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The Destruction of Bacterial Spores Upon Compressional Pressure

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타정 압력에 의한 세균포자의 파괴현상

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Abstract

The tolerance of useful bacterial spores to the conditions of tablet making, specifically, the destruction of bacterial spores upon compressional pressure was investigated.

The damage of bacterial spores occurred mainly during the tabletting. The bacterial spores obeyed a logarithmic destruction rate upon compressional pressure. The spore destruction rate was dependent upon the strains of microorganism. The Decimal Reduction Pressure, designated as P-value, were 2.9 *ton/cm²*, 2.6 *ton/cm²* and 2.1 *ton/cm²* for the spores of *Bacillus subtilis*, *Bacilus coagulans* and *Clostridium butyricum*, respectively, and 1.7 *ton/cm²* for the vegetative cell of *Streptococcus faecalis*.

The spore destruction upon compressional pressure was influenced by the type of filler. The P-value of the spore of *B. coagulans* was 2.8 *ton/cm²* in the lactose filler, but 2.0 *ton/cm²* in the starch filler. The number of viable spores was inversely proportional to the hardness and density of tablet, in case that the same type of filler was used. The starch filler, which resulted in the lower hardness and lower density of tablet, caused higher spore destruction rate compared with the lactose filler.

Introduction

Tabletting of useful microorganisms in food and pharmaceutical products is a widely used and well mechanized process. Microorganisms are mixed with filler, which has a good tablet forming property, and compressed in a mould[1,3]. One of the important criteria of the process is the survival of the added microorganisms through the selected conditions of tablet making. Bacterial spores are often used for this purpose because of their high tolerance to low water activity, high ionic strength, high osmotic pressure and other mechanical forces[4,5].

This study examined the tolerance of bacterial spores to the conditions of tablet making. Specifically, the destruction of bacterial spores upon compressional pressure was investigated. The effect of different types of filler on the spore destruction rate was studied, and the mechanism of spore des-

truction upon compression was considered.

Materials and Methods

Test microorganisms

Spores of *Bacillus coagulans* (lactic acid forming bacteria), *Bacillus subtilis* (amylase producing bacteria) and *Clostridium butyricum* (butyric acid producing bacteria) were tested. The vegetative cell of *Streptococcus faecalis*, which is considered to be tolerant to the conditions used for tablet making, was also tested in order to compare the tolerance of vegetative cells to that of bacterial spores.

Spore preparation

Bacillus coagulans was grown in a 30 *l* jar fermentor (L. E. Marubishi Co., Ltd., Japan) with 12 *l* yeast extract-peptone medium of pH 5.0 at 45°C. The broth medium was agitated and aerated at a rate of 200 rpm and 1 *v/v*/min, respectively. The number of cell reached to 15~20 x 10^8/*ml* after 12~14 hrs of cultivation, and 90% of the cells formed spores. The spores were matured for 6~10 hrs before the harvesting by centrifugal separation. *Bacillus subtilis* was grown in Roux flask for surface culture using soybean extract broth medium of pH 6.8 at 37°C[6,7]. *Clostridium butyricum* was grown anaerobically in a 30 *l* jar fermentor with 16 *l* starch molasses medium of pH 6.8 at 37°C[8]. Similarly, *Streptococcus faecalis* was grown anaerobically with molasses medium of pH 6.8 at 37°C.

Fillers

Mixtures of lactose (Meggle Milchindustrie GMBH & Co., West Germany) and corn starch (Pungjin Chemical Co., Korea) were used as filler. The ratio of the two components in the mixture was varied. Lactose filler was designated to a mixture containing 70% lactose and 30% corn starch, and starch filler was named to a mixture containing 40% lactose and 60% corn starch.

Tablet making

The bacterial spores harvested were mixed with filler and granulated by kneeding on a basket type granulator. The granules were dried at 60°C for 1 hr and ground into powder. The final concentration of the bacterial spores was adjusted to 1~4 x 10^8 spores per *g* powder during the final mixing. The granulated powder was compressed into tablet with a 24-punch rotary tabletting machine (Kilian & Co., West Germany). The term tabletting is specifically used for this process. The compressional pressure was varied from 500 to 3,500 kg/cm^2 by adjusting the pressure-spring handle. The accuracy of the testing pressure level was checked by the pressure-tablet height relationship of lactose filler published by Yumioka[11]. The flow diagram for the tablet making of bacterial spores is shown in Fig. 1.

Viable cell count

The of viable cell number was determined by the methods of Postage[9]. The number of viable cell of *B. coagulans* was determined by BCP plate count method, *B. subtilis* by starch agar plate count method, and *Cl. butyricum* and *Str. faecalis* by roll-tube method using FTM agar.

Physical properties of tablet

The hardness of tablet was measured by a spring

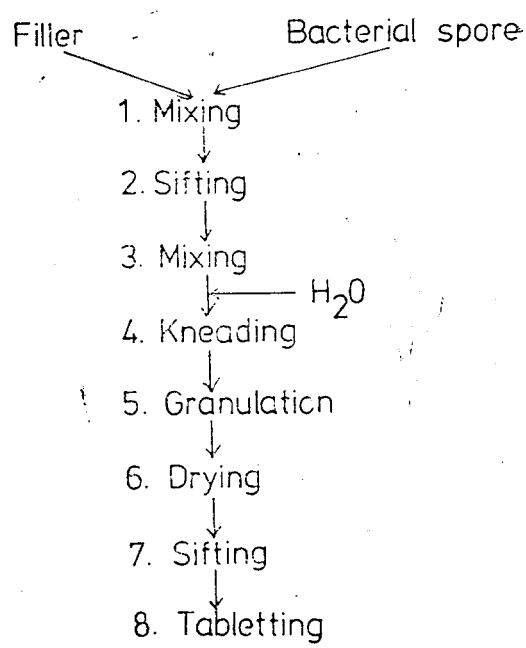

Fig. 1. Flow diagram for the production of tablet containning bacteria spores

lever type hardness tester. The bulk density of the filler was determined by measuring the weight of the filler packed by tapping in a container of known volume. The density of tablet was calculated from the weight and size of tablet.

Results

Spore destruction pattern during tablet making

The destruction pattern of bacterial spores during the tablet making indicated that the major spore destruction occurred during tabletting. As shown in Fig. 2, 10% of the added spores were damaged during granulation, whereas 60~70% of the added spores were destroyed during tabletting. The type of filler did not affect the degree of spore damage during granulation. On the other hand, a significant difference in spore destruction was observed during tabletting depending on the type of filler used.

Destruction rate of bacterial spores upon compressional pressure

Fig. 3 shows the destruction curve of bacterial spores in lactose filler compressed at different pressures for a constant time (0.42 sec). The declining straight lines on the semi-logarithmic plot indicates that the bacterial spores has a logarithmic destruction rate upon compressional pressure. The destruction curve of bacterial spores fitted to the following equation:

$$\log C_1 = \log C_0 - KP_1$$

where C_1 is the viable spore concentration after

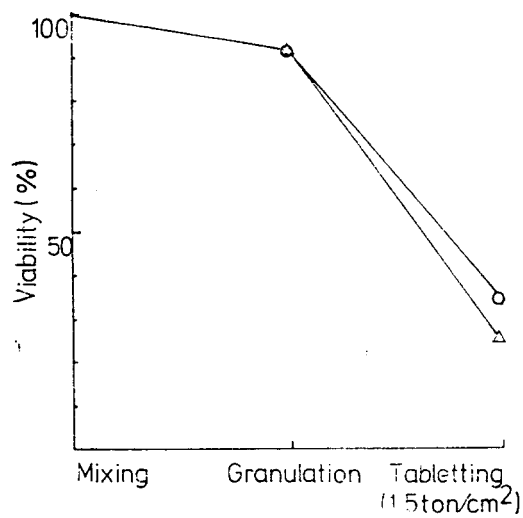

Fig. 2. Destruction of the spores of *B. coagulans* during tabletting process
○—○ : lactose filler; △—△ : Starch filler

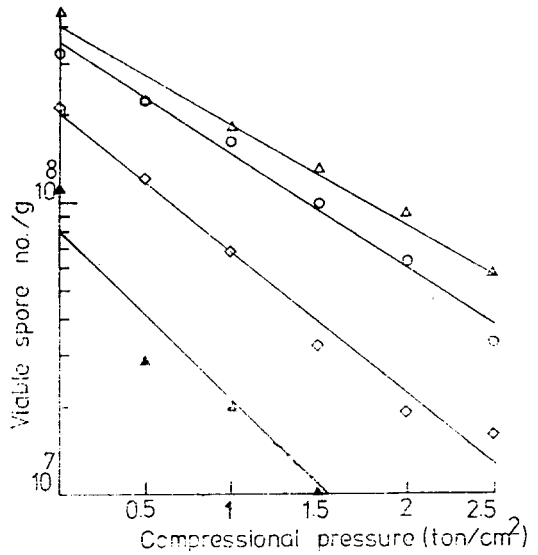

Fig. 3. Destruction curve of bacterial spores in lactose filler upon compressional pressure
△—△ : *B. subtilis*; ○--○ : *B. coagulans*; ◇—◇ : *Cl. butyricum*; ▲—▲ : *Str. faecails*

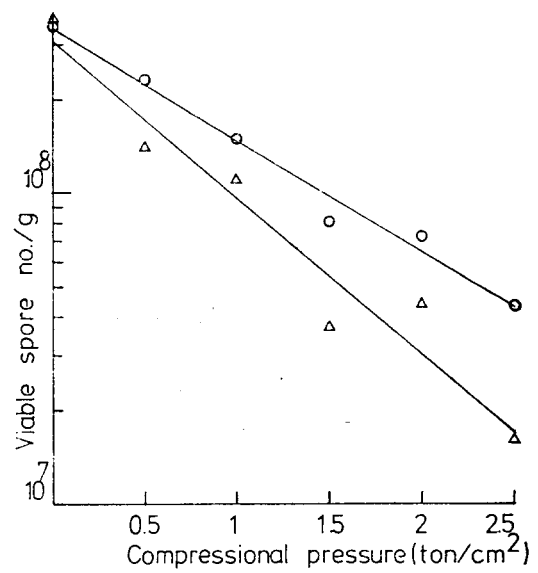

Fig. 4. Destruction curve of *B. coagulans* spores in different fillers upon compressional pressure
○—○ : lactose filler; △—△ : starch filler

Table 1. Regression analysis for the spore destruction curves upon compressional pressure and the P-value of the spores

Strains	Slope(K)	Standard deviation of regression	Correlation coefficient	P-value(*ton/cm²*)
B. subtilis	0.3406	0.0579	−0.9911	2.9
B. coagulans	0.3869	0.0480	−0.9931	2.6
Cl. butyricum	0.4750	0.0720	−0.9896	2.1
Str. faecalis	0.5838	0.0358	−0.9833	1.7

compression at pressure P_1, C_0 is the initial spore concentration, and K is a constant, which is the slope of the curve. This equation is similar to that for the chemical reaction kinetics, except for that the changes in spore concentration is considered as a function of pressure instead of time. As shown in Table 1, the slope of spore of spore destruction curve upon compressional pressure changed by the species of bacteria. The slope increased in the order of *B. subtilis*, *B. coagulans*, and *Cl. butyricum*. The slope of the vegetative cell of *Str. faecalis* was significantly steeper than those of bacterial spores.

In order to describe the tolerance of bacterial spores to the compressional pressure quantitatively, the Decimal Reduction Pressure, P-value, is introduced by taking the reciprocal of the slope of the destruction curve. The P-value is defined as the increment of pressure necessary to reduce the number of living spores to 1/10 of the initial concentration. The P-value of the spores of *B. subtilis* was 2.9 *ton/cm²*, *B. coagulans* 2.6 *ton/cm²*, and *Cl. butyricum* 2.1 *ton/cm²*. The P-value of the vegetative cell of *Str. faecalis* was 1.7 *ton/cm²*, which was distinguishably lower than those of bacterial spores.

Effect of the type of filler on spore destruction

Fig. 4 shows the destruction curve upon compressional pressure of the spore of *B. coagulans* in lactose and starch fillers. The slope of the curve changed by the type of filler used. As shown in Table 2, the slope of strach filler was significantly greater than that of lactose filler. The P-value of the spore of *B. coagulans* in lactose filler was 2.8 *ton/cm²*, while that of the same spore in starch filler was 2.0 *ton/cm²*.

The effect of the type of filler on spore destruction was further analyzed in terms of the hardness and the density of tablet. Fig. 5 shows the changes in spore destruction and the hardness of tablet upon compressional pressure. For the lactose filler, the hardness of tablet increased proportionally with the compressional pressure. It resulted in the inversely proportional relationship between hardness of tablet and the number of spores survived after compression. On the other hand, the hardness of the tablet made by starch filler was not proportionally increased with the compressional

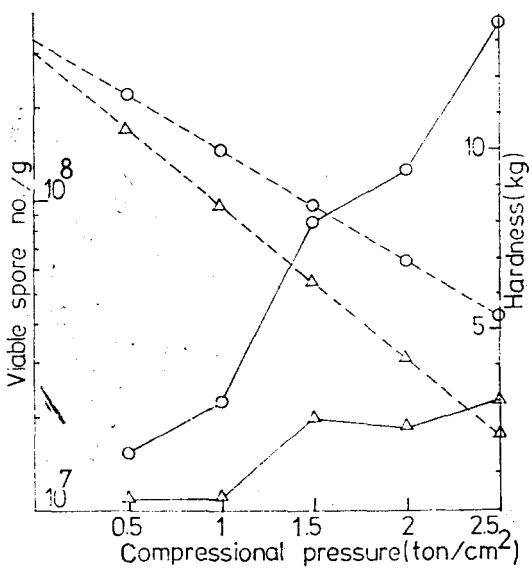

Fig. 5. Relationship between compressional pressure and hardness of tablets made by different fillers
○ : lactose filler; △ : starch filler; —— : hardness; ····· : viable spore no. of *B. coagulans*

pressure. The hardness of the tablet made by starch filler was lower than that of lactose filler, although the spore destruction in starch filler was greater than that in lactose filler. Thus, the inversely proportional relationship between the hardness of tablet and viable spore number after compression was valid only for the same type of filler.

The bulk densities of lactose filler and starch filler were 0.8586 g/cm^3 and 0.7752 g/cm^3, respectively. Fig. 6 shows the changes in the tablet density upon compressional pressure. The viable spore number appeared to be inversely proportional to the tablet density for the same type of filler. However, the tablet density of starch filler, which caused greater spore destrutction, was lower than that of lactose filler.

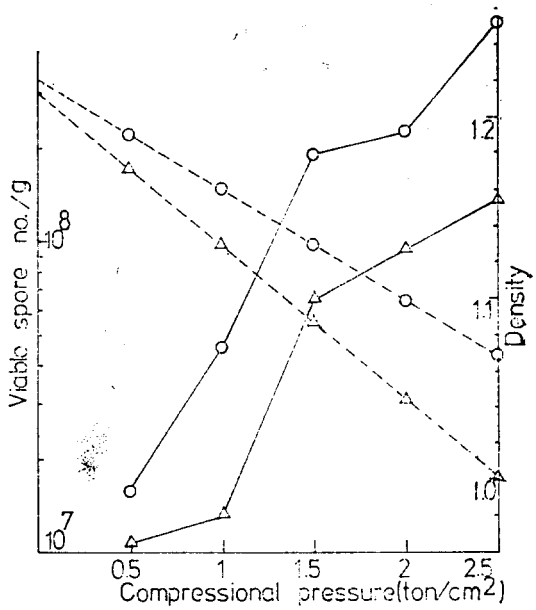

Fig. 6. Relationship between compressional pressure and density of tablets made by different fillers
○ : lactose filler; △ : starch filler;
····· : viable spore no. of *B. coagulans*;
—— : density

Discussion

The thermal destruction of bacterial spores has been widely studied for the development of thermal processing of food[10]. However, little attention has been paid to the destruction behavior of bacterial spores upon pressure. This study found a logarithmic dstruction rate of bacterial spores upon compressional pressure.

The term destruction rate used in this study is not related to the chemical reaction kinetics, which is usually calculated on the basis of time. Instead, the spore destruction rate was calculated on the basis of pressure with a fixed compression time. Therefore, the P-value is conceptually different from the D-value or Z-value for the thermal resistance of microogranisms. The P-value is to be changed by the compression time and the type of filler. However, when these conditions are identical the P-value can be used for a parameter representing the resistance of bacterial spores to the compressional pressure. In this study, the initial number of spore was fixed at one level of the concentration. The destruction rate at varied levels of the initial spore concentration is presently under investigation in order to generalize the spore destruction behavior upon compressional pressure.

The effect of the type of filler on the spore destruction rate is of industrial importance. This will also provide a clue for developing the mechanism of spore destruction upon compressional pressure. The lactose powder used in this study was multi-shaped particles having 1~20 μm of axial length. The corn starch was uniform spherical particles of 10~20 μm in diameter. The lactose filler which contained 70% lactose and 30% corn starch would

Table 2. Regression analysis for the spore destruction curve of *B. coagulans* in different fillers and their P-values

Fillers	Slope(K)	Standard deviation of regression	Correlation coefficient	P-value(ton/cm^2)
Lactose filler	0.3573	0.045	−0.9915	2.8
Starch filler	0.4979	0.1352	−0.9679	2.2

have lactose as the continuous phase in the compressed tablet, whereas the starch filler which had 40% lactose and 60% corn starch would have starch as the continuous phase in the tablet. When the starch concentration exceeded the level necessary to form the starch as the continuous phase, the tablet formation was unsatisfactory. The volume of tablet became larger and cracking of tablet occurred. The greater tablet height with the strach filler will result in the longer compression time in the rotary tabletting machine.

The selection of materials for filler is, therefore, important for the survival of bacterial spores during tabletting. The present study suggests that the proper combination of fillers having different shapes and mechanical properties can improve the survival of useful bacterial spores during tabletting. The mechanism of spore destruction and the interaction between bacterial spore and fillers during compression need further study.

요 약

본 연구는 유용 세균의 포자를 정제화(tabletting)하는 과정에서 포자의 내구성 특히 타정 압력에 대한 세균 포자의 파괴율을 규명하려 하였다.

Bacillus coagulans, *Bacillus subtilis* 및 *Clostridium butyricum*의 세가지 포자 형성균의 포자와 포자를 형성하지 않는 세균인 *Streptococcus faecalis*의 영양세포에 대하여 시험하였다.

정제제조 과정에서 포자의 파괴는 주로 타정과정에서 일어나며 세균의 포자는 타정압력에 대하여 대수적 파괴율을 나타내었다. 세균포자의 압력에 의한 파괴율은 세균의 종류에 따라 달라지며 이것을 정량적으로 나타내기 위하여 Decimal Reduction Pressure라는 개념을 도입하여 P-value라 칭하고 각 세균 포자의 P-value를 구하였든 바 *B. subtilis*의 포자는 2.9 *ton/cm²*, *B. coagulans*의 포자는 2.6 *ton/cm²*, *Cl. butyricum*의 포자는 2.1 *ton/cm²*의 값을 각각 나타내었다. 반면, *Str. faecalis*의 영양세포는 1.7 *ton/cm²*의 낮은 값을 나타내었다.

세균포자의 파괴율은 사용하는 부형제에 따라 영향을 받았으며 동일한 *B. coagulans*의 포자라도 lactose filler에서는 그 P-value가 2.8 *ton/cm²*이었으며 starch filler에서는 2.0 *ton/cm²*이었다.

동일한 부형제를 사용하였을 때 생존 포자수는 정제의 경도와 밀도에 역비례하였다.

그러나 경도와 밀도가 낮은 starch filler에서는 lactose filler에 비교하여 더 높은 포자 파괴율을 보였다.

결론적으로 부형제의 종류는 정제 형성뿐만 아니라 세균 포자의 생존에도 중요한 요인이며, 물리적 성질이 서로 다른 부형제를 알맞게 혼합함으로서 타정시 유용세균포자의 생존율을 크게 향상시킬 수 있다.

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