ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study on the Optimal Fermentation Condition of *Paenibacillus kribbensis* CX-7 Strain

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Manuscript Info

Manuscript History:

Key words:

Paenibacillus, signal factor experiment, orthogonal test, the optimal culture condition

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Abstract

Mineral potassium is the main source of potassium absorbed by plants. *Paenibacillus*strain has the function of solubilization soil minerals and releasing soluble potassium. In order to utilize the soil microorganismsfurther, the optimal condition of *Paenibacillus kribbensis* CX-7 strain screened from the wheat soil of Shanxi of China has been studied. Through signal factor experiment and orthogonal test, the best medium ratio has been get, which is 5% starch, 1.5% soybean powder, 0.07% $MgSO_4$, 0.05% NaCl and 0.05% yeast extract. The optimal culture condition is seed age 20 h, medium volume 30mL/250mL, seed value 4%. Theoretical bases has been provided for the industrial manufacturing and agricultural application of CX-7 strain.

Introduction

Potassium absorbed from the soil by cereal crops is belong to non-exchange potassium and it is mainly come from potassium minerals, so the potassium minerals in the soil are the main source to supply potassium (Bao SH, et al. 1982, Xie JC, 1985). For, the majority minerals in the soil can not dissolved in the water and the plant can not absorb directly, so how to develop and utilize the minerals in the soil is the key project for many researchers.

Paenibacillus strains have the effect of activating the insoluble potassium mineral and releasing soluble potassium (He JQ et al.1999, Alexandra B. T. 1955) which can be absorbed by crops directly. At the same time, Al, Si, Mn, Zn etc. Elements can be released (Li YM ea al. 2007, Li YF, 1994). So it can be used for bio-fertilizer producing, which has the ability of decreasing the dosage of chemical potassium and phosphate fertilizer (Zhang AM et al. 2003), improving the quality of crops and increasing the yield of crops(Ash C et al. 1993, Ross N et al. 2001, Gong GS et al. 2009).

Separation, screening, identification and practical applications of *Paenibacillus* strains were more reported in domestic and overseas literature (Chen HK 1981, Lin QM et al. 2000, Zahra MK et al. 984). But study on the fermentation technology and culture condition were seldom reported. But in the developing and practical application fields, fermentation of CX-7 strain level not only affect the cost of production, but also affect the effect of applying (Yin YX et al. 1985, Jiang XJ et al. 2000). It is worthy to be studied.

By single factor test and orthogonal test, the optimal culture and culture condition of spore forming of *Paenibacillus kribbensis* CX-7 strain has been studied (Zhang AM et al. 2013, Lian B 1998). Theoretical basis has been get for its practical application.

Material and Methods

Fermentation of *Paenibacillus kribbensis* CX-7 strain

Slant culture: The preserved CX-7 strain was inoculated to the slant culture(beef extract 5.0 g, peptone 5.0 g, NaCl 5.0 g, distilled water 1000 mL) and cultivated for 36-48h in 30℃condition.

Seed liquid culture: CX-7 slant seed was inoculated to 250ml flask containing 50mL seed medium (starch 5.0 g, sucrose 2.0 g, yeast extract 0.5 g, K_2HPO_4 2.0 g, $(NH_4)_2SO_4$ 0.8 g, $MgSO_4$ 0.5 g, distilled water 1000 mL) and cultivated for 15h in the condition of temperature 30℃ and shaking table rotating speed180r/min.
Fermentation: Seed liquid was inoculated to 500mL flask containing 50mL fermentation culture (yeast extract 0.7 g, peptone 1.0 g, starch 2.0 g, K_2HPO_4 1.0 g, $(NH_4)_2SO_4$ 0.2 g, $MgSO_4$ 0.2 g, distilled water1000 mL) and cultivated in the different condition.

The growth curve of CX-7 strain
CX-7 slant seed was inoculated to 50mL fermentation medium and cultivated in the condition of shaking table rotating speed180 r/min and temperature 30℃. The growth curve was drawn with culture time as abscissa against OD (610 nm) as ordinate. The fermentation period can be determined simultaneously.

Measurement of biomass and spore forming rate of CX-7 strain
Biomass of CX -7 strain was determine by hemocytometer and spore forming rate was counted by microscope observing. 3 times repeat were done and the spore forming rate was express as average value (x)± standard deviation(s).

Single factor experiment of CX-7 strain
Different carbon source, nitrogen source and inorganic salts were substitute for that (starch, peptone, $MgSO_4$ et al.) in the fermentation medium (yeast extract 0.7 g, peptone1.0 g, starch 2.0 g, K_2HPO_4 1.0 g, $(NH_4)_2SO_4$ 0.2 g, $MgSO_4$ 0.2 g, distilled water1000 mL) and spore forming rate were measured to determine the optimal carbon source, nitrogen source and inorganic salts in the media.

Orthogonal experiment of CX-7 strain
In the basis of the results of single factor experiment, different level of carbon source, nitrogen source and inorganic salts was designed according to orthogonal table $L_9(3^4)$. The best optimal media was determined by measuring spore forming rate in the experiment.
The detail experiment conditions were: the culture time 72 h, seed value 6% , 50 ml in 500 mL total volume, the temperature of the shaking table 30℃, rotating speed 180 r/min.

Determining of the optimal culture conditions of CX -7 strain
In the basis of the results of the optimal medium experiment, the optimal culture conditions were determined by orthogonal experiment. Seed age was designed in 3 level as 16 h, 20 h and 24 h. Medium volume in the 500 mL flask were 50 ml, 100 mL and 125 mL separately. Initial pH were 6, 7 and 8 respectively. Please see table 1.

Tab.1 Orthogonal test of culture conditions

Test NO.	Seed age/h	Medium volume/mL	Seed value/%	pH
1	16	50	6	6
2	16	100	8	7
3	16	125	10	8
4	20	50	8	8
5	20	100	10	6
6	20	125	6	7
7	24	50	10	7
8	24	100	6	8
9	24	125	8	6

Results and Discussion

The growth curve of CX-7 strain
CX-7 slant seed was inoculated to 500mL flask containing 50mL fermentation medium and cultivated in the condition of shaking table rotating speed180r/min and temperature 30℃. The growth curve was drawn with culture time as abscissa against OD as ordinate. Please see Fig. 1.
From Fig. 1 we can see that delay period is 0-6h, logarithmic growth phase is 6-16h,
Stable period is 18h later in the growth process of CX -7 strain. In the logarithmic growth phase, the bacillus grow rapidly, the size of cell is more consistent, metabolism is vigorous.

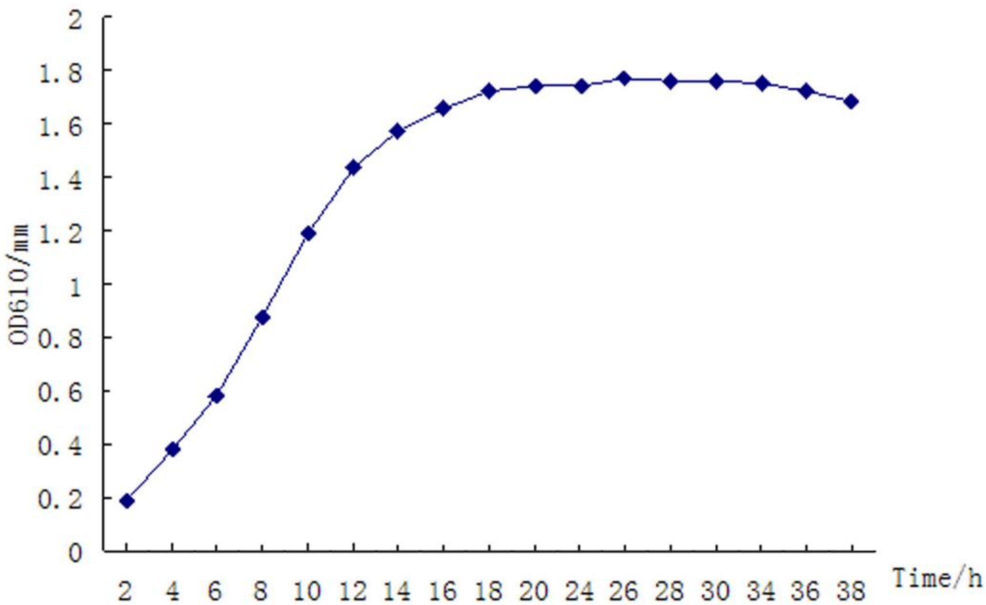

Fig.1 The growth curve of strain CX-7

Screening of carbon source of CX-7 strain

CX-7 strain sporulation rate experiment were done using glucose, sucrose, maltose, soluble starch, wheat bran, corn flour and dextrin as carbon source. The results can be seen in the Fig. 2.

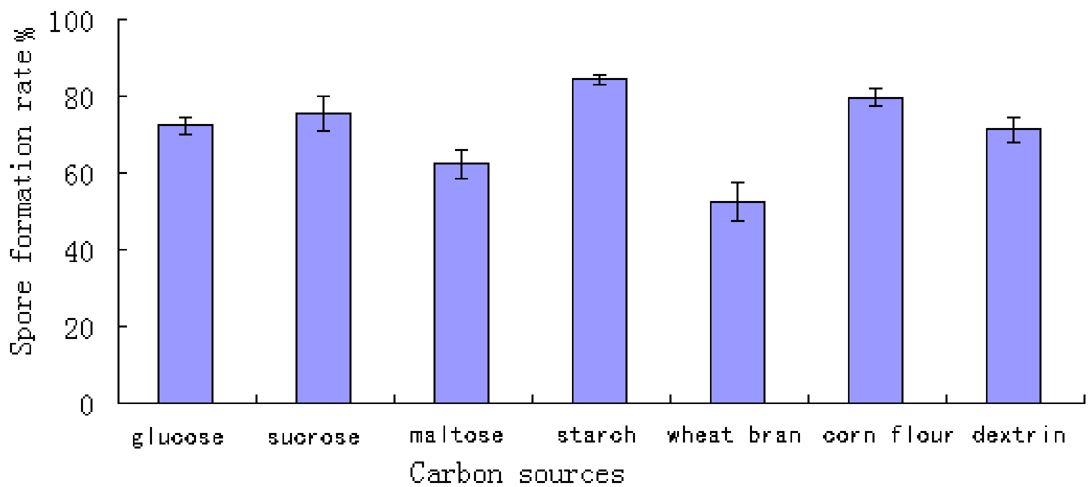

Fig.2 Effect of different carbon sources on spore formation rate of CX-7 strains

Screening of nitrogen source of CX-7 strain

Sporulation rate experiments of CX-7 strain were done using peptone, tryptone, casein, yeast extract, soybean powder, urea and $(NH_4)_2SO_4$ as nitrogen source. The results can be seen in the Fig. 3.

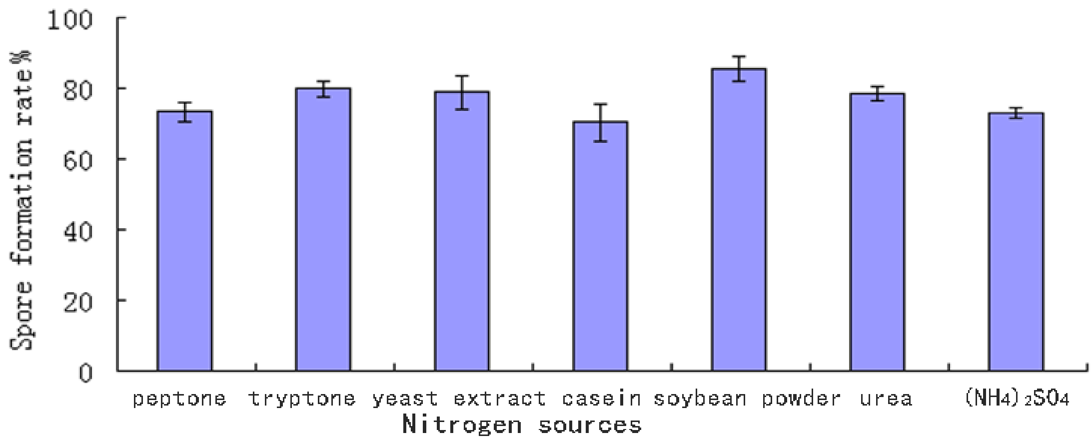

Fig.3 Effect of different nitrogen sources on spore formation rate of CX-7 strains

Screening of inorganic salts

Sporulation rate experiments of CX-7 strain were done using $MgSO_4$, $CaSO_4$, $FeCL_3$, $ZnSO_4$, $MoSO_4$, NaCl, $MnSO_4$ and $(NH_4)_2SO_4$ as inorganic salts. Please see Fig. 4.

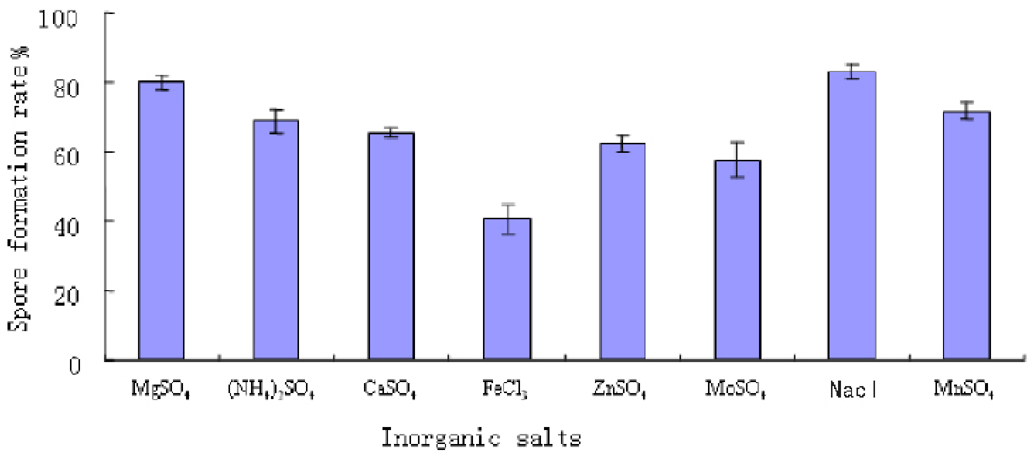

Fig.4 Effect of different inorganic salts on spore formation rate of CX-7 strains

In Fig.4 we can see that spore forming rate is 80% and 93% using $MgSO_4$ and NaCl as inorganic salts in the medium. It is more than that of others as inorganic salts.

In addition, we can see that the spore forming rate will be descend if there is no K_2HPO_4 in the medium in the experiments. Thus, phosphate is the essential inorganic salts in the process of spore forming of CX-7 strain.

The best carbon source, nitrogen source and inorganic salts of CX-7 strain were determined by signal factor experiments, which were starch, soybean powder, NaCl, MgSO4 and K_2HPO_4 separately.

Determination of optimal medium of CX-7 strain

In the basis of the results of signal factor experiments, different level carbon source, nitrogen source and inorganic salts were designed using orthogonal table $L_9(3^4)$. Starch (carbon source) was designed in 3 level, which was 2%, 5% and 7%. Soybean powder (nitrogen source) was designed in 3 level, which was 0.5%, 0.7% and 1.5%. $MgSO_4$ and NaCl (inorganic source) wa s designed in 3 level, which was 0.02%, 0.05% and 0.07%.

The sporulation rate of CX-7 strain can be reach by three times repeated in each treatment. The results can be seen in the table 2 and table 3.

Tab.2 Orthogonal test table $L_9(3^4)$

NO.	Starch (A)	Soybean (B)	$MgSO_4$(C)	NaCl(D)
1	2%	0.5%	0.02 %	0.02 %
2	2 %	0.7 %	0.05 %	0.05 %
3	2 %	1.5%	0.07 %	0.07 %

4	5 %	0.5 %	0.02 %	0.02 %
5	5 %	0.7 %	0.05 %	0.05 %
6	5 %	1.5%	0.07 %	0.07 %
7	7 %	1.0 %	0.02 %	0.02 %
8	7 %	2.0 %	0.05 %	0.05 %
	7 %	5.0 %	0.07 %	0.07 %

Tab. 3 Orthogonal test results of different medium ratio

Test No.	Carbon 'ces	Nitrogen source	$MgSO_4$	NaCl	Spore forming rate
1	2	0.5	0.02	0.02	82.51±0.33
2	2	0.7	0.05	0.05	77.52±0.83
3	2	1.5	0.07	0.07	89.86±0.95
4	5	0.5	0.05	0.07	76.67±5.42
5	5	0.7	0.07	0.02	86.48±0.15
6	5	1.5	0.02	0.05	94.13±0.19
7	7	0.5	0.07	0.05	85.90±1.98
8	7	0.7	0.02	0.07	62.85±0.18
9	7	1.5	0.05	0.02	79.73±2.37
K1	83.3	79.83	79.74	82.91	
K2	85.76	83.3	77.97	85.85	
K3	76.16	87.91	87.41	76.46	
Range R	9.6	8.08	9.44	9.39	
Excellent combination	A2	B3	C3	D2	

Note: K stands for average. R stands for range.

From table 3, we can see that effect of spore forming rate is significant using different concentration carbon source, nitrogen source and inorganic salts in the medium. The range of different factor were $R_A>R_B>R_C>R_D$. The best combination is $A_2B_3C_3D_2$ through biostatics of orthogonal test results. Thus, the optimal medium ratio is 5% starch, 1.5% soybean powder, 0.07% $MgSO_4$ and 0.05% NaCl.

Determination of the optimal culture condition

According to the orthogonal test table1, spore forming rate can be get in the different culture condition using the optimal medium(5% starch, 1.5% soybean powder, 0.07% $MgSO_4$ and 0.05% NaCl) in the experiment. The results can be seen in the table 4.

From table 4, we can see that the effect of spore forming rate of different factor is A>B>C>D. Seed age is significant factor and medium volume is the second factor of four factor. So we can draw a conclusion that there is relationship between spore forming rate and seed age and dissolved oxygen. The optimal culture condition of CX-7 strain is seed age 20h, medium volume 30mL/250mL, seed value 4%. The spore forming rate can be reach 97.78% in the above culture condition.

Tab. 4 Orthogonal test results of different culture condition

Test no.	Seed age (A)/h	Medium volume(B)/mL	Seed value(C)/%	pH (D)	Spore forming rate/%
1	16	30	4	6	91.60±0.34
2	16	50	6	7	86.04±0.63
3	16	100	8	8	91.49±0.37
4	18	30	8	7	92.87±0.35
5	18	50	4	8	79.81±0.30
6	18	100	6	6	92.65±0.11
7	20	30	6	8	98.31±0.22
8	20	50	8	6	95.22±0.18

9	20	100	4	7	96.64±0.17
K1	89.93	94.22	93.29	89.46	
K2	88.26	87.08	87.66	92.51	
K3	96.65	93.56	89.57	92.89	
Range R	8.39	7.14	5.62	3.43	
Excellent Combination	A3	B1	C1	D3	

Conclusion

Through the preliminary studies on the carbon source, nitrogen source, inorganic salts and culture condition of CX-7 strain by signal experiment and orthogonal test showed that the optimal medium ratio of the spore forming rate is 5% starch, 1.5% soybean powder, 0.07% $MgSO_4$, 0.05% NaCl and 0.05% yeast extract. The optimal culture condition is seed age 20 h, medium volume 30mL/250mL, seed value 4%.

The foundation has been provided for the industrial production of CX-7 strain. The development of CX-7 strain in the agriculture can be increased in the basis of the results of this paper.

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