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Research Article

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Laboratory Scale Comparative Studies of Penicillin Antibiotic Production from Various Carbon Sources

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Abstract Comparative penicillin antibiotic yields or production from various carbon sources was studied in the laboratory. Surface culture method for assessment of growth of the fungus, *Pencillium notatum* and penicillin yields was employed. The basic nutrient medium; corn steep liquor, standard salts with initial pH of 4.0 were used throughout the study. The basic medium was supplemented with 3 percent (3%) and 6 percent (6%) of the various carbon sources; glucose, lactose, cornstarch, glycerol, brown sugar, sorbitol and corn dextrin respectively. Penicillin yields for 7-days period at 3% concentration of glucose, brown sugar, glycerol, cornstarch, corn dextrin, and lactose were 66mg/ml, 79 mg/ml, 52mg/ml, 146mg/ml, 146mg/ml and 146mg/ml respectively. Similarly, at 6% concentration penicillin yields were 50mg/ml, 45mg/ml, 40mg/ml, 42mg/ml and 105mg/ml respectively. At 3% concentration, lactose, cornstarch, corn dextrin and brown sugar were more beneficial for penicillin production, glycerol being definitely inferior. Increases in pH of the broth were less rapid in the lactose, starch and dextrin cultures compared to those of glucose, sucrose and glycerol cultures. But at 6% concentration, there was less fluctuation in pH in the cultures containing lactose than those of glucose, sucrose, glycerol or sorbitol respectively. Under these conditions, there was no corresponding increase in penicillin yields as the concentration was increased from 3% to 6%. However, at both 3% and 6% concentrations, cultures containing lactose gave the highest penicillin yields and therefore should be the substrate of choice even in industrial application.

Keywords Penicillium notatum, surface culture, glucose, lactose, cornstarch, sorbitol, brown sugar

Introduction

The production of penicillin by the surface culture of *Penicillium notatum* is the foundation upon which large new industries have been built [1]. Penicillin antibiotics were first produced by cultivation of fungus *Penicillium notatum Westling* on the surface of a liquid nutrient such as Czapek-Dox glucose medium [2]. Although cultivation of mould in submerged culture now appears to be more economical and more practical from an industrial stand point, surface cultures were utilized to produce the penicillin that first effected the remarkable clinical cures which indicated that the large investment of time and money made during the past years would be justified.

Previous investigators of penicillin production have not been successful in developing a highly productive medium [3-7], the discoverer of the drug refers only to the use of a "nutrient broth", whereas Clutterbuck *et al.* [8] used a modified Czapek-Dox medium. Although the yields of penicillin obtained by this group and by Fleming were not calculated in terms of the Oxford unit adopted later, they were undoubtedly very low, at least when compared with present standards. Abraham *et al.* [9] using the same modified Czapek-Dox medium with the addition of small amounts of a crude yeast extract, obtained only 2 to 6 Oxford units per ml. However, recent experiments have led to an increase in the yields of penicillin from the range of 2 to 6 milligram per ml to as much as 160 to 230 milligram per ml. This increase in yield has been achieved primarily by the proper selection of organisms and nutrients, including the use of corn steep liquor and the use of lactose as the principal carbohydrate.

Materials and Methods

The experiments were conducted by the cylinder-plate method originated by Abraham *et al.* [9] and modified by Schmidt and Moyer [10]. Spores for laboratory inoculations were grown on both agar slants and Petri dish cultures. These spores were used for the inoculation of production cultures. The production cultures were grown in 250ml Pyrex Erlenmeyer flasks containing 50ml of the nutrient medium. In each experiment, a sufficient number of flasks were employed so that duplicate cultures could be harvested and assayed on several consecutive days. All production cultures were incubated at 24 °C. The production culture medium contained: carbon sources (glucose, brown sugar, lactose, glycerol, cornstarch and dextrin), 3.0g per 100ml; corn steep liquor, 10g per 100ml; standard salt ($MgSO_4.7H_2O$, 0.250g; KH_2P0_4, 0.50g; $NaN0_3$, 3.0g; $ZnSO_4$. $7H_20$, 0.044g; and $MnSO_4$. $4H_20$, 0.004g per liter of the final medium). The production culture medium was further supplemented with 6.0g per 100ml of the same carbon sources to produce 3 percent and 6 percent concentration of the various carbon sources respectively. The nutrition media were adjusted to initial pH, 4.0. The production culture media were further supplemented with various percentages of crude sources of starch for comparison with pure or refined carbon sources on penicillin yields and fungus growth and pH adjusted to 4.0.

Results

The results obtained from the comparison of various carbon sources each of which was used in 3% concentration in corn steep-liquor standard salt medium is presented in Table 1. In the corn steep-liquor, there was sufficient assimilable carbohydrate, particularly glucose and dextrin to support fairly good fungus growth with moderate penicillin production. Lactose, cornstarch and dextrin were equally good for penicillin production, glycerol being definitely inferior.

Table 1: Penicillin production from various carbon sources at 3% concentration

Carbon Source	Culture Age (Days)				
	3	4	5	6	7
	Penicillin Yield (mg/ml)				
Control (no added carbon)	27	45	41	36	27
Glucose	18	54	91	95	65
Brown sugar	7	40	85	102	79
Lactose	30	63	112	138	146
Glycerol	14	43	80	74	52
Corn stach	35	85	122	140	45
Corn dextrin	28	73	91	125	46

The changes in pH of the medium containing various carbon sources at 3% concentration are presented in Table 2. The pH of the broth increased gradually in lactose, starch and dextrin cultures as observed in glucose, sucrose and glycerol cultures.

Table 2: Penicillin Production from Various Carbon Sources at 3% Concentration

Carbon Source	Culture Age (Days)				
	3	4	5	6	7
	pH of filtrate				
Control (no added carbon)	6	7.5	8	8.1	8.3
Glucose	4.6	6.1	7.3	8.1	8.2
Brown sugar	4.3	5.4	7	7.8	8.1
Lactose	4.7	5.7	6.7	7.4	7.8
Glycerol	4.8	6.2	7.5	8.1	8.2
Corn stach	4.9	6.6	7.1	7.4	7.7
Corn dextrin	4.7	6	6.7	7.4	7.7

Table 3, presents a further comparison of the various carbon sources at 6% concentration. Cultures containing lactose gave the highest penicillin yields in both 3 and 6% concentrations within the days of observation.

Table 3: Penicillin Production from Carbon Sources at 6% Concentration

Carbon Source	**Culture Age (Days)**				
	3	**4**	**5**	**6**	**7**
	Penicillin Yield (mg/ml)				
Control (no added carbon)	40	40	40	40	10
Glucose	78	86	98	105	133
Brown sugar	25	25	23	40	14
Lactose	30	34	33	42	18
Glycerol	30	35	40	45	58
Corn stach	30	40	45	50	30
Corn dextrin	40	40	40	40	10

The pH is presented in Table 4, with lactose broth culture being lower than those containing glucose, sucrose, glycerol or sorbitol.

Table 4: Penicillin Production from Various Carbon Sources at 6% Concentration

Carbon Source	**Culture Age (Days)**				
	3	**4**	**5**	**6**	**7**
	pH of filtrate				
Control (no added carbon)	8	8	8.1	8.2	8.4
Glucose	7	7	7.1	7.2	7.8
Brown sugar	7	7	8	8.1	8.4
Lactose	8.1	8.1	8.1	8.1	8.4
Glycerol	7	7.1	7.5	7.5	8.3
Corn stach	7	7.1	7.5	7.6	8.4
Corn dextrin	8	8	8.1	8.2	8.4

In the corn steep liquor there was sufficient assimilable carbohydrate, mainly glucose and brown sugar to support fairly good fungus growth (Table 5).

Table 5: Penicillin Production from Various Carbon Sources at 6% Concentration

Carbon Source	**Culture Age (Days)**				
	3	**4**	**5**	**6**	**7**
	pH of filtrate				
Control (no added carbon)	0.21	0.22	0.45	0.51	0.41
Glucose	0.11	0.12	0.13	1.14	1.15
Brown sugar	0.12	0.45	1.21	1.31	1.13
Lactose	0.11	0.46	1.21	1.28	1.07
Glycerol	0.21	0.47	1.34	1.48	1.32
Corn stach	0.12	0.13	0.56	1.31	1.14
Corn dextrin	0.21	0.22	0.45	0.51	0.41

The result of crude sources of starch is presented in Table 6. Ground corn, ground wheat and granular wheat flour were as satisfactory as lactose for penicillin production (Table 6).

Table 6: Penicillin Production from Medium Containing Ground Corn and Wheat

Carbon Source	**g/100ml**	**Culture Age (Days)**				
		3	**4**	**5**	**6**	**7**
		pH of filtrate				
Control (no added carbon)	4.3	46	92	95	109	140
Glucose	6	55	80	120	150	145
Brown sugar	4.5	46	98	110	127	140
Lactose	7.4	50	85	115	140	140
Glycerol	3.7	53	90	112	138	119
Corn stach	3	56	68	102	127	127
Corn dextrin	3	64	85	109	143	147

A comparison of penicillin production, carbohydrate utilization, pH change and growth rate on the glucose and lactose medium is presented in Figure 1. The maximum penicillin yields of 146 and 84 mg/ml from lactose and glucose media respectively were attained between fifth and sixth days, after which there was a decrease in the penicillin content of the broth. The total reducing power of the medium was determined after acid hydrolysis and was calculated as glucose. The cultures on the glucose medium grew slightly faster than those on the lactose medium which corresponds with the observation that the glucose was consumed more rapidly than the lactose. The pH of the glucose cultures during the first 3 to 5 days was lower than that of the lactose cultures, but after the fourth day the reverse was true.

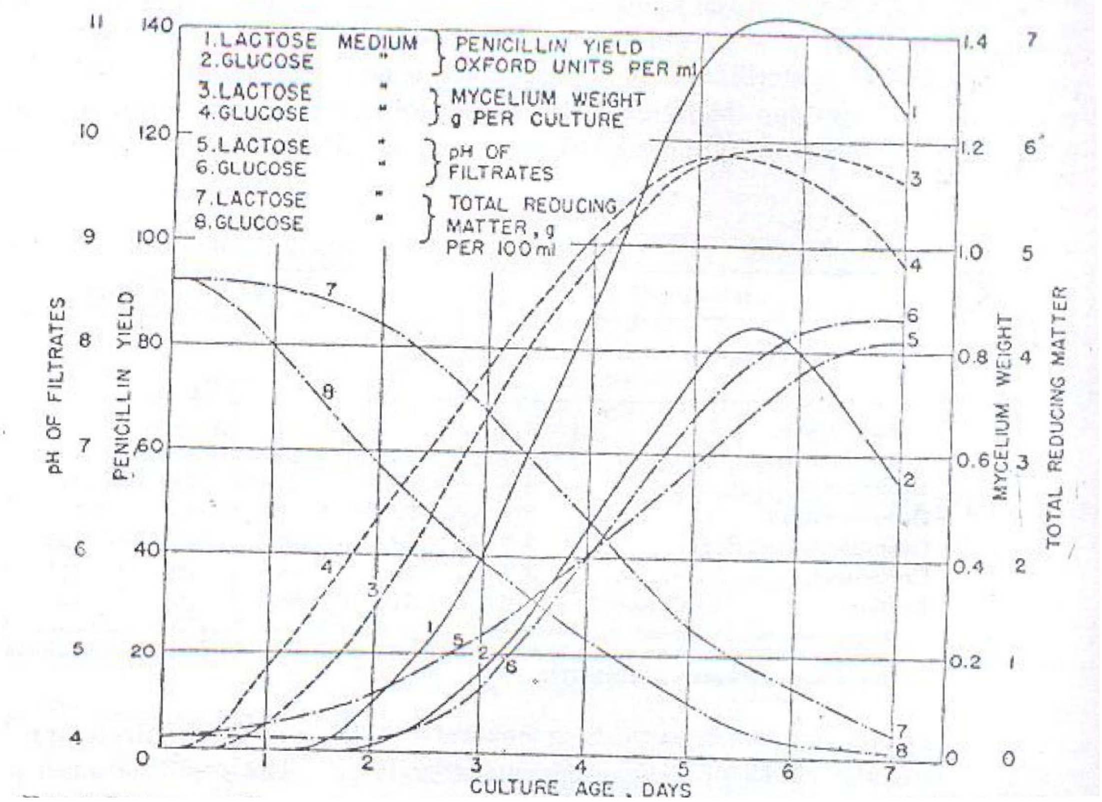

Figure 1: Penicillin production, growth, changes in pH and carbohydrate utilization in glucose and lactose media.

Discussion

A culture medium for good penicillin production must not only provide nutrients suitable for rapid fungus growth and penicillin formation, but must also provide conditions in which the penicillin is not too rapidly destroyed.

No significant differences in penicillin production could be observed between cultures containing commercial glucose and those containing brown sugar. When the Czapek-Dox medium (without corn steep liquor) was used, there was a marked difference in penicillin production in response to various carbon sources [2, 8]. However, we observed a marked effect of various carbon sources added to the basic medium containing corn steep liquor on the growth of the fungus and penicillin yields (Table 1). This result compares well with the report of other researchers [11,12]. Earlier, Fleming [2] (1929) the discoverer of the drug, refers only to the use of a "nutrient broth", whereas Clutterbuck *et al*. [8] used a modified Czapek-Dox medium. Abraham *et al.* [9] using the same modified Czapek Dox medium with the addition of small amounts of crude yeast extract obtained only 2 to 6 Oxford units per ml of Penicillin. The yields of penicillin obtained by this group and by Fleming were undoubtedly very low, at least when compared with the range of 58 mg/ml to 146 mg/ml obtained in our results using the same standard salts and employing various carbon sources in corn steep liquor (Tables 1 and 3 and Fig. 1).

Besides suitable nutrient medium, other factors influencing the stability of penicillin during fermentation are temperature and pH. Several investigators have shown that penicillin inactivation is a function of temperature and pH and that the greatest stability in solution is exhibited at pH 6.0 [13-15]. In this work, the use of 24 °C and initial pH of 4.0 revealed that the growth period were not optimum for penicillin stability as previously reported [7]. The data obtained in the course of these nutritional investigations indicate that penicillin was produced during the active growth phase of the fungus and not an autolytic product released at the cessation of growth. As shown in Figure 1, penicillin formation can be detected by the time the pH has risen to 5. At this pH and temperature the half-life of pure penicillin was about 60 hours [16]. Near-optimum growth-pH-Penicillin relationships are believed to exist when the greater part of the fungus growth occurs between pH 5 and 7.5. In the lactose medium (Figure 1), 72% of the growth occurred between pH 5.0 and 7.0, and in the glucose cultures only 31% of the growth occurred in this range, 69% of it occurred below pH 5.0.

Several factors are associated with increase in the pH during fermentation and could be related with the amount of corn steep liquor, the amount and kind of carbon source applied. This corroborates earlier reports of other investigators [4, 17-19]. There was more rapid increase in pH in lactose cultures during the first part of the fermentation than in the glucose cultures and these differences may be attributed to availability of the carbon sources (Tables 2 and 4). Lactose in the presence of corn steep liquor was more slowly utilized than glucose which appears to be readily available and assimilable carbohydrate than lactose. This confirms earlier findings that carbon sources such as lactose and starch appears to prolong the productive life of the cultures [14]. This study also demonstrates that the role of the corn steep liquor in maintaining a suitable pH appears pivotal to the maintenance of a vigorous fungus growth.

Conclusion

Penicillium notatum is now widely used for the industrial production of penicillin antibiotic by the laboratory surface culture method. The yield of penicillin production in surface cultures by *Penicillium notatum* using various carbon sources in corn steep liquor greatly increase the penicillin yields as we observed in the study. The use of lactose is found to give higher penicillin yields than can be obtained with glucose, brown sugar, sorbitol, or glycerol. The use of *Penicillium notatum* in culture medium containing corn steep liquor and lactose has given penicillin yields of 146 in 7 days.

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