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Studies on the effect of carbohydrates and fat compound suited to the best environment condition for the penicillin production

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Abstract

The performance of potato starch, maltose and dextrin of the constituent of the medium was suited to the best environment condition than lactose medium for penicillin production. These experiments were reported that the lactose is replaced by other carbohydrates and fat compound. This study was done exclusively on shake flask method. Glucose, sucrose and glycerol were unable to supply the essential requirement for the mold. Because they are exhausted at rapid rate, such medium would given a low yield of penicillin production when they are used to completely replace the lactose in medium. Potato starch, maltose and dextrin were able to supply the essential requirement for the mold, because they are exhausted at slow rate. Thus the maximum yield of penicillin can be obtained when they are used to completely replace the lactose in medium. The present investigation was observed that potato starch, maltose and dextrin were suited to the best environment condition for penicillin production.

1. Introduction

The penicillin fermentation processes were mainly conducted with the easily available raw materials such as cotton seed meal, lactose or sucrose. Penicillin was produced when a suitable medium is fermented by *Penicillium chrysogenum* in the presence of supplemented precursor. *Penicillium chrysogenum* is an aerobic fungi (mold) that is widely distributed in nature and is often found living on food and in indoor environment. Lactose is used as very good carbon source in fermentation (Moyer and Coghill 1995). Jarvis and Johnson (2001) were in investigated that the relationship between sugar utilization and penicillin formation in penicillin fermentation.

Carbohydrates are commonly used as carbon source in penicillin fermentation. Jarvis and Johnson (2001) were in investigated that the relationship between sugar utilization and penicillin formation in penicillin fermentation. Glucose is simple sugar, and is fermented at very rapid rat in penicillin fermentation. Glucose may be used as satisfactory substitute to lactose, if feed rate are used, higher Penicillin yield was obtained than lactose (Stanbury 1997). Sucrose may be used as substitute to lactose, if fed rate are used and were described that considerably higher penicillin yield than lactose could be obtained in synthetic medium when sucrose are continuously added to fermentation.

Maltose are a group of low molecular weight carbohydrates produced by the hydrolysis of starch, it is one of the disaccharide. They are water soluble, white solids that are optically active. Maltose is fermented at very low rate in fermentation. They may be used as carbon source in penicillin fermentation. Starch is an obtained from grains (maize), tubers (potato). They are water soluble, white solids. It is fermented at very low rate in fermentation. Starch is used as a major carbon source for Glutamic acid production. Dextrins are group of low-molecular-weight carbohydrates produced by the hydrolysis of starch. They are water soluble, white to slightly yellow solids that are optically active. Dextrins are used as widespread in industry, as thickening agent in food processing and as binding agent in pharmaceuticals. Glycerol is a chemical compound with the formula $HOCH_2CH$ (OH) CH2OH. This colorless, odorless, viscous liquid is widely used in pharmaceutical formulations. Also commonly called glycerin or glycerin, it is a sugar alcohol, and is sweet-tasting and of low toxicity. Glycerol is also used as a sugar substitute. In this regard, it has approximately 27 calories per teaspoon and is 60% as sweet as sucrose.

Although it has about the same food energy as table sugar, it does not raise blood sugar levels These experiments were reported here present that the lactose is replaced by other carbohydrates and fats .This study was done exclusively on shake flask fermentation processes.

2. Materials and Methods

Culture

Penicillium chrysogenum ATTC 46583 was obtained from Microbiology department, SPIC Pharma**,** Cuddalore as spore suspension.

Inoculum Development

The first step in the fermentation process is inoculation of cultures. The main purpose of this and subsequent inoculum development steps is to increase the concentration of fungal mycelium (biomass) to give a population which can be added to the next step to assure that each step will be reasonably short and the large-scale equipment is used efficiently. Inoculum development stages are typically conducted at around 25ºC in shake cultures and agitated vessels.

Colony selection

Ampoules containing pan lab strain were broken and serially diluted in distilled water. 0.1ml of sample from each dilution was taken and inoculated in lactose corn steep agar (LCSA) plate. LCSA plates were incubated at 25C, which is optimum for the growth of fungi.

After 13 days green coloured colony of 5-10mm size was observed on the plates. Pick out such as a type of colony, which is considered the best. The colony was made to 2 ml and taken in 10 tubes, this colony transferred to rice flask method.

Rice flask method

The rice is used recently as media for inoculums development. One Kg of rice was soaked into water for 15 min and air dried for 15 min. The amount of water present in rice after soaking was calculated by the following formula,

$$\frac{\text{Weight diff before and after soaking}}{\text{Total wet weight}} \times 100$$

The optimum moisture should be 22.25%. The picked colony was added to the flask with rice. The flask was incubated for 13 days in thermostatic chamber, where a constant temperature maintained. The flask was mixed thoroughly on 6th and 9th day of incubation for uniform growth. 200ml of Tween 60(0.05%) was added to each flask on 13th day and washed away the green colored spores formed on rice medium. The green spores would be suspended in water. This suspension was transferred to sterile flask. The viable count and sterility test were checked.

Sterility test

The sterility of spores were checked for determine the other fungal and bacterial contamination. The sterility media was prepared (Sabourauds Dextrose Agar for Fungi, Nutrient Agar & Nutrient Broth for Bacteria). The tubes and plates were autoclaved at 121ºC for 30 minutes. After sterilization, the spore suspension was transferred into test tubes and plates. The NB tubes and NA plates were incubated at 37ºC for 24 hours and SDA plates also incubated at 25ºC for 48 hours. If red color turns to yellow color (NB), would be confirmed Bacterial contamination in this spore suspension. If any other colony were observed in SDA, directly identified under the microscope.

Viable count test

This Viable count test can be used to check for viable spores. The spore suspension was serially diluted with Tween 60 up to 10^5 dilutions. 0.1ml were spread plated in viable count medium and incubated at 25ºC for 5 days. White colored spores are formed (1-2mm). The number of colonies formed was counted. After checked, the inoculums was transferred to shake flask method.

Shake Flask Method

Seed medium

Seed medium were used for enhancing the germination of spore, for inoculation into fermentation medium. All the ingredients of seed medium were dissolved in small amount of Deminiralized water and made up to required volume with Deminiralized water.

The natural pH was noted as 5.32 and 50ml 0f seed medium were distributed into each flask. All flasks were autoclaved at 121ºC for 15 min. After sterilization, the pH was noted as 6.4.

Inoculation

The spore load for 50ml of seed medium were calculated by the following formula,

$$\frac{\text{Spore load} \times \text{volume of seed media}}{\text{Viability of inoculums}}$$

$$\frac{9 \times 10 \text{ spores/ml} \times 50 \text{ ml}}{2.25 \times 10}$$

0.225ml of spore suspension of *Penicillium chrysogenum* was transferred to each 50ml of sterile seed medium. All flask were incubated at 25ºC for 54 hrs on shaker at 240rpm. After 54 hrs, the packed mycelia volume (PMV) and pH, pellet morphology was studied. PMV were noted as 30%.

PMV = (10- amount of supernatants) ×10%

= (10-7) × 10% = 30%

Fermentation Medium

The fermentation medium commonly known as production medium for fermentation processes. The precursor phenoxy acetic acid (POAA) was dissolved in small amount of demineralized water and then pH was adjusted from 2.7 to 6.5 by using 1N of NaOH solution. After pH adjustment, all ingredients were dissolved and make up to required volume with demineralized water. The pH was checked as 6.62 and 50ml of fermentation medium was distributed into each shake flask. The fermentation medium with lactose was assumed as control for determining the test trials. 0.5ml of corn oil was added to each shake flask as antifoaming agent. All shake flask were autoclaved at 121˚C FOR 30 min and after sterilization, pH were checked as 6.7.

Seeding

When seed media are ready, 5nl of seed media was transferred to 50ml of production media under aseptic condition. The pH and packed mycelia volume (PMV) as 5.14, 30% and pellet morphology was observed under the Microscope. After seeding, were incubated in shaker at 240rpm for 164 hrs.

PMV= (10- amount of supernatants) ×10%

Hatvesting

After 164 hrs incubation, the pH and pellet morphology were checked. Broth was filtered by using Whattman filter paper No.5. Supernatant of broth given to QC for analyzing of penicillin V activity.

Analyticals Method

The activity of penicillin V and residual of POAA was analyzed by HPLC method. Depends upon the curve area, the amount of penicillin V present in broth was calculated by using the following formula,

Amount of pen-V=1527×con.of pen-V ×dilution

=X u/ml

The residual POAA was calculated by using the following formula,

=con. Of POAA ×dilution

=X mg/ml

3. Result and Discussion

The parameters of production medium are given in the table 1. The activity of pen-V and residual POAA OF 25% tested carbon source with lactose are given in the table 1. The maximum rate of penicillin production was obtained when 25% of all tested carbon source were used with lactose. The medium containing 25% of glucose, sucrose, dextrin, maltose, glycerol, and potato starch with lactose had higher yield than control such as respectively 34102 IU/ml, 37717 IU/ml, 39435 IU/ml, 36572 IU/ml, 36648 IU/ml, 36610 IU/ml, and the residual POAA as respectively 0.062 mg/ml, 0.075mg/ml, 0.08mg/ml, 0.08mg/ml, 0.05mg/ml, and 0.035mg/ml. Jarvis and Johnson (2001), found that optimal penicillin was obtained, if glucose and lactose are present in such ratio that proper quantity of mycelium is formed from the readily available glucose before the mold is forced to use the slowly fermented lactose and proved by this experiment.

Table: 1. The parametrs and HPLC result of 25% other carbon sources with lactose containing medium

Numbers of Samples	Parameters of Carbon Source	Pen-V IU/ml	POAA mg/ml	pH	PMV (%)
PM1	Lactose 100%(Control)	35,388	0.05	6.06	35
PM2	Lactose 75% + Glucose 25%	34,102	0.06	5.50	30
PM3	Lactose 75% +Sucrose 25%	37,717	0.07	5.32	35
PM4	Lactose 75% +P.Starch 25%	36,610	0.03	6.04	38
PM5	Lactose 75% +Glycerol 25%	36,648	0.05	5.50	30
PM6	Lactose 75% +Dextrin 25%	39,435	0.08	6.01	39
PM7	Lactose 75% +Maltose 25%	36,572	0.08	5.90	36

The activity of pen-V and the residual POAA of 50% tested carbon source with lactose are given in the table 2. The increased yield of penicillin V was obtained when 50% of Dextrin, Potato Starch, Maltose, Glycerol, Glucose, and Sucrose were used with lactose. The increased yield of pen-V was obtained when 50% of Dextrin, Potato Starch, Maltose, and Glycerol with lactose were used with lactose than control. They had higher yield such as respectively 42794 IU/ml, 33060 IU/ml, 31227 IU/ml, 30616 IU/ml and residual POAA as 0.10mg/ml, 0.08mg/ml, 0.03mg/ml and 0.05mg/ml. Jarvis and Johnson (2001), found that optimal penicillin was obtained, if glucose and lactose are present in such ratio that proper quantity of mycelium is formed from the readily available glucose before the mold is forced to use the slowly fermented lactose and proved by this experiment.

Table: 2. The parametrs and HPLC result of 50% other carbon sources with lactose containing medium

Numbers of Samples	Parameters of Carbon Source	Pen-V IU/ml	POAA mg/ml	pH	PMV (%)
PM1	Lactose 100%(Control)	35,388	0.05	6.06	35
PM8	Lactose 50% + Glucose 50%	27,532	0.05	6.52	35
PM9	Lactose 50% +Sucrose 50%	29,471	0.03	6.71	39
PM10	Lactose 50% +P. Starch 50%	36,616	0.05	6.00	39
PM11	Lactose 50% +Glycerol 50%	33,060	0.08	5.92	33
PM12	Lactose 50% +Dextrin 50%	42,794	0.10	6.20	35
PM13	Lactose 50% +Maltose 50%	41,277	0.03	6.12	36

The activity of pen-V and the residual POAA of 75% tested carbon source with lactose are given in the table 3. The increased yield of penicillin was obtained when 75% of Maltose, Dextrin and Potato. Starch was used with lactose in medium. It's had higher yield such as respectively 34625 IU/ml, 34014 IU/ml, 32831 IU/ml, and residual POAA as 0.05mg/ml, 0.03mg/ml and 0.075mg/ml. The decreased yield was obtained when 75% of Glucose, Sucrose, and Glycerol were used with lactose. It's had lowest yield such as respectively, 5459 IU/ml, 4721 IU/ml, 1714 IU/ml and residual POAA as 4.65mg/ml, 4.32mg/ml and 1.55mg/ml.

Table: 3. The parametrs and HPLC result of 75% other carbon sources with lactose containing medium

Numbers of Samples	Parameters of Carbon Source	Pen-V IU/ml	POAA mg/ml	pH	PMV (%)
PM1	Lactose 100%(Control)	35,388	0.05	6.06	35
PM14	Lactose 25% + Glucose 75%	5,459	5.00	5.21	40
PM15	Lactose 25% +Sucrose 75%	4,721	4.65	5.43	41
PM16	Lactose 25%+P.Starch 75%	37,831	0.07	7.32	37
PM17	Lactose 25% +Glycerol 75%	1,714	1.55	7.80	44
PM18	Lactose 25% +Dextrin 75%	41,041	0.03	6.66	35
PM19	Lactose 25% +Maltose 75%	40,625	0.03	6.72	30

The activity of pen-V and the residual POAA of 100% tested carbon source with lactose are given in the table 4. The increased yield was obtained when 100% of Potato Starch, Maltose and Dextrin were used to completely replace the lactose. Because it is exhausted at slow rate by *P. chrysogenum*. They had higher yield such as respectively 33518, 30960, 31101 and residual POAA as 0.08mg/ml, 0.08mg/ml, and 0.03mg/ml. As described by Minoda, Y. (1986), starch is used as major carbon source for Glutamic acid production. They may be used as satisfactory carbon source in penicillin fermentation.

Table: 4. The parametrs and HPLC result of 100% others carbon source without lactose containing medium

Numbers of Samples	Parameters of Carbon Source	Pen-V IU/ml	POAA mg/ml	pH	PMV (%)
PM1	Lactose 100% (Control)	35,388	0.05	6.06	35
PM20	Glucose 100%	3,702	6.02	6.73	40
PM21	Sucrose 100%	4,848	7.03	4.03	43
PM22	P.Starch 100%	37,364	0.03	6.92	35
PM23	Glycerol 100%	8,704	2.90	8.20	40
PM24	Dextrin 100%	38,944	0.08	6.71	30
PM25	Maltose 100%	36,219	0.08	7.23	35

The decreased yield was obtained when 100% of Glucose, Sucrose, and Glycerol were used to completely replace the lactose in medium. They had lowest yield such as respectively 4702, 4848, 4904 and residual POAA as 2.90mg/ml, 3.03mg/ml and 2.17mg/ml. Because they are exhausted at rapid rate by *P. chrysogenum*. All the harvested broth had pH between 4-8. 100% sucrose had lowest pH as 4.6 and 1005 glycerol had higher pH as 8.2. Johnson (1953) were found previously considerably

higher Penicillin yield than lactose control, could be obtained in a synthetic medium when glucose or sucrose are continuously added to fermentation. (Stanbury 1997).

The glucose, glycerol and sucrose served as one of the carbon source in fermentation processes. It was exhausted at rapid rate by *P. chrysogenum*. Therefore which support a very rapid rate of fungal growth. Obviously glucose, glycerol and sucrose had been used to completely replace the lactose in medium then the growth phase would have continued until all the glucose was exhausted. Such a medium would have given a very low yield of penicillin. Soltero, F.V and Johnson, MJ (2007), were found previously considerably higher penicillin yield than lactose, could be obtained in a synthetic medium when glucose or sucrose are continuously added to fermentation.

Starch when heated in the sterilization processes, it gelatinizes, giving rise to very viscous liquids. But only concentration of starch up to 2% can be used as carbon source in an antibiotic fermentation. (Stanbury, 1997). Obviously Potato starch, Dextrin and Maltose had been used to completely replace the lactose in medium that provide the higher yield of penicillin than control. Therefore Potato starch, Dextrin and Maltose can be used to completely replace the lactose in medium for penicillin fermentation.

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