MK-10 ($H_4$)] were different from those of strain No. 9482.

Thus, strain No. 9482 was classified in the genus *Kitasatosporia* from the resemblance of the cell wall composition of these two strains. But, there are many differences between strain No. 9482 and *K. setae* in the following points. *K. setae* had LL-DAP as a major type (80%) in the aerial mycelium, while strain No. 9482 had less amount of LL-DAP (30%). Although *K. setae* contains galactose and rhamnose as a whole-cell sugar, strain No. 9482 contains galactose and large amounts of mannose (Table 6). Cell wall type of *K. setae* (incubated in the modified GY medium) was type I from the whole-cell analysis, while that of strain No. 9482 was type II. Strain No. 9482 produces melanoid pigment in tyrosine agar. In the morphological characteristics, strain No. 9482 did not develop fragmented vegetative mycelium in agar media. Strain No. 9482 (incubated in the modified GY medium) developed into fragmented short mycelium (Fig. 5). The sporophores of strain No. 9482 formed hooked and spiral spore chains (Fig. 3).

As results, strain No. 9482 was identified as a new species in the genus *Kitasatosporia*, named *Kitasatosporia kifunense* sp. nov., referring to the soil obtained from which the organism was isolated.

A description of the new species are as follows.

*Kitasatosporia kifunense* sp. nov.

Morphology

Sporophores are simply branched and form straights, hooks, or spirals. No sporangia, zoospores, or synnemata is formed. The spores are cylindrical, and $0.6\sim0.7\times1.2\sim1.3$ $\mu$m in size. Spore surfaces are smooth.

Color of Colonies

The color of the aerial mycelium is in the gray series on oatmeal agar, or on yeast extract - malt extract agar.

Color of Reverse Side of Colonies

The vegetative growth of the organism is yellowish brown on yeast extract - malt extract agar or inorganic salts - starch agar.

Color in Medium

No soluble pigment is produced.

Physiological Characteristics

Starch is hydrolyzed. The optimum growth temperature is 30°C. The growth-permissible temperature range is from 25°C to 33°C. Melanoid pigment is produced in tyrosine agar. Carbohydrates such as glycerol, D-glucose, mannitol, D-trehalose, L-arabinose, and maltose are utilized for growth.

Cell Wall Composition

*meso*-DAP, glutamic acid, alanine, mannose, galactose, and a small amount of LL-DAP and glycine are contained in the cell wall preparations. The cell wall is of type II.

Aerobic. Habitat: Soil. Type strain: Strain No. 9482. A culture of this strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the accession number FERM-P 8526.

## Biological Activity

As reported, FR-900494 restored the immune suppression caused by immunosuppressive factor *in vitro*. In addition, we have examined the effect of FR-900494 on antibody formation to sheep red blood cells (SRBC) in immuno-deficient mice which was treated with immunosuppressive factor. The administration of FR-900494 restored the capacities of the immuno-deficient mice to produce antibody against SRBC (data not shown). Further studies on biological activities of FR-900494 are now progress.

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