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Journal of Chemical and Pharmaceutical Research, 2016, 8(8):491-494

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Hyaluronan production in *Streptococcus thermophilus*

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ABSTRACT

The strain of hyaluronan-producing bacteria that was Streptococcus thermophilus was used to study the hyaluronan production. We used the Plackett-Burman design of optimum multifactorial experiments to estimate the level effects of different factors such as peptone, meat extract, yeast extract, glucose, tween 80, K_2HPO_4, sodium acetate, ammonium citrate, magnesium sulphate and manganese sulphate on hyaluronan production. As a result, pepton (0.2%), meat extract (0.2%), manganese sulphate (0.0001%), temperature (40^oC), K_2HPO_4 (0.04%), yeast extract (0.1%), glucose (0.4%), under which the hyaluronan production in Streptococcus thermophilus was obtained. The predicted maximum yied of hyaluronan in Streptococcus thermophilus was 86 mg/l.

Keywords: hyaluronan, Plackett-Burman, *Streptococcus thermophilus*

INTRODUCTION

Hyaluronan (HA) is a commercially valuable medical biopolymer increasingly produced through microbial fermentation [1]. HA has been used in wound healing, and analgesic effects. It is also being used as a vehicle for drug delivery. HA is produced through the extraction from rooster comb or fermentation of group C *Streptococcus* that synthesizes HA as part of their outer capsule. In rooster comb, HA is complexed with proteoglycans, making its isolation tediously and costly. However, HA produced from pathogenic *Streptococci*, may have the potential to be contaminated with pathogen factors [2]. Up to now, there was the study on HA in *Streptococcus thermophilus* showing the highest safety [3]. Although HA biosynthesis was known 40 years ago, the amount of HA is still too small. HA produced from microorganisms was too little. There for more approaches on non-pathogenic bacteria have been studied. It has been reported that S*treptococcus thermophilus* that produced useful exopolysaccharides (EPSs) including HA from traditional dairy food products. But the quantitative is low and each study can only apply in the country of this study. *Lactococcus lactis* have been studied to produces hyaluronic acid production by recombinant technology [4].

Optimization of the hyaluronan production from microorganisms to improve the yield is necessary. The optimization of the hyaluronan production based on Plackett-Burman matrix [5] in hyaluronan-producing *Streptococcus thermophilus* was not focused. The optimization of the fermentation process to get the high yield and large scale are very ideal in the industry of hyaluronan production. Plackett-Burman matrix was used to design the medium that gave a low cost and could explain the interactions and estimation of many factors affecting on HA production.

In the study, we optimized the factors based on Placket-Burman design and the experiments were done to determine the hyaluronan yield by *Streptococcus thermophilus*.

EXPERIMENTAL SECTION

Bacterial strains, plasmids, and growth conditions

Streptococcus thermophilus MR10 isolated from yorgurts [6]. The nutrients as peptone, glucose, yeast extract, tween 80, K_2HPO_4, sodium acetate, ammonium citrate, magnesium sulphate, manganese sulphate, carbazole were from Merck. The nutrients were prepared in the stocks for optimization according to these concentrations as glucose 6%, yeast extract 1.5%, K_2HPO_4 1.5%, meat extract 1.5%, peptone 1.5%, MnSO4 0.1%, MgSO4 0.1%, tween 80 0.5%, amonium citrate 0.5%, acetate sodium 0.5%

Optimization of HA production

To maximize the production of hyaluronan, the MRS medium was selected as the basal medium. The Plackett-Burman strategy [5] was implemented along with the polynomial function to analyze the relationship between each ingredient with the HA productivity. There were 11 factors used in 15 experiments (Table 1 and Table 2).

Table 1: The income variables and theirs levels for the Placket – Burman DOE

Income Variables	Symbols	Code levels		
		-1	0	+1
Peptone	A	0 g/l	10 g/l	20 g/l
Meat	B	0 g/l	10 g/l	20 g/l
Yeast	C	0 g/l	5 g/l	10 g/l
Glucose	D	0 g/l	20 g/l	40 g/l
Tween 80	E	0 g/l	1 ml/l	2 ml/l
K_2HPO_4	F	0 g/l	2 g/l	4 g/l
CH_3COONa	G	0 g/l	5 g/l	10 g/l
$(NH4)_2C_6H_6O_7$	H	0 g/l	2 g/l	4 g/l
$MgSO_4$	J	0 mg/l	200 mg/l	400 mg/l
$MnSO_4$	K	0 mg/l	50 mg/l	100 mg/l
Temperature	L	34^{o}C	37^{o}C	40^{o}C

Table 2: The Plackett-Burman design and the experimental response values

Experiments	A	B	C	D	E	F	G	H	J	K	L
1	-1	1	1	1	-1	-1	-1	1	-1	1	1
2	-1	-1	-1	1	-1	1	1	-1	1	1	1
3	1	1	-1	1	1	1	-1	-1	-1	1	-1
4	-1	-1	1	-1	1	1	-1	1	1	1	-1
5	0	0	0	0	0	0	0	0	0	0	0
6	1	-1	1	1	-1	1	1	1	-1	-1	-1
7	-1	1	-1	1	1	-1	1	1	1	-1	-1
8	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
9	1	-1	1	1	1	-1	-1	-1	1	-1	1
10	0	0	0	0	0	0	0	0	0	0	0
11	-1	1	1	-1	1	1	1	-1	-1	-1	1
12	1	1	-1	-1	-1	1	-1	1	1	-1	1
13	1	-1	-1	-1	1	-1	1	1	-1	1	1
14	0	0	0	0	0	0	0	0	0	0	0
15	1	1	1	-1	-1	-1	1	-1	1	1	-1

Uronic acid measurement

The HA amount produced was determined according to the uronic acid content by reaction with carbazole based on the British Pharmacopeia 2007, monograph 1472. The color changes into purple red that indicates the positive result of uronic acid.

RESULTS AND DISCUSSION

Optimization of HA concentration produced from *Streptococcus thermophilus*

The impact level of 11 factors of the culture medium toward the amount of hyaluronan produced from *S. thermophilus* was depicted in table 3.

Table 3: Factors of the culture medium and their impacts

Factor		Concentration -1	Concentration 0	Concentration +1	Impact	p-value
Peptone	A	0 g/l	10 g/l	20 g/l	34.02	<0.0001
Meat	B	0 g/l	10 g/l	20 g/l	25.11	<0.0001
Yeast	C	0 g/l	5 g/l	10 g/l	2.91	0.0003
Glucose	D	0 g/l	20 g/l	40 g/l	1.75	0.0007
Tween 80	E	0 g/l	1 ml/l	2 ml/l	-9.02	<0.0001
K2HPO4	F	0 g/l	2 g/l	4 g/l	2.93	0.0003
CH_3COONa	G	0 g/l	5 g/l	10 g/l	-8.64	<0.0001
$(NH4)_2C_6H_6O_7$	H	0 g/l	2 g/l	4 g/l	-0.78	0.0035
MgSO4	J	0 mg/l	200 mg/l	400 mg/l	-3.34	0.0002
MnSO4	K	0 mg/l	50 mg/l	100 mg/l	7.29	<0.0001
Temperature	L	34°C	37°C	40°C	6.74	<0.0001

While factor with negative impact cause the decreasing in the amount of hyaluronan produced from *S. thermophilus* and positive impact cause the increasing in the amount of hyaluronan produced from *S. thermophilus*. Based on table 3, we concluded that peptone had strongest impact on the the amount of hyaluronan yield produced from *S. thermophilus*. The actual and predicted hyaluronan yield (µg/ml) by *S. thermophilus* was shown in table 4 and analyzed as in figure1.

Table 4: The actual and predicted hyaluronan yield (µg/ml) by *S. thermophiles*

Experiments	actual value	predicted value
1	86.075	86.1
2	13.475	13.5
3	71.65	71.7
4	0	0
5	6.575	6.59
6	11.85	11.81
7	10.175	10.15
8	0	0
9	13.625	13.64
10	6.275	6.29
11	40.075	40.1
12	76.85	76.9
13	6.7	6.9
14	6.525	6.54
15	62.2	62.1

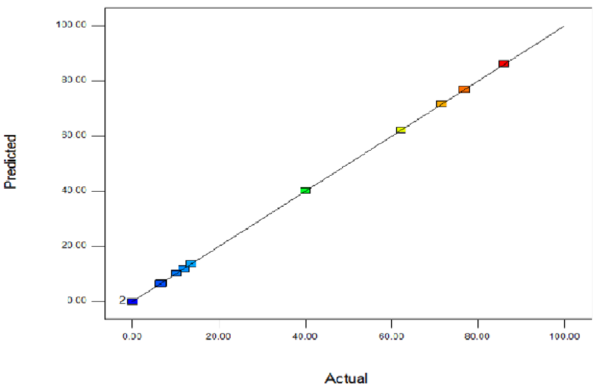

Figure 1: Comparisons between predicted values and actual values in hyaluronan production by *Streptococcus thermophilus*

As expressed in the above figure, the actual values (allocated at the horizontal axis) and the predicted value (allocated at the vertical axis) were match together, proved that the established model is valid within the selected range (from -1 level to +1 level). On the other hand, predicted and actual R^2 calculated for the equation were similar to each other. The effect of each factor was expressed from highest to lowest order as peptone, meat extract, manganese sulphate, temperature, di potassium hydrogen phosphate, yeast extract, glucose (table 4). The highest yield of hyaluronan produced by *Streptococcus thermophilus* was 86 mg/l under peptone (0.2%) condition.

CONCLUSION

Based on the Plackett-Burman design and optimium experiments, we calculated that pepton (0.2%), meat extract

(0.2%), manganese sulphate (0.0001%), temperature (40^{o}C), K_2HPO_4 (0.04%), yeast extract (0.1%), glucose (0.4%), under which the hyaluronan production in *Streptococcus thermophilus* was obtained. The maximum yield of hyaluronan in *S. thermophilus* was 86 mg/l.

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