

BRIEF COMMUNICATION

Immunologic cross-reactions of thermophilic actinomycetes isolated from home environments

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A thermophilic actinomycete was isolated from the furnace humidifier of a patient with hypersensitivity pneumonitis. Rabbit antiserum prepared by immunizing the animals with the spore extract of this microorganism showed a high titer of precipitins against the extract but failed to cross-react with antigens of other thermophilic actinomycete species, such as Thermoactinomyces vulgaris or Micropolyspora faeni. However, a number of sera from patients with farmer's lung or bagassosis contained precipitins against the thermophilic actinomycetes isolated from the furnace humidifier. These findings suggest that the thermophilic actinomycetes isolated from home environments may also be found in other surroundings and may belong to some less common or not yet identified species. Because of the lack of cross-reactivity among the individual thermophiles, patients suspected of having hypersensitivity pneumonitis due to inhalation of thermophilic actinomycetes should be tested for precipitins with the use of the microorganism isolated from their own environment.

Thermophilic actinomycetes have been implicated as causative agents in several forms of hypersensitivity pneumonitis, including farmer's lung,^{1, 2} bagassosis,³ and mushroom worker's lung.⁴ In all these conditions the susceptible individuals are exposed to dusts derived from decomposing organic materials containing thermophilic microorganisms. Recently, thermophilic actinomycetes were isolated from heat-exchangers of air-conditioning systems in office buildings^{5, 6} and from humidifiers of residences⁷⁻⁹; in all these cases some of the occupants of the rooms served by the contaminated appliances have developed symptoms of hypersensitivity pneumonitis. Subsequently, thermophilic actinomycetes were isolated from furnace humidifiers in a number of other homes.* Since on preliminary testing the sera of these patients^{5, 7} failed to cross-react

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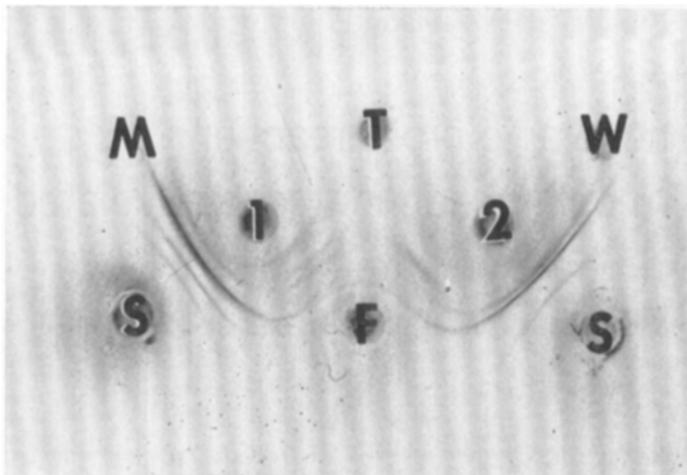


FIG. 1. Immunodiffusion pattern of 2 rabbit sera against the spore (S) and filament (F) extracts of a thermophilic actinomycete, isolated from a humidifier, against *T. vulgaris* (T) and the Marshfield (M) and Wales (W) extracts of *M. faeni*.

with extracts of several known species of thermophilic actinomycetes, it was decided to investigate whether the thermophilic microorganisms isolated from homes and offices are specific for this environment or whether the sera from patients with hypersensitivity pneumonitides of similar etiology, such as farmer's lung or bagassosis, cross-react with these thermophilic actinomycetes.

MATERIALS AND METHODS

Aliquots (5 to 10 ml.) of the contaminated water obtained from the furnace humidifier of a patient with hypersensitivity pneumonitis were incubated in peptone-soy broth at 56° C. for 3 to 4 days. The filamentous growth of the actinomycetes was then plated out on peptone-soy agar and kept at 56° C. until spores developed on the surface of the agar and no contamination with other microorganisms was apparent. The spore material was then again transferred to peptone-soy broth and grown at 56° C. for 3 to 4 days. The spore growth of the isolates was skimmed from the surface of the medium, rinsed 3 times with distilled water on a Buechner funnel, sonified in the cold for 15 minutes, and lyophilized. The filamentous cell material grown in the medium was also washed with distilled water and processed separately in a manner similar to that of the spores. The nitrogen content of the lyophilized material was determined by Kjeldahl's method¹⁰ and was found to be 87 mg. per gram of the dried spores.

Antibodies to the spore material were produced in rabbits by injecting them intramuscularly in the subscapular region with 30 mg. of the lyophilized material in complete Freund's adjuvant. The injections were repeated at 2 week intervals for 6 weeks; at this time the animals demonstrated good titers of precipitating antibody against the spores.

Antigen extracts from pure cultures of *Thermoactinomyces vulgaris* and *Micropolyspora faeni* were obtained from Mr. F. Wenzel, Marshfield Clinic, Marshfield, Wisconsin. Sera from patients with bagassosis, as well as crude extracts of bagasse, were supplied by Dr. John Salvaggio, Louisiana State University School of Medicine, New Orleans, Louisiana. Sera of patients with farmer's lung and additional extracts of *M. faeni* were obtained from Dr. Jonathan Edwards, Sully Hospital, Sully, Penarth, Wales, Great Britain. All antigenic extracts were used at the concentration of 10 mg. of lyophilized material per milliliter saline.

The presence of precipitins in the human and animal sera against the various thermophilic extracts was tested by a micromodification of the double-diffusion technique, as described previously.¹¹

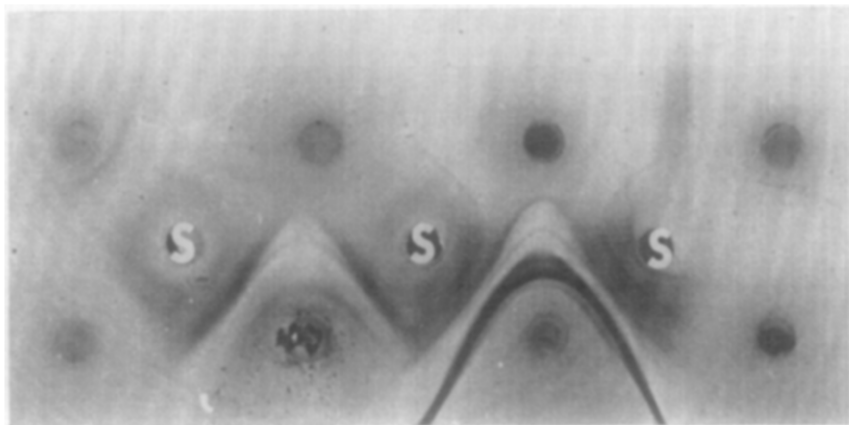


FIG. 2. Immunodiffusion pattern of 8 farmer's lung sera reacting with the spore extract of a thermophilic actinomyces isolated from a furnace humidifier. The 3 central wells (S) contain the spore extract, the 8 peripheral wells the farmer's lung sera.

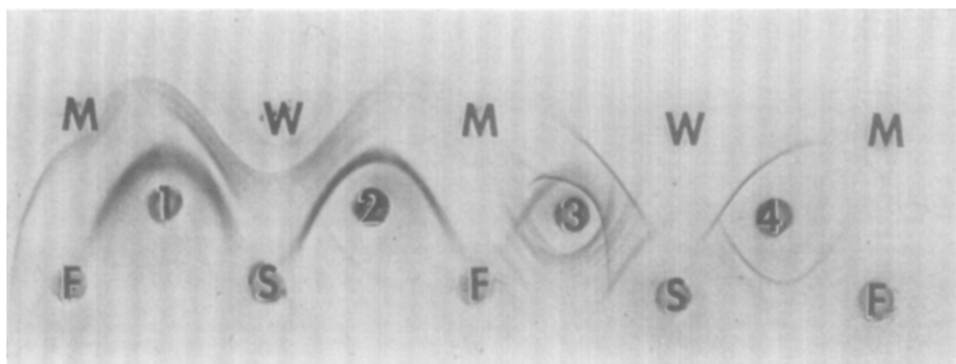


FIG. 3. Precipitin patterns obtained with farmer's lung sera (1 to 4) reacted against spore (S) and filament (F) extracts of a thermophilic actinomycete from a humidifier and Marshfield (M) and Wales (W) extracts of *M. faeni*.

RESULTS

The results of immunodiffusion studies using rabbit antisera against the spore extract are illustrated in Fig. 1. The animals developed multiple precipitins against the spore material which cross-reacted with the filament extract from the same organism. The precipitin pattern of the two tested materials indicated that the spore and filament preparations had common antigens. However, the rabbit antiserum did not react with either of the two extracts from pure cultures of *M. faeni* or with the *T. vulgaris* extract.

Ten of 27 farmer's lung sera tested against the spore extract showed precipitins. The intensity of the reaction and number of precipitin bands varied, as showed in Fig. 2. While some of the sera demonstrated multiple precipitin lines against the extract of the thermophilic actinomycetes isolated from the humidifier, other sera showed only one precipitin band. All farmer's lung sera were also tested against the extracts of *M. faeni* obtained from two the different sources. All 27 reacted with the extract from Wales, and 23 reacted with the

extract from Marshfield. As shown in Fig. 3, various types of precipitin patterns were seen with the two *M. faeni* extracts reacted with the farmer's lung sera, indicating a difference either in the antigenic composition of the extracts or in the reactivity of the farmer's lung sera to the individual *M. faeni* antigens. No cross-reactions or lines of identity were observed with the antigens of the thermophiles from the humidifier. Six of the 27 farmer's lung sera also reacted with the *T. vulgaris* extract. Of the 4 sera from patients with bagassosis tested against the spore extract, 3 gave precipitin reactions which were, however, too light for photographic reproduction. Nine sera were tested from patients suspected to have hypersensitivity pneumonitis due to inhalation of thermophilic actinomyces in their home or office surroundings. Three of them had weak precipitin lines against the present spore and filament extracts but not against *T. vulgaris* or *M. faeni*, while the other 4 reacted only with spore extracts prepared from the actinomyces which were isolated from the patients' surroundings. The remaining 2 also reacted against the extract of *M. faeni* obtained from Wales.

DISCUSSION

The results of the present study indicate that sera of some patients with farmer's lung or bagassosis (hypersensitivity pneumonitis associated with thermophilic actinomyces) contain precipitins against the thermophilic microorganisms isolated from the home environment. It would seem, therefore, that either these thermophiles are not specific for the human habitats and can also be found in agricultural and other surroundings, or that the various thermophilic organisms contain similar antigens. Since no bands of identity between the various thermophilic extracts could be identified with the use of sera from patients with known hypersensitivity pneumonitis (Fig. 3), the former possibility seems to be more likely.

The presently available information is not sufficient to decide whether these thermophilic isolates represent an entirely new species. While no lines of identity were found on testing the reactive rabbit and human sera against *M. faeni*, *T. vulgaris*, and, more recently, *T. viridis*,* the results obtained with the two different extracts of *M. faeni* and farmer's lung sera indicate that the immunologic response to similar antigenic extracts may differ from one sensitive patient to another. However, the possibility should also be considered that the two extracts were prepared under different conditions, resulting in quantitative and qualitative differences in their antigenic compositions. Additional work on antigenic composition of the isolates, as well as more sophisticated techniques of cell wall analysis and histochemical staining, will be needed to decide the question of the identity of the thermophilic actinomyces isolated from home and office environments. In a preliminary report on a patient with hypersensitivity pneumonitis due to thermophilic contamination of a home environment,⁸ the offending species was identified as *T. vulgaris*.

Our data thus suggest that the thermophilic actinomyces contaminating home and office environments may belong to several different species, some of

*Unpublished observation.

them not yet definitely identified. Therefore, sera of patients suspected of having a hypersensitivity pneumonitis due to home or office contamination should be preferably tested against the thermophilic microorganisms isolated from the patient's environment, if such isolation is possible. Furthermore, one should be aware that the presence of precipitins in the serum is not a positive diagnostic indication of hypersensitivity pneumonitis, since a large proportion of individuals exposed to organic dusts may develop precipitins without any symptoms of the disease.¹² The demonstration of precipitins in the serum should be complemented with other typical respiratory, laboratory, and possibly roentgenographic findings¹³ before a definite diagnosis of hypersensitivity pneumonitis is entertained.

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