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Regular Article

Optimization of process parameters of cyclodextrin glycosyltransferase isolated from novel mutated *Bacillus* sp. TPR71HNA6 by Taguchi orthogonal array method

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Cyclodextrin glycosyl transferase CGTase) production by B*acillus* SP. TPR71HNA6 under shake flask culture was optimized following Taguchi orthogonal array (OA) design of experiment. An OA layout of L16 was constructed with five most influensive factors on CGTase like pH, initial temperature, agitation speed, time of incubation and inoculum concentration at three levels for the proposed experimental design. According to the experimental plan (L-16) the experiments were conducted and the data revealed significant variation in the enzyme production. Minimum and maximum CGTase production values were observed to be 19 and 89 U/mL under the above fermentation conditions. The observed variation certainly indicates the imperative role of optimization of all fermentation-related factors in achieving the best possible yields. With the help of statistical tool by optimizing the process variables without altering the nutritional compounds and their concentration an overall 39% of enzyme yield was improved.

Keywords: CGTase, Taguchi method, Optimisation, *Bacillus*

Cyclodextrin glycosyltransferase (CGTase or 1, 4 - α-D-glucopyranosyl transferase) is an extracellular enzyme, which degrades starches into cyclodextrin (CDs) molecules via cyclisation reaction. Cyclisation happens when a linear oligosaccharide (starch) chain is cleaved and the new reducing end sugar is transferred to the non-reducing end sugar of the same chain. Therefore cyclodextrins are cyclic oligosaccharides consisting of 6-12 units of glucose joined by the α-1, 4-linkages. CGTases also catalyse two intermolecular transglycosylation reactions: coupling, in which a cyclodextrin ring is cleaved and transferred to an acceptor maltooligosaccharide substrate and disproportionation, in which a linear malto-

oligosaccharide is cleaved and the new reducing end sugar is transferred to an acceptor maltooligosaccharide substrate. Besides these reactions, the enzyme has a weak hydrolysing activity (Bart et al., 2000). Cyclodextrins with 6, 7 and 8 glucose units are most common and also known as α -, β- and γ- cyclodextrin, respectively.

CGTases with varying properties are produced by bacteria mainly belonging to the *bacillus* genus, by submerged culture in a complex medium (Bart et al., 2000). Some of the known sources of CGTase producers are *Bacillus macerans*, *Bacillus subtilis*, *Bacillus steaothermophillus*, *Bacillus megaterium*, *Klebsiella pneumonia* and *Micrococcus* species. Alkalophilic micro-organism is also known to produce unusual enzyme that can be used in industrial and other processes. All the three known CGTases (Bart et al., 2000) produce a mixture of cyclodextrins (and linear malto-oligosaccharides) when incubated with starch. However a CGTase, which only produces a single type of cyclodextrin, is industrially favourable. Based on the industrial usage of the cyclodextrin a β-cyclodextrin producing CGTase was isolated from the mutated *Bacillus* sp. TPR71HNA6 and preliminary studies were conducted. The nutrient parameters were screened based on the Plackett-Burman design. In the present study the various environmental parameters were optimised based on the Taguchi experimental design.

Success in experiments and/or technology mainly depends on a properly designed process or product. The Taguchi method of design of experiments is a simple statistical tool involving a system of tabulated designs (arrays) that allows a maximum number of main effects to be estimated in an unbiased (orthogonal) fashion with a minimum number of experimental runs. It has been applied to predict the significant contribution of the design variable(s) and the optimum combination of each variable by conducting experiments on a real-time basis. The modeling that is performed essentially relates signal-to-noise ratio to the control variables in a 'main effect only' approach. This approach enables both multiple response and dynamic problems to be studied by handling noise factors. Taguchi principles and concepts have made extensive contributions to industry by bringing focused awareness to robustness, noise and quality. This methodology has been widely applied in many industrial sectors; however, its application in biological sciences has been limited (Sreenivas Rao et al., 2008; Prakasham et al., 2010). To the best of our knowledge this is first report on the CGTase production optimization by using the Taguchi experimental design.

Characterization and optimization for each factor of growth, nutritional requirement, and production yields are essential requirements before the selected strain is used for further investigation (Satish and Prakasham, 2010). The yield improvement of CGTase, in general, by any microbial system depends on the physiological, nutritional, and biochemical nature of the microbe employed, and these factors vary from organism to organism. Keeping this in view, preliminary investigations were made to understand various requirements for CGTase production by mutated *Bacillus* sp. TPR71H NA6.

MATERIALS AND METHODS

Microorganism and Culture Conditions

In the present study a mutated *Bacillus* sp. TPR71HNA6 (Gen Bank No: FN993946) was used. This culture was stored in a nutrient agar slants and subcultured periodically once a week.

Estimation of CGTase activity

Enzyme activity was measured by decrease of phenolphthalein colour intensity. Enzyme assay was carried out according to the Kaneko et al., 1987 method. The reaction mixture containing 1mL of 40mg of soluble starch in 0.1M potassium phosphate buffer (pH 6.0) and 0.1mL of the crude enzyme from the culture was incubated in water bath at 60°C for

10min. The reaction was stopped with 3.5mL of 30mM NaOH. Finally, 0.5mL of 0.02% (w/v) phenolphthalein in 5mM Na_2CO_3 was added and mixed well. After leaving the mixture to stand for 15min at room temperature, the reduction in colour intensity was measured at 550nm. A blank lacking the enzyme is tested simultaneously with each batch of samples. One unit of enzyme activity was defined as the amount of enzyme that forms 1µgm of β-CD from soluble starch in 1min.

Optimization by using Taguchi method

Taguchi methodology for optimization can be divided into four phases, viz. planning, conducting, analysis and validation. Each phase has a separate objective and contributes towards the overall optimization process. Taguchi methodology for optimization has been represented in the form of a flowchart as shown in Fig 1.

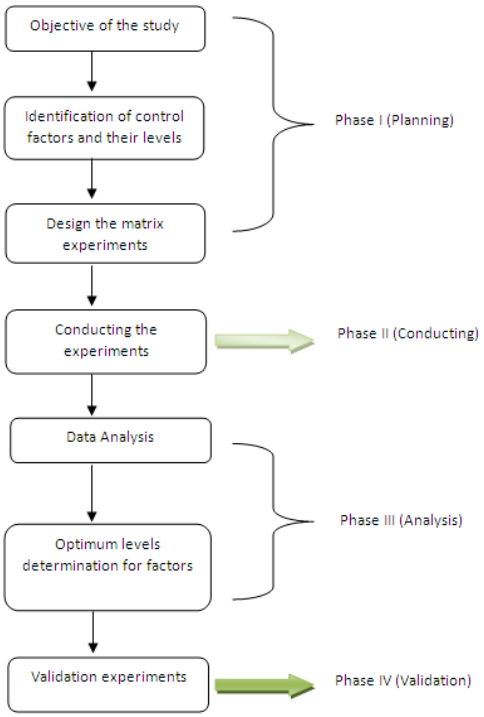

Fig 1. Experimental protocol of DOE for CGTase production by mutated *Bacillus* sp. TPR71H NA6

Table 1. Selected Bioprocess parameters and their assigned Levels

	Variables		Levels			
S. No	**Real**	**Coded**	**1**	**2**	**3**	**4**
1	pH	X1	6	7	7.5	9
2	Temperature (°C)	X2	25	30	33	37
3	Agitation speed (rpm)	X3	200	220	240	260
4	Incubation time (hrs)	X4	24	36	48	60
5	Inoculum size (mL)	X5	1	3	5	7

Phase I—Planning

Phase 1 is the first step, this determines the various factors to be optimized in the culture conditions that have critical effect on the CGTase yield. Based on the preliminary studies, five fermentation factors viz pH, initial temperature, agitation speed, time of incubation and inoculum concentration which are having significant influence on the enzyme yield, were selected for the present study. A Taguchi experimental design was employed to optimize these factors. In the present study a 16 experimental orthogonal array (OA) was used. All five factors were chosen at four levels. Table 1 depict the selected process parameters and their levels and Table 2 depicts the L16 Taguchi experimental plan. In the design each column consisted of a number of conditions depending on the levels assigned to each factor.

Phase II –Conducting the Experiments

Mutated bacterial strain was incubated in 100 mL of medium containing starch 2% (w/v), yeast extract 0.5% (w/v), K_2HPO_4 0.5% (w/v) and $MgSO_4$ 0.04% (w/v). The process parameters such as pH, inoculum size, agitation speed, incubation

temperature and time were varied according to the Taguchi design. After a specific time as per experimentation the flasks were removed from the shakers and the culture was centrifuged at 10,000rpm for 10min to remove the biomass and other insoluble substrates from the culture. After centrifugation the supernatant liquid was collected and estimated for CGTase activity.

Phase III-Analysis of experimental data (AED)

The experimental data obtained was processed using Qualitek-4 software with bigger-is-better quality characteristics for the determination of the optimum culture conditions for the enzyme yield; to identify individual factor influence on the CGTase production; and to estimate the performance (fermentation) at the optimum conditions. In Taguchi's method, quality is measured by the deviation of a characteristic from its target value and a loss function [L(y)] is represented by

$$L(y) = k * (y - m)^2 \text{ ---------- (2)}$$

Where k = the proportionality constant,

m = the target value and

y = the experimental value obtained for each trial.

In case of bigger-is-better quality characteristics, the loss function can be written as

$$L(y) = k^* (1/y^2) \text{ ---------- (3)}$$

And the expected loss function can be represented by

$$E [L(y)] = k * E (1/y^2) \text{ ---- (4)}$$

Where $E (1/y^2)$ can be estimated from a sample of n as

$$\frac{\sum_{i=1}^{n}[1/y_i^2]}{n} \text{ ---------- (5)}$$

Phase IV- Validation of experiment

In order to validate the methodology, fermentation experiments were further performed for CGTase production, using the obtained optimized culture conditions.

Table 2. L-16 Taguchi experimental plan with coded values

S. No	X1	X2	X3	X4	X5
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	2	1	2	3	4
6	2	2	1	4	3
7	2	3	4	1	2
8	2	4	3	2	1
9	3	1	3	4	2
10	3	2	4	3	1
11	3	3	1	2	4
12	3	4	2	1	3
13	4	1	4	2	3
14	4	2	3	1	4
15	4	3	2	4	1
16	4	4	1	3	2

RESULTS AND DISCUSSION

According to the experimental plan (L-16) the experiments were conducted and the data revealed significant variation in the enzyme production (Table 3). Minimum and maximum CGTase production values were observed to be 19 and 89U/mL under the above fermentation conditions. The observed variation certainly indicates the imperative role of optimization of all fermentation-related factors in achieving the best possible yields. This type of variation in enzyme yields has also been noticed in other metabolite optimization investigations (Mahalakshmi et al., 2009).

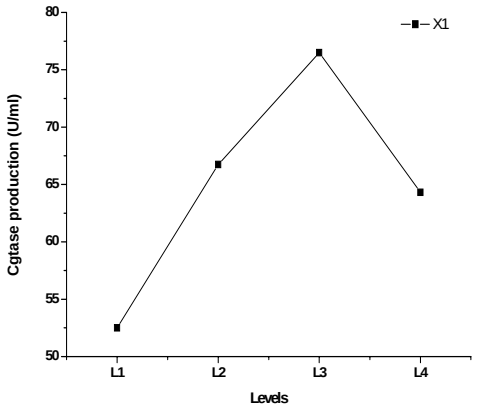

Fig 2. Impact of pH on CGTase production by mutated *Bacillus sp.*TPR71HNA6

The average effect of the factors at the assigned levels on the CGTase production by mutated *Bacillus sp.* TPR71H NA6 was shown in Table 4. At individual level stage the highest effect on enzyme production was noticed with agitation speed at 2nd level (78.25) followed by pH and temperature at 3rd level (76.50), incubation time at 1st level (75.50). Among all, inoculum size at 3rd level (67.25) has highest effect on CGTase production by mutated *Bacillus* sp. TPR71H NA6. (Fig 2-6)

Table 3. Experimental Setup (L-16 Orthogonal Array) along with CGTase produced by the mutated *Bacillus* sp. TPR71H NA6

S. No	X1	X2	X3	X4	X5	CGTase activity
1	6	25	80	24	1	54
2	6	30	90	36	1.5	68
3	6	33	120	48	2	74
4	6	37	150	60	2.5	24
5	7	25	90	48	2.5	19
6	7	30	80	60	2	85
7	7	33	150	24	1.5	45
8	7	37	120	36	1	41
9	7.5	25	120	60	1.5	67
10	7.5	30	150	48	1	89
11	7.5	33	80	36	2.5	76
12	7.5	37	90	24	2	74
13	9	25	150	36	2	73
14	9	30	120	24	2.5	71
15	9	33	90	60	1	46
16	9	37	80	48	1.5	87

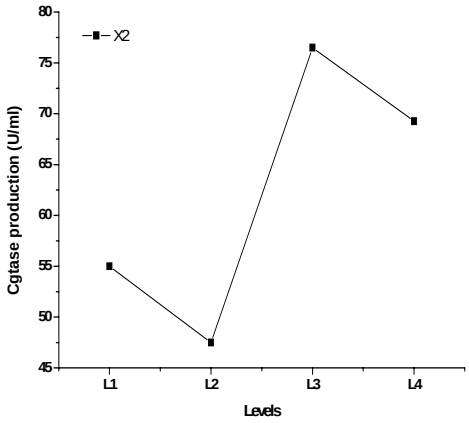

Fig 3. Impact of incubation temperature on CGTase production by mutated *Bacillus* sp.TPR71HNA6

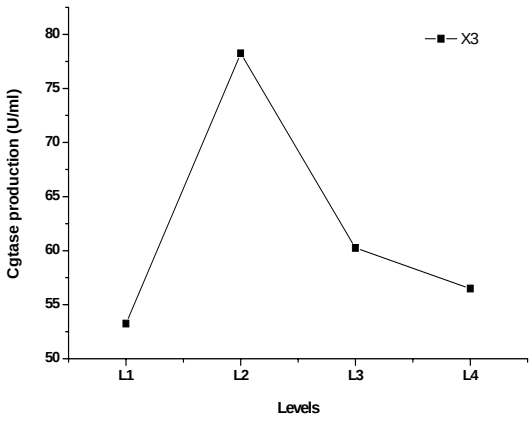

Fig 4. Impact of agitation speed on CGTase production bymutated *Bacillus* sp. TPR71HNA6

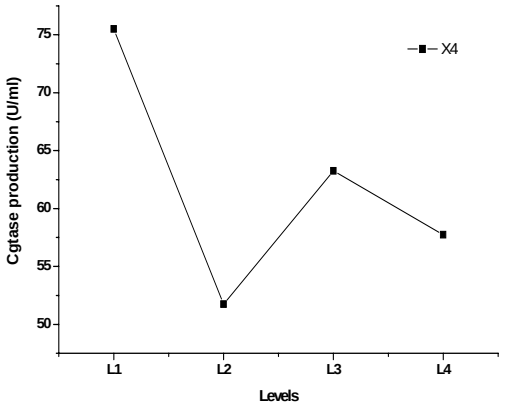

Fig 5. Impact of incubation time on CGTase production by mutated *Bacillus* sp.TPR71HNA6

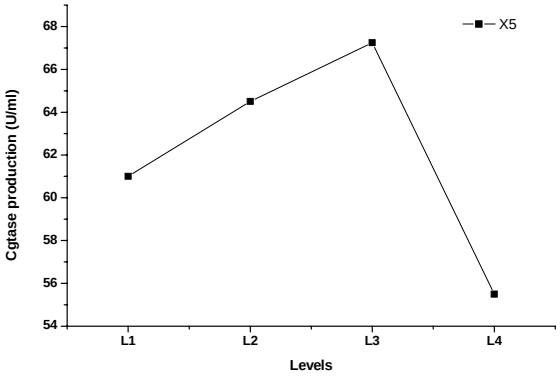

Fig 6. Impact of inoculum size on CGTase production by mutated *Bacillus* sp. TPR71HNA6

Table 4. CGTase production by mutated *Bacillus sp.*TPR71HNA6 at Different Assigned Levels

	Level 1	**Level 2**	**Level 3**	**Level 4**	**L2-L1**
X1	52.5	66.75	76.50	64.32	14.25
X2	55.0	47.50	76.50	69.25	-7.50
X3	53.25	78.25	60.25	56.50	25.00
X4	75.5	51.75	63.25	57.75	-23.75
X5	61.0	64.50	67.25	55.50	3.50

The difference among various levels (L2-L1) of each factor indicates the relative influence of the affect. The larger the difference, the stronger is the influence. Among all selected factors agitation speed shows the stronger influence, followed by incubation time, pH, temperature and inoculum concentration indicating that the mutated microorganisms need a proper aeration for effective CGTase production and the incubation time also plays a vital role in the production of the enzyme (Table 4).

The pH optimum varies with the different *Bacillus* sp. It was observed that pH between 7-10 is suitable for the CGTase production by various Bacillus sp. Alves-Prado et al., 2007 reported that *Bacillus clausii* E16 HR1 produce CGTase at pH 9.9. pH 7.8 was found to be suitable for the CGTase production by *Bacillus* sp.G1 (Ibrahim et al., 2005), *Bacillus firmus* (Gawande and Patkar, 1999) and *Kiebsiella pneumoniae* AS-22 (Gawande et al., 1998). Rosso et al., 2002 observed that pH 8.3 is suitable for the CGTase production from *Bacillus circulans* DF 9R.

The incubation temperature varies with the microbial species. Gawande and Patkar 1999 observed that *Klebsiella pneumonia* AS-22 produce the higher titre of CGTase at 28^{o}C. *Bacillus circulans* DF 9R produce CGTase in wide incubation temperature range of 32-42^{o}C. The maximum activity was observed at 37^{o}C (Rosso et al., 2002). The obtained incubation time results were good in agreement with the literature reports. Many authors reported that *Bacillus* sp produce the maximum CGTase after 48-50 hours (Rosso et al., 2002; Jamuna et al., 1993; Stefanova et al., 1999; Alves-Prado et al., 2007). Rosso et al., 2002 studied the effect of aeration on the CGTase production and the authors suggested that higher aeration is required for the effective CGTase production by *B. circulans* DF 9R.

Interaction influence of various factors on CGTase production

To have better insight of the overall enzyme production process and to understand further the bacterial strain, interaction effects of selected factors were analyzed individually. Any individual factor may interact with any or all of the other factors, creating the possibility of the presence of a large number of interactions. This kind of interaction is possible in the Taguchi DOE. Estimated interaction severity index (SI) of the factors under study helps to determine the influence of two individual factors at various levels of the interactions (Table 5). In Table 5, the "columns" represent the locations to which the interacting factors are assigned and optimum indicates the best suitable level of the factors. Interaction SI presents 100% of SI for 90° angle between the lines, and 0% SI for parallel lines.

Table 5. Interaction influence of selected factors on CGTase production

S. No	Interacting factors pairs	SI	Column	Optimum levels
1	X2*X4	57.14	2	3,4
2	X3*X1	38.99	7	4,2
3	X2*X3	37.14	3	3,2
4	X5*X1	25.96	1	3,2
5	X4*X5	20.00	7	4,3
6	X3*X5	15.71	6	2,3
7	X2*X5	12.85	5	3,3
8	X3*X4	12.85	1	2,4
9	X4*X1	12.38	6	1,2
10	X2*X1	12.28	4	4,2

From Table 5, it is observed that temperature and incubation time (at 3rd and 4th levels) have the highest interaction influence (SI = 57.14%) followed by agitation speed and pH (at 4th and 2nd levels) with 38.99%. From the above data it was noticed that the factors level are varied at individual levels and interaction levels. Agitation speed (X3) showed highest effect at 3rd level in individual level where as it showed highest influence with pH at 4th level. In the interactions the agitation showed the best condition at 4th or 2nd level, interaction at 3rd level was not observed. Similarly incubation time showed highest influence at 1st level where as it showed highest SI with temperature at 4th level. The data indicates that when there is an interaction of the factors it can influence their concentration also. The agitation speed has highest impact at individual level it showed varied interaction SI values ranging from 38.99 to 12.85% with other interaction components such as pH (38.99%) and incubation time (12.85%). The interaction of highest and low influential parameters (agitation speed and inoculum concentration) showed only 15.71% SI. The interaction influence of pH and temperature has the least interaction influence (12.28%). From this data it was observed that the CGTase production was influenced by the various fermentation factors and their concentration at individual and interaction with other factors.

ANOVA

The contribution of individual factors is the key for the control to be enforced on the fermentation process. In the Taguchi approach, analysis of variance (ANOVA) is used to analyze the results of the OA experiment and to determine how much variation each factor has contributed. From the calculated ratios (*F*), it can be determined that all factors and interactions considered in the experimental design are statistically significant at 95% confidence

limit, indicating that the variability of experimental data explained in terms of significant effects. By studying the main effects of each of the factors, the general trends of the influence of the factors toward the process can be characterized. The characteristics can be controlled such that a lower or a higher value in a particular influencing factor produces the preferred result. Thus, the levels of factors to produce the best results can be predicted. ANOVA with the percentage of contribution of each factor with interactions are shown in Table 6.

From table 6 it was observed that incubation temperature has more contribution (30.831 %) on overall fermentation process for effective CGTase production. After temperature, pH (24.408%) has a high effect followed by agitation speed and incubation time on CGTase production by mutated *Bacillus* sp. TPR71HNA6. Inoculum concentration has the least significance (4.55%) on the enzyme production. The observed very low error contribution (0.002%) indicates the accuracy of the experimental data. Figure 7 shows the pie-chart of contribution of all factors along with the error on the CGTase production by mutated *Bacillis* sp. TPR71HNA6.

Table 6. ANOVA Table

S. No	Factors	DOF	SS	V	F-ratio	Pure Sum	Percent contribution
1	X1	2	1653.187	826.593	8265937.5	1653.187	24.408
2	X2	3	2088.187	696.026	6960625	2088.187	30.831
3	X3	3	1495.687	498.562	4985625	1495.687	22.083
4	X4	3	1227.687	409.229	4092291.66	1227.687	18.126
5	X5	3	308.187	102.729	1027291.66	308.187	4.550
	Other/error	1	-0.001	-0.001			0.002
	Total	15	6772.937				100

DOF: Degree of freedom; SS = sum of squares; V= variance

Table 7. Optimum conditions and performance of selected factors on CGTase production by mutated *Bacillus* sp.TPR71HNA6

S. No	Column	Level	Contribution
1	X1	3	14.437
2	X2	2	16.187
3	X3	1	13.437
4	X4	3	5.187
5	X5	3	14.437
Total Contribution from all factors			63.684
Current Grand average of performance			62.062
Expected Result at optimum condition			125.747

Optimum conditions and validation of the experiments

Optimum conditions and their performance in terms of contribution for achieving higher CGTase yield are shown in Table 7. The Taguchi DOE design, used in the present study for the maximizing the production of CGTase from mutated *Bacillus* sp.TPR71HNA6, revealed the effect of interaction of varying factors. Higher levels of CGTase activity can be achieved with the obtained optimization culture conditions: pH at 7.5; incubation temperature 30^{o}C; agitation speed 220rpm; incubation time is 48h and inoculum size 5mL. The expected CGTase yield at these optimum conditions is 125.74U/mL with total contribution from all the factors being 63.684U/mL with grand average performance of 62.062U/mL. Figure 8 shows the optimum performance with major factors contribution of individual factors on CGTase production. From this graph it was observed that incubation temperature and pH have a major role in the synthesis of CGTase by mutated *Bacillus* sp.TPR71HNA6. By conducting the experiments at these conditions 124U/mL of CGTase was obtained. It was noticed that

an overall 39% enhancement in the enzyme production/ yield was achieved. Table 8 depicts the optimum process conditions for the CGTase production by various microorganisms.

The expected improvement on overall CGTase production by this bacterial strain under submerged fermentation could be viewed from figure 9. The validation of experimental data at predicted optimum conditions revealed 35U/mL activity was improved. This experimental data revealed that with help of L-16 taguchi experimentation the CGTase yield was enhanced from 89 to 124U/mL (39% improvement) with the modified culture conditions. This work investigated the cultivation conditions for the fermentation of mutated *Bacillus* sp. TPR71H NA6 in a shake-flask culture for effective CGTase production. The effects of various operational variables such as initial pH, incubation temperature, inoculums size, incubation time and agitation speed, on the efficiency of fermentation were optimized by Taguchi assay method. With the help of statistical tool by optimizing the process variables without altering the nutritional compounds and their concentration an overall 39% of enzyme yield was improved.

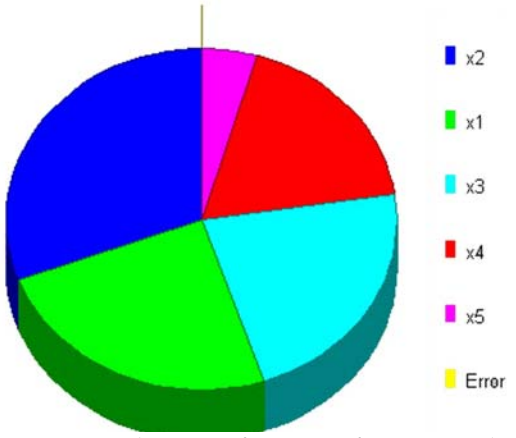

Fig 7. Contribution of various factors on the CGTase production by mutated *Bacillus* sp. TPR71HNA6

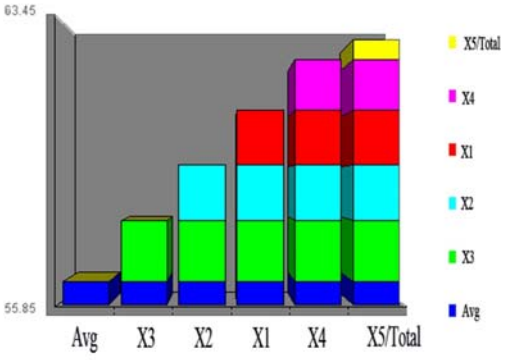

Fig 8. Optimum performance with major factors contribution

Table 8. Summary of CGTase production by various types of micro-organisms and fermentation conditions compared to the present study

Micro-organism	pH	Temperature (°C)	Incubation time (hrs)	Ref
B. circulans C31	7.2	37	96	Pongsawadi and Yagisawa, 1988
B. amyloliquefaciens AL 35	7.0&9.0	37	48	Yu et al., 1988
B. sphaericus	7.0	30	24	Oguma et al., 1990
Klebsilella oxytoca 19-1	7.0	37	216	Lee et al., 1992
Bacillus sp.	10.0	37	25	Georganta et al., 1993
B. Halophilus INMIA-3849	7.0-7.2	37	48	Abelian et al., 1995
Alkalophilic bacteria	10.0	37	120	Salva et al., 1997
Alkalophilic *Bacillus* sp.	10.0	30	96	Varavinit et al., 1997
B. firmus	10.0	37	48	Yim et al., 1997
B. amyloliquefaciens	6.5	37	24	Abdel-Naby et al., 2000
Bacillus sp. G1	6.0	37	24	Tien, 2001
Bacillus sp. TS1-1	10.32	37	20	Khairizal et al., 2004
B. stearothermophillus HR1	7.54	55	24	Roshanida et al., 2004
Bacillus sp. TPR71HNA6	7.5	30	48	Present study

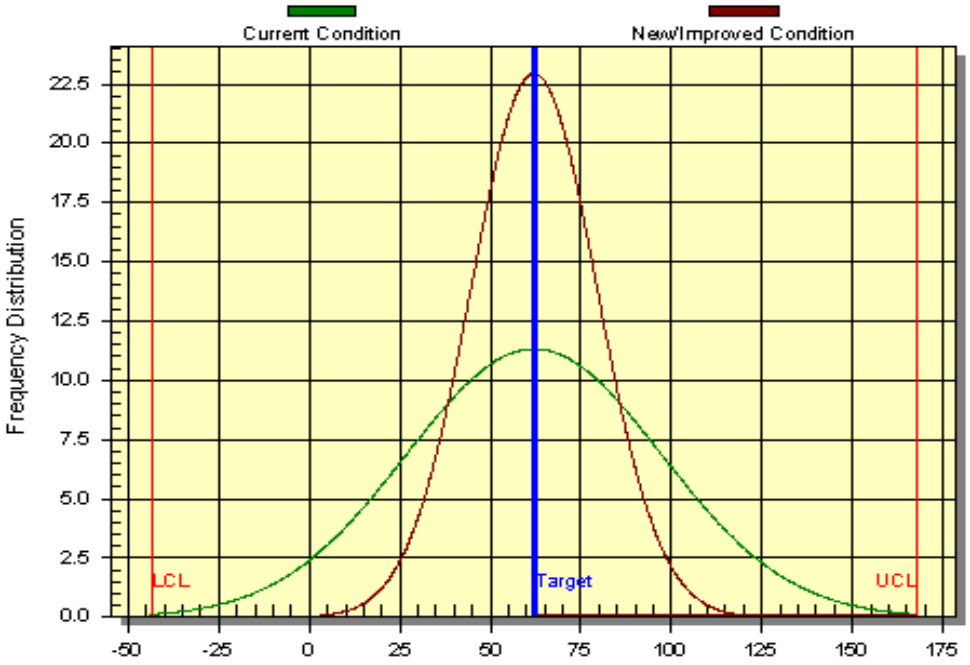

Fig 9. Performance distribution on CGTase production by mutated *Bacillus* sp. TPR71HNA6 at current and improved conditions

References

Abdel-Naby MA, Reyad MR, Adel-Fattah AF. (2000) Biosynthesis of cyclodextrin glucosyltransferase by immobilized *Bacillus amyloliquefaciens* in batch and continuous cultures, Biochem. Eng. 5: 1-9.

Abelian VA, Adamian MO, Abelian LA, Balayan AM, Afrikian EK. (1995) A new cyclodextrin glucanotransferase from alkalophilic *Bacillus*. Biochem Moscow 60(6): 891-7.

Alves-Prado HF, Bocchini DA, Gomes E, Baida LC, Contiero J, Roberto IC, and Silva RDA. (2007) Optimization of cyclodextrin glucanotransferase production from *Bacillus clausii* E16 in submerged fermentation using response surface methodology, Appl. Biochem. Biotechnol. 137-140:1-12: 27-40.

Bart AV, Joost CMU, Bauke WD and Lubbert D. (2000). Engineering of cyclodextrin glycosyltransferase reaction and product specificity. Biochem. Biophys. Acta. 1543: 336-360.

Gawande BN and Patkar AY. (1999) Application of factorial designs for optimization of cyclodextrin glycosyltransferase production from *Klebsiella pneumoniae* AS-22. Biotech Bioeng. 64, 168-173.

Gawande BN, Singh RK, Chauhan AK, Goel A, Patkar AY. (1998) Optimization of cyclomaltodextrin glucanotransferase production from *Bacillus firmus*. Enzyme Microb. Technol. 22: 288-91.

Georganta G, Kaneko T, Nakamura N, Kudo T and Horikoshi K. (1993) Isolation and Partial Properties of Cyclomaltodextrin Glucanotransferase-producing Alkaliphilic *Bacillus spp.* from a Deep-sea Mud Sample. Starch 45: 95–99

Jamuna R, Saswathi N, Sheela R and Ramakrishna SV. (1993) Synthesis of cyclodextrin glycosyltransferase by *Bacillus cereus* for the production of cyclodextrins. Appl. Biochem. Biotechnol. 43: 163-176.

Kaneko T, Kato T, Nakamura N, Horikoshi K. (1987) Spectrophotometric determination of cyclization activity of –cyclodextrin forming cyclodextrin

glucanotransferase. J. Jpn. Soc. Starch Sci. 34: 45-8.

Lee JH, Choi KH, Choi JY, Lee YS, Kwon IB and Yu JH. (1992) Enzymatic production of a-cyclodextrin with the cyclomaltodextrin gluconotransferase of *Klebeiella oxytoca* 19-1. Enzyme Microb. Technol. 14:1017-1020.

Mahalaxmi Y, Sathish T, Prakasham RS. (2009) Development of balanced medium composition for improved rifamycin B production by isolated Amycolatopsis sp. RSP-3. Lett Appl Microbiol 49(5): 533-538.

Khairizal MM, Rosli MI, Roshanida AR, Noor Aini AR, Nik Azmi NM, Osman H, Suraini AA, Kamarulzaman K. (2004) Production of cyclodextrin glucano-transferase (CGTase) from alkalophilic *Bacillus* sp. TS1-1: media optimization using experimental design, Enzyme Microb. Technol. 35 (2004) 467–473.

Oguma T, Kikuchi M, Mizusawa K. (1990) Purification and some properties of cyclodextrin-hydrolyzing enzyme from *Bacillus sphaericus*. Biochem. Biophys. Acta. 1036:1–5.

Pongsawasdi P, Yagisawa M. (1988) Purification and some properties of cyclomaltodextrin glucanotransferase from *Bacillus circulans*. Agric. Biol. Chem. 52(5): 1099–103.

Prakasham RS, Sathish T and Brahmaiah P. (2010) Biohydrogen production process optimization using anaerobic mixed consortia: A prelude study for use of agro-industrial material hydrolysate as substrate. Bioresour. Technol. 101: 5708-5711.

Roshanida AR, Rosli MI, Mohd Ghazali MN, Ismail AF, Osman H, Kamarulzaman K. (2004) Optimisation of growth medium for the production of cyclodextrin glucanotransferase from *Bacillus sterothermophilus* HR1 using response surface methodology Process Biochem. 39: 2053-2060.

Rosso AM, Ferrarotti SA, Krymkiewicz N, Nude BC. (2002) Optimisation of batch culture conditions for cyclodextrin glucanotransferase production from Bacillus circulans DF 9R. Microb. Cell Factories 1: 3–12.

Salva TJG, Bittencourt de Lima V and Pagan AP. (1997) Screening of alkalophilic bacteria for cyclodextrin glycosyltransferase production. Rev. Microbiol 28: 157-164.

Sathish, T. and R.S. Prakasham. (2010) Enrichment of glutaminase production by *Bacillus subtilis* RSP-GLU in submerged cultivation based on neural network - genetic algorithm approach, J. Chem. Technol. Biotechnol. 85 (1): 50-58.

Sreenivas Rao R, Ganesh Kumar C, Prakasham RS, PhilHobbs J. (2008) The Taguchi methodology as a statistical tool for biotechnological applications: A critical appraisal. Biotechnol. J. 3(4): 510-523.

Stefanova ME, Tonkova AI, Miteva VI and Dobreva EP. (1999) Characterization and cultural conditions of a novel cyclodextrin glucanotransferase producing *Bacillus stearothermophilus* strain. J. Bas. Microbiol. 39(4): 257-263.

Tien SF. 2001. Isolation of Alkalophilic *Bacillus* sp. G1 and the Production of Cyclodextrin Glucanotransferase (CGTase). Universiti Teknologi Malaysia: Master Thesis.

Yim DG, Sato HH, Park YH, Park YK. (1997) Production of cyclodextrin from starch by cyclodextrin glycosyl-transferase from *Bacillus firmus* and characterization of purified enzyme. J. Indust. Microbiol. Biotechnol. 18: 402-5.

Yu KC, Aoki H and Misawa M. (1988). Specific alpha cyclodextrin production by a novel thermostable cyclodextrin glycosyltransferase. Applied Microbiol. Biotech. 28: 377-379.

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