Assessment of the Pathogenicity of Bacteria Used in the Production of Single Cell Protein

Minoru Yoshida and Hajime Minato

National Institute of Animal Health, Kannon-dai, Yatabe, Tsukuba, Ibaraki 305, Japan

Received May 30, 1986

It has been required that strains of yeast and bacteria to be used for the production of single cell protein should be carefully checked as to their potential pathogenicity beforehand. A procedure for assessing the potential pathogenicity of yeasts was proposed after 9 years research work,1,2 which was briefly introduced in this journal.3) Thereafter, an attempt was made to apply the proposed procedure to the assessment of the potential pathogenicity of bacteria.1) The applied procedure in brief is as follows:

One-tenth ml per head of a suspension containing 10^8 colony forming units of washed vegetative cells is inoculated into the tail veins of 10 specific pathogen-free female mice of about 5 weeks of age. The mice are sacrificed after 14 days, and the liver, kidneys, and brain are collected. The surface of an agar culture plate is rubbed with a section of each of the organs, and then the plates are incubated, after which the colonies on the surface are counted. Specimens of the organs are prepared for histopathological examination.

The counts of colonies on the plates are given scores from 0 to 4; A score of 0 for no colonies, 1 for less than 10 colonies, 2 for less than 50, 3 for less than 100, and 4 for over 100. The results of histopathological examination are also given scores from 0, for no apparent change, to 4, for the severest change in the organs. The sum of the scores for the 3 organs gives the total score for an individual mouse. A total score of 12 is given for a mouse that dies within 14 days, and one of 15 for a mouse that dies within the first 5 days. The discrimination function, Z, is calculated using Eq. (1),

\[ Z = 9.228x_1 + 1.616x_2 - 30.158 \] (1)

where \(x_1\) is the average total score for 10 mice for the remaining living cells in the organs, and \(x_2\) that for the histopathological change. A strain of bacteria with a positive \(Z\) value belongs to Group I, and one with a negative \(Z\) value to Group II.

In Fig. 1, the 21 strains of bacteria tested are plotted against \(x_1\) and \(x_2\). The discrimination line is also shown in Fig. 1, which was obtained using Eq. (1) taking \(Z\) as zero. The strains to the upper right of the discrimination line belong to Group I, and those to the lower left to Group II.

On quick and simple judgement based on the \(x_1\) value alone, the strains could be discriminated at a point of \(x_1 = 2.8\), with a 95% fiducial interval of from 4.1 to 1.5. Strains having average scores for 10 mice of less than 1.5 can be handled as usual in the traditional fermentation industry, since they belong to Group II. Those having average scores of over 4.1 should not be used in the fermentation industry, since they belong to Group I. If someone wants to use one of the latter because it has highly beneficial characteristics, it should be handled very...
The pathogenicity of 7 among 21 strains of bacteria tested is briefly given in Table I. Although the hosts and symptoms of the diseases produced by these known pathogens are quite different, the responses of mice inoculated with cells of these strains were similar. The 4 strains numbered 1 to 4 were very virulent as they killed all of the mice within 5 days after the inoculation. Those numbered 5 to 7 killed no mice within 5 days after the inoculation, but killed some of them during the 14 day experimental period. The 7 strains located at the upper right of the discrimination line in Fig. 1 belong to Group I.

The responses of mice inoculated with cells of a strain, of which the pathogenicity was unknown but which belonged to Group I, were similar to those mentioned above, indicating that the strain resembled the pathogens in some way. Therefore, it was strongly suspected that the strain had potential pathogenicity.

"Salmonella mbandaka Le Minor" L-7464) was a strain submitted for serological typing, which was reportedly isolated from a group of diseased chickens. However, the symptoms the chickens suffered from and the isolation procedure were not reported, so the pathogenicity of the strain was doubtful. As shown in Fig. 1, the strain, numbered 8 and belonging to Group I, is strongly suspected to be pathogenic.

On the other hand, the responses of mice inoculated with cells of the other 13 strains were different. The scores were such that the strains were located at the lower left of the discrimination line in Fig. 1. No mouse inoculated with these cells died during the 14 day experimental period. These strains showed low possibility of having potential pathogenicity.

Two strains of Pseudomonas aeruginosa, pathogenic IID 1130 and IID 1005, of weak pathogenicity, both of which were from the Institute of Medical Science, the University of Tokyo, were also tested. As shown in Fig. 1, the former, numbered 1, belongs to Group I, and the latter, numbered 11, belongs to Group II. Thus, the proposed procedure is useful for distinguishing into two strains with the same taxonomic characteristics. Pseudomonas aeruginosa is known to be pathogenic, causing mainly suppurative diseases. However, the findings revealed that not all strains of P. aeruginosa are pathogenic. Pathogenicity is thus not specific to species but specific to a strain.

REFERENCES