Factors influencing the production of kanamycin by *Streptomyces canus*

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(Eingegangen am 25. 11. 1970)

The production of kanamycin by three species of *Streptomyces* and four fermentation media was studied. *Streptomyces canus* in medium No. 2 gave the highest yields of kanamycin after 72 hrs. of fermentation. The presence of both molasses and glucose in the fermentation medium was an important factor for the production of kanamycin. The best concentration of molasses and glucose were 15.0 and 10.0 g/l, respectively. Replacement of peptone by equal amounts of inorganic and organic nitrogen sources was not suitable for the production of kanamycin, and peptone in a concentration of 5.0 g/l gave the best results.

Kanamycin was discovered by HAMAO UMEZAWA in 1956. The antibiotic has exhibited a therapeutic effect on bacterial diseases including tuberculosis, especially on infections caused by resistant microorganisms which were main subject of chemotherapy studies, and has been widely used for the treatment of acute bacterial infections. The microorganisms (*Streptomyces kanamyceticus*) was isolated from a soil sample collected at Nagano prefecture. Other strains of *Streptomyces* species exhibited more or less identical characteristics of *S. kanamyceticus* and also were able to produce kanamycin. These strains are *S. mitakaensis* and *S. canus*.

Many investigators studied the fermentative production and biosynthesis of kanamycin (*UMEZAWA et al. 1957, 1963, HAYASHI and DAIGAKU 1959, MAEDA et al. 1957, and KOJIMA et al. 1968, 1969*). Since kanamycin is a competitive item for several industrial organizations, most of the factors influencing the fermentative production have not been published. The aim of the present study is to explore the different factors controlling the production of kanamycin by fermentation. Among the factors studied are: the constituents of the medium, incubation period, and environmental conditions.

**Material and methods**

*Streptomyces* species: *S. kanamyceticus*, *S. canus*, and *S. mitakaensis* were maintained on a medium containing the following ingredients (g/l): Glucose 20.0, NaNO₃ 2.0, KH₂PO₄ 1.0, KCl 0.5, MgSO₄ × 7 H₂O 0.5, FeSO₄ × 7 H₂O 0.005, agar 20.0, distilled water. The sterile slants were inoculated aseptically with the microorganisms and incubated at 27—30 °C for 10 days to obtain good growth. The slants were kept in a refrigerator at 5 °C.

Fermentation media: The media are composed of the following ingredients (g/l): Medium No. 1: Soybean meal 10.0, glucose 10.0 (technical grade), NaCl 5.0, and CaCO₃ 1.0. Medium No. 2: Peptone (technical grade) 5.0, glucose (technical grade) 10.0, and molasses 20.0. Medium No. 3: Starch 20.0, KCl 0.5, MgSO₄ × 7 H₂O 0.5, K₂HPO₄ 1.0, NaNO₃ 2.0, and
FeSO$_4$ 0.005. Medium No. 4: Soybean meal 12.0, peptone 3.0, glucose 10.0, starch 20.0, NaCl 3.0, CaCO$_3$ 2.0, KCl 0.5, MgSO$_4$ $\times$ 7 H$_2$O 7 0.5, and K$_2$HPO$_4$ 1.0.

The best fermentation medium was selected as basal medium. The different constitutive media were prepared and portioned into 250-ml-Erlenmeyer flasks, each containing 30 ml. The initial pH values of the fermentation media were adjusted to 7.5-8.0. The flasks were plugged and sterilized at 21.5 lb/sq. in. for 20 min. When the flasks had obtained room temperature, the cultures were inoculated aseptically with standard inocula of one ml of spore suspensions. The inoculated flasks were incubated at 27-30 °C for 120 hrs. At the end of the incubation period, the mycelial dry weight and the final pH of the fermented cultures were determined. The capacities of the microorganisms for the production of kanamycin were tested with *Bacillus subtilis* NRRL B-543 (Abou-Zeid and Shehata 1969). The amount of antibiotic produced was calculated from a biological standard curve.

![Fig. 1](image_url)

Fig. 1. Selection of the favourable medium and *Streptomyces* species for the production of kanamycin

**Results and discussion**

Selection of the strain producing most kanamycin and of the fermentation medium

The three species of *Streptomyces* produced different yields of kanamycin in the fermentation media (Fig. 1). *S. canus* proved to be the most productive
Production of kanamycin

Strain in the fermentation medium No. 2. The descending order of the fermentation media was medium No. 2 > medium No. 4 > medium No. 1 > medium No. 3. The initial pH of the fermentation media was 7.5 to 8.0 and at the end of the incubation period, the pH shifted towards different values from 7.1 to 8.3, depending on the medium used. The antibiotic production was closely connected with the mycelial growth, therefore, maximal mycelial growth was exhibited in case of *S. canus*, while with the other two microorganisms the growth was small compared with the mycelial growth produced in medium No. 2.

In the case of *S. mitakaensis* the yield of the antibiotic was less than the amount produced by *S. canus* and no antibiotic was produced in medium No. 4. The final pH in the case of *S. mitakaensis* also shifted towards variable values depending on the constituents of the fermentation media.

Medium No. 2 and *S. canus* were selected for further experiments.

Biochemical changes in the fermentation medium during the biosynthesis of kanamycin

The antibiotic was produced after 48 hrs. and the yield reached its maximum at 96 hrs., later a slight decrease was observed. Therefore, the incubation period

![Fig. 2. Effect of different carbon sources on the production of kanamycin by *S. canus*](image-url)
of kanamycin production was divided into two phases; in the first phase, the microorganism built its microbial constituents to face the necessary metabolic activities and in the second phase, the biosynthesis of kanamycin came into action. The mycelial growth of *S. canus* reached its maximum at 72 hrs., above which a slight decrease in the mycelial yield was obtained. The final pH of the medium No. 2 shifted towards variable values depending on the incubation period, ranging from 6.0 to 7.9 at the end of the incubation. The microorganism utilized the carbon sources from the first hours of the incubation period and the utilization increased with increasing length of the incubation period, reaching its optimum at 96 hrs., above which a decrease in the amount of the consumed sugars was recorded after 120 hrs. of incubation period. The utilization of the nitrogen source also reached its maximum at 96 hrs., above this time only a slight increase in the total nitrogen was recorded. This slight increase may be due to the partial lysis of the microbial cells. Therefore, for further experiments the incubation period was 72 hrs.

**Effect of initial pH of the fermentation medium**

The data showed that the adjustment of the fermentation medium to pH 8.0 was most suitable for the production of kanamycin. The microorganism could not produce the antibiotic at pH 3.0 and 5.0, while above these values biosynthesis increased with the pH value reaching its maximum at pH 8.0, above this value a decrease was recorded. The final pH depended on the initial adjustments. The mycelial growth was observed between pH 5.0 and 9.0, and it increased the pH value. Therefore, the basal fermentation medium was adjusted to pH 8.0 before sterilization.

**Effect of different carbon sources**

The basal fermentation medium contained molasses and glucose as carbon sources. When glucose was replaced by other carbon sources, different yields of kanamycin were produced depending on the carbon source added, but still glucose was the best carbon source besides molasses for the production of kanamycin. When the basal medium was depleted from the carbon sources except molasses, the microorganism produced about 137 μg/ml compared with 475 μg/ml in the complete medium with molasses and glucose. Without carbon sources the microorganism produced about 9 μg/ml. The descending order of the different carbon sources for the production of kanamycin was glucose > fructose > cellobiose > starch > raffinose > maltose > sucrose > galactose > lactose > arabinose > medium without glucose > medium without glucose and molasses (Fig. 2).

**Influence of different concentrations of glucose**

The production of kanamycin increased with the glucose concentration reaching its maximum at 10.0 g/l, above which it decreased. Therefore, 10.0 g/l of glucose was used in the production of kanamycin. At a high level of glucose, the final pH shifted towards neutrality (pH 7.0), while at a low level it was directed towards alkalinity (pH 8.3). The mycelial growth also reached its maximum at 10.0 g/l.
Effect of different concentrations of molasses

The results of these experiments indicated that the presence of molasses was an essential factor for the production of kanamycin. When the medium was depleted from molasses and glucose remained in the medium, the yield of the antibiotic was dropped from 475 to 85 μg/ml. On adding molasses to the medium, the yield of the antibiotic increased reaching its maximum at 15.0 g/l molasses. At the end of the incubation period, the final pH of the fermentation medium shifted towards values between pH 7.0 at high contents and pH 8.2 at low contents of molasses.

Effect of different nitrogen sources

The basal fermentation medium contained peptone as nitrogen source. If peptone was replaced by an equivalent amount of different inorganic and organic nitrogen sources, the results indicated that peptone was an essential constituent of the medium and the inorganic nitrogen sources used were unfavourable for the production of kanamycin. Moreover, ammonium acetate and ammonium monohydrogen phosphate failed completely to be nitrogen sources for the production of kanamycin. The final pH of the fermented cultures shifted towards different values (pH 7.3 to 5.9). Potassium nitrate, sodium nitrate, ammonium sulphate, and ammonium nitrate favoured the microbial growth but depressed the antibiotic yield, while ammonium acetate and ammonium monohydrogen phosphate were unsuitable for both processes.

Tryptone and yeast extract were unfavourable for the production of kanamycin; corn steep liquor, meat extract, and soybean meal failed completely as nitrogen sources for the production of kanamycin, but favoured the mycelial growth.

Influence of different concentrations of peptone

The production of kanamycin reached its maximum at 5.0 g/l peptone. At the end of the incubation period, the pH shifted towards values between 6.4 and 7.8 depending on the concentration of peptone. The mycelial growth was increased with the increase of peptone concentration.

References


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