Pakistan J. Sci. Ind. Res., Vol. 15, No. 3, June 1972

UTILIZATION OF MOLASSES IN THE PRODUCTION OF A NEW PEPTIDE ANTIBIOTIC PRODUCED BY STREPTOMYCES SPECIES

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(Received August, 13, 1971)

Abstract. Egyptian molasses with other ingredients was used for the production of a new peptide antibiotic produced by *Streptomyces* species, isolated from Egyptian soils. The presence of potassium ferrocyanide or EDTA in the fermentation medium reduced the toxic effect of molasses by reducing availability of trace elements by forming insoluble salts. Also the addition of starch, $NaNO_3$ and KH_2PO_4 to the fermentation medium increased the antibiotic yield.

In surveying the potentialities of *Streptomyces species* from Egyptian soils,[1] an organism attracted our attention due to its high destructive effect on some bacteria. The isolation and purification of the active metabolite present in the fermentation broth of *Streptomyces species* revealed that it is a new peptide antibiotic.[2]

This work deals with the utilization of Egyptian molasses with other ingredients in the production of the new peptide antibiotic.

Maintenance of Streptomyces species. The organism was maintained on the following ingredients (g/l): glucose 20.0, $KH_2P\ O_4$1.0, KCl 0.5, $MgSO_4\ .7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.005, agar 30.0, and water to 1 litre. The inoculated slants were incubated at 27–30°C for 10 days to permit luxuriant growth. The slants later were kept in a refrigerator at 5°C.

Fermentation Medium. The crude molasses was diluted with distilled water and centrifuged to remove mud, suspended matter and other impurities. The crude molasses contain about 50% sugars. The following concentrations of sugar were obtained by dilution of molasses with distilled water (g/l): 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 and 100. The initial pH of each diluted amount was adjusted to 7.0. Triplicate Erlenmeyer flasks were used for each concentration, each flask containing 50 ml. The flasks were plugged, sterilized at 121°C for 15 min. After cooling, the flasks were inoculated with a standard inoculum of spore suspension of *Streptomyces species* of 10 days old. The flasks were then inserted on a rotary shaker of 200 rev/min at 28°C for 72 hr. At the end of the incubation period, the flasks were removed and analysed for the determination of final pH, sugar consumption,[3] mycelial dry weight and antibiotic produced.[4]

Results and Discussion

The results (Fig. 1) indicated that refined or clarified molasses proved to be suitable as a fermentation medium. The production of antibiotic increased gradually with an increase in the sugar content till it reached its maximum concentration (158.5 mg/100 ml) when 6.0–7.0 g/100 ml sugars was used, above which a decrease in the production of the antibiotic was recorded. The mycelial growth increased with an increase in the sugar content, till it reached 0.89 g/100 ml when the sugar content was 9.0 g/100 ml. The final pH ranged from 7.5–7.9. In all cases the pH shifted towards the alkaline side and reached its maximal alkalinity at 7.9 when the organism produced high yields of the antibiotic.

Effect of Different Concentrations of $NaNO_3$. The results (Fig. 2) indicated that when the concentration of $NaNO_3$ was 0.1–0.3 g/100 ml, the antibiotic production was increased from 158.5 mg/100 ml to 223.9 mg/100 ml. Higher concentrations of $NaNO_3$ (more than 0.4 g%) depressed the antibiotic production. The mycelial growth showed no great

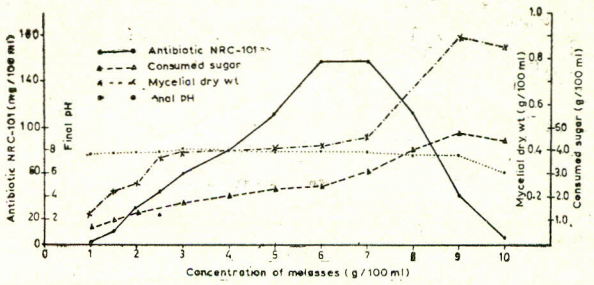

Fig. 1. Effect of molasses concentrations on the production of a new peptidec antibiotic by *Streptomyces species.*

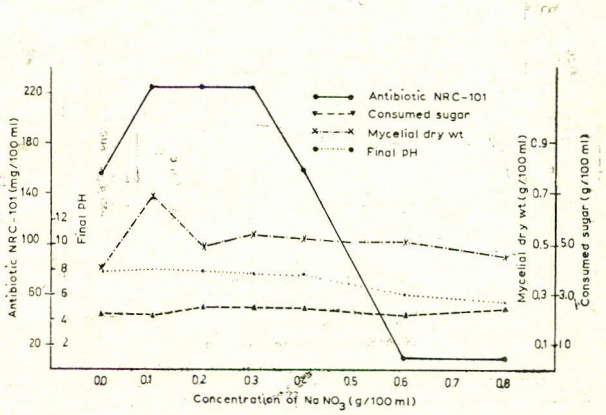

Fig. 2. Influence of $NaNO_3$ concentrations on the production of a new peptide antibiotic by *Streptomyces species.*

variabilities, but it slightly increased when low concentrations of $NaNO_3$ were added. The rate of sugar consumption was more or less the same in all conditions. The final pH shifted to the alkaline side, but when higher concentrations of $NaNO_3$ were used, the final pH value shifted to the acidic side.

Effect of Different Concentrations of KH_2PO_4. From the results (Fig. 3) it is obvious that phosphate inhibited the antibiotic production and the increase in phosphate concentration led to a decrease in the antibiotic production. The control (without phosphate) gave a better antibiotic production and least amount of mycelial dry weight. The increase in phosphate concentration was accompanied by an increase in the mycelial growth. The sugar consumption increased with an increase in the phosphate concentration, reaching its optimum at 4.11 g/100 ml, above which a decrease in the sugar consumption was recorded. The final pH was 8.0 with the untreated cultures, but it slightly decreased till it reached 7.2, when the concentration of phosphate was 0.6 g/100 ml.

Effect of Different concentrations of starch. No detectable increase was observed in the antibiotic production, (Fig. 4) when starch was added in low concentrations (0.2 g%). The yield of the antibiotic, however, increased and reached its optimum at 0.4 g% starch concentration, above which a decrease in the production of the antibiotic was recorded. The mycelial growth increased slightly from 0.59 g% to 0.72 g%, with the increase in starch concentration. The sugar consumption exhibited an increase with the addition of starch. The final pH shifted towards the alkaline side.

Effect of the Reduction of Inorganic Salts Present in Molasses on the Antibiotic Production Using Potassium Ferrocyanide. The most usual method for the reduction of metal content of molasses was the treatment of molasses with K_4 [Fe(CN)$_6$]. Ferrocyanide was added before sterilization in different concentrations up to 1.0 g/litre molasses medium. The treatment was done at pH 7.0.

The results (Fig. 5) indicated that the antibiotic production was highly affected when the molasses medium was treated with $K_4[Fe(CN)]_6$. Concentrations of 0.01–0.02 g% did not affect the antibiotic production, but higher concentrations of 0.03–0.05 g% increased in the yield of the antibiotic. At the concentration of 0.04 g% ferrocyanide, the yield was about 80% more than control cultures. This is mainly due to the reduction of the toxic effects of the metals present in molasses. The mycelial growth varied with the variation of the ferrocyanide concentration, but 0.1 g% ferrocyanide depressed the mycelial formation. The rate of sugar consumption was almost the same, while the final pH exhibited no

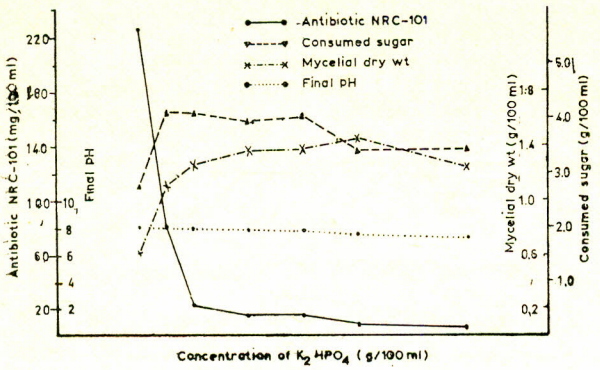

Fig. 3. Influence of KH_2PO_4 concentrations on the production of a new peptide antibiotic by *Streptomyces species.*

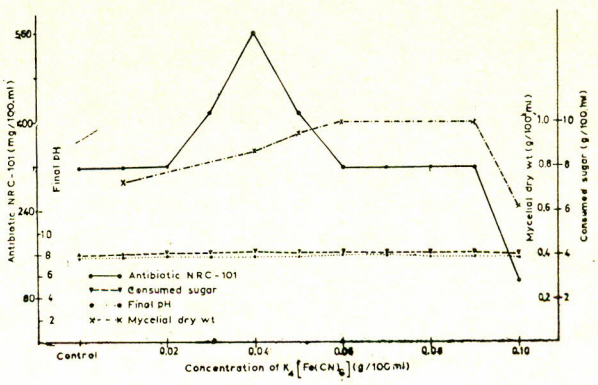

Fig. 5. Reduction of inorganic salts by $K_4[Fe(CN)_6]$.

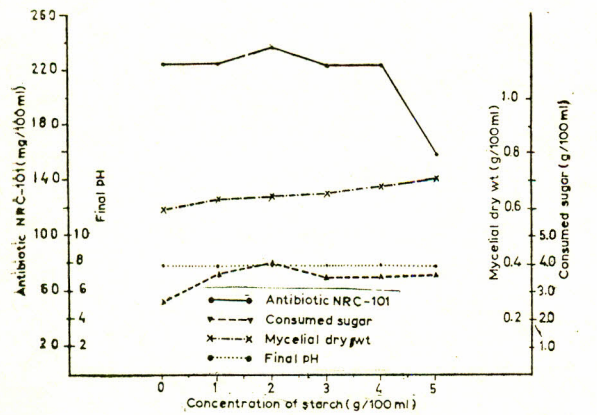

Fig. 4. Influence of starch concentrations on the production of a new peptide antibiotic by *Streptomyces species.*

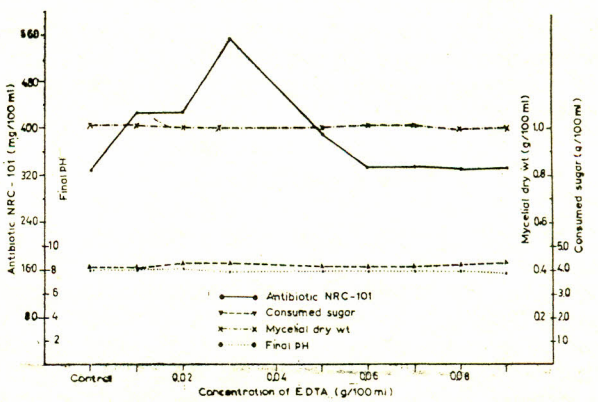

Fig. 6. Reduction of inorganic salts by EDTA.

great variation, but it was shifted towards the alkaline side.

Effect of the Reduction of the Inorganic Salts Present in Molasses on the Production of the Antibiotic NRC-101 using EDTA. The results (Fig. 6) showed that EDTA highly affected the antibiotic production. The yield of the antibiotic increased with an increase in EDTA concentration, till it reached 524.4 mg/100 ml at a concentration of 0.03 g/100 ml of EDTA. The mycelial growth showed no great variation when various concentrations of EDTA were used. The rate of sugar consumption showed no detectable increase, while the final pH was shifted towards the alkaline side.

The lack of the total nitrogen present in the black strap molasses led to investigate the influence of supplementary different concentrations of sodium nitrate to the fermentation medium (molasses medium) for the production of the antibiotic NRC-101. Suitable concentration of sodium nitrate was 1.0 g% and percentage increase in the yield of the antibiotic was about 29.4 g% more than the control. Therefore, the optimal concentrations of molasses and sodium nitrate were taken into consideration in the next experiments. This increase in the antibiotic production is correlated to the fact that molasses did not contain nitrogen sources which were sufficient for the growth of the microorganism and the antibiotic production.

The influence of dipotassium monohydrogen phosphate on the antibiotic production was investigated. The addition of dipotassium monohydrogen phosphate decreased markedly the antibiotic production and a further increase in its concentration greatly reduced the antibiotic formation by the organism. This inhibition in the fermentation production of the antibiotic may be due to the level content of phosphorus present in the black strap molasses.

The influence of different concentrations of starch was studied on the production of the antibiotic. Suitable concentration of starch favouring the antibiotic production was 4.0 g/litre. This indicated that the microorganism could utilize starch as carbon source beside molasses. The increase in the antibiotic production was about 41.2% more than the molasses medium.

The effect of different concentrations of some compounds such as $K_4[Fe(CN)_6]$ and sodium salt of ethylenediamenetetraacetic acid on the reduction of some elements, which may enhance the fermentation production of the antibiotic NRC-101 by the experimental organism, was carried out.

The ferrocyanide effect was ascribed to the removal of trace elements, as it is known that ferrocyanide forms an insoluble salt with ferric ion and probably with other metals as copper, manganese, zinc, aluminium, titanium and inorganic phosphorus.

The influence of the different concentrations of ferrocyanide indicated that 0.4 g/litre of potassium ferrocyanide was most suitable for optimum yield of antibiotic NRC-101.

The increase in the antibiotic production was about 77.8%, more than the antibiotic produced in the molasses medium without adding $K_4[Fe(CN)_6]$. The stimulatory influence of $K_4[Fe(CN)_6]$ may be due to the interaction between ferrocyanide and some toxic trace elements forming insoluble salts and, hence, the inhibiting influence may be removed or at least reduced.

The most favourable concentration of the sodium salt of EDTA for the fermentation production of the antibiotic NRC-101 was 0.3 g/litre. The increase in the antibiotic production was about 58.8% more than in the control culture. The stimulatory effect of EDTA on the production of the antibiotic NRC-101 may be due to the control of toxic elements in the molasses medium by chelation.

The stimulatory effect of $K_4[Fe(CN)_6]$ was more effective than EDTA. The increase in the antibiotic produced in molasses medium treated with $K_4[(Fe(CN)_6]$ was about 7.2% more than that produced in the molasses medium treated with EDTA.

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