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Optimization of Agrase production by alkaline *Pseudomonas aeruginosa* ZSL-2 using Taguchi experimental design

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ABSTRACT

The culture conditions for the production of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2 were optimized using One-Factor-At-A-Time combined with orthogonal array design. One-Factor-At-A-Time method investigates the effect of time, temperature, NaCl, carbon sources, nitrogen sources and pH on agarase production. The optimized culture conditions obtained from the statistical analysis were temperature of 30 °C, pH 8.5, NH_4NO_3 2 g L^{-1} and agar 3 g L^{-1}. The L_9 orthogonal array design was used to select the fermentation parameters influencing the yield of agarase. The order of the factors affecting the fermentation process was found to be NH_4NO_3 > pH > agar > temperature, with temperature playing a significant role on the agarase production ($p < 0.10$). The higher yields than those in basal media culture were obtained in the final optimized medium with activity of 0.439 ± 0.013 U ml^{-1}. Extracellular agarase hydrolysed agar into a range of oligosaccharides which were analysed by LC-ESI-MS spectrometry as anhydrogalactose, galactose, agarobiose, agarotetrose and agarohexaose.

***Keywords*:** Agar, Agarase; *Pseudomonas aeruginosa* ZSL-2; L_9 orthogonal array design; LC-ESI-MS

1. INTRODUCTION

In recent years, bioactivity studies have demonstrated that the oligomer derived from agar or agarose exhibit a variety of physiological activities like hepatoprotective potential (Chen et al. 2006), anti-oxidation (Wang et al. 2004 [36]) and have potential applications in the food, cosmetic (Kobayashi et al. 1997 [1]) and medical industries.Moreover, agarases can be used to prepare protoplasts and extract biological substances such as unsaturated fatty acid, vitamins, carotenoids, and betaine, among others, from algae (Araki et al., 1998 [35]).

The extent of these activities is also correlated with the degree of polymerization (DP) of the galactosyl groups on the neoagaro oligosaccharides (NAOS) and agaro oligosaccharides (AOS). Neoagarobiose (N2) was reported to possess moisturizing and whitening effects on melanoma cells [1]. Neoagarotetraose (N4), derived from porphyran, was reported to be utilized *in vitro* by intestinal bacteria, which stimulated the growth of *Bacteroides*, as well as *Eubacterium* and *Lactobacillus* [2]. Recently, the prebiotic

effectiveness of NAOS with DP 4-12 has been confirmed both *in vivo* and *in vitro*. They showed augmented growth of *Bifidobacterium* and *Lactobacillus* [3].

Agar oligosaccharide can be attained by many methods, including chemical degradation and enzyme hydrolysis. A special enzyme hydrolysing the agar is agarase (agarose 4-glycanohy-drolase, E.C.3.2.1.81). Based on the mode of action on agarose, agarases are classified into two groups α-agarase and β-agarase which hydrolyse α-1,3 linkages and β-1,4 linkages in agarose, respectively. Agarases are mainly applied in the production of oligo-saccharides from agar. Agarases can also be used to degrade the cell walls of marine algae for the preparation of protoplasts (Chen et al. 1994; Dipak kore et al. 2005). It was also reported that the agarases were used to reclaim DNA from agarose gel (Finkelstein and Rownd 1978; Burmeistera and Lehrachb 1989). Efforts are undertaken recently towards finding more aga-rases with a high activity from the environment.

Agarases are mainly obtained from marine bacteria. To date, a number of micro-organisms have been reported to secrete agarase, mainly in a marine environment, either in the sea water, in marine sediments or associated to red algae [4,5]. The agarase producing bacteria are also isolated from nonmarine environments, like the ones from low-land river [6], soil [7,8] and plant root [9]. There are limited number of studies which have investigated the optimization of culture conditions for agarase-producing microbes [10-16]. Optimal culture conditions for agarase-producing *Pseudomonas sp.* by statistical approach have not been reported.

In present investigation we report optimization of fermentation conditions for the production of agarase by two steps. First, optimization by "One-Factor-At-A-Time" (OFAT) involves changing one of the independent variables, temperature, pH, nitrogen source etc. while fixing the others at certain levels, and second, further optimization by Taguchi experimental design. The basic principle of the Taguchi method serves as screening filters that examine the effects of many process variables and identify those factors that have major effects on process by using a few experiments and also LC-ESI-MS analysis of agar degraded products.

2. MATERIALS AND METHODS

2. 1. Microorganism

An agarolytic *Pseudomonas aeruginosa* ZSL-2 strain isolated from marine environment was inoculated in minimal mineral salts (MMS) medium composed of 0.38 g/L K_2HPO_4; 0.20 g/L $MgSO_4$; 0.05 g/L $FeCl_3$; 1.0 g/L NH_4NO_3 and 3.0 g/L agar. This strain was routinely maintained and stored at 4 °C on MMS-agar slants in the laboratory. Seed culture was prepared from 24h grown culture on MMS liquid broth at 180 rpm and 37 °C as reported earlier [17].

2. 2. Agarase assay

Agarase activity was determined by neocuproine method as described by Dygert et al., [18]. The assay mixture of 1ml contained 20 mM Tris-HCl buffer (pH 8.0), 0.5 ml of agrose (0.1 % w/v in Tris-HCl buffer) and 50 µl suitably diluted enzyme at 40 °C. One unit of the enzyme activity was defined as the amount which liberates 1µmol galactose equivalent per minute under assay condition. The protein content of the enzyme solution was determined by the Lowry method using Bovine serum albumin as standard protein [19].

2. 3. Optimization of culture parameters for the production of extracellular agarase by "one-factor-at-a-time" (OFAT)

The optimization of fermentation conditions were carried out based on stepwise modification of the governing parameters for agarase production. They include fermentation period, pH, temperature, NaCl, carbon source and nitrogen sources. The optimum culture condition obtained in previous step was used in next step.

2. 3. 1. Effect of culture conditions on agarase production

The *P. aeruginosa* ZSL-2 was inoculated into the MMS medium supplemented with agar (0.3 % w/v) and incubated. A 1ml exponential growth phase culture was inoculated into a fresh 250 ml Erlenmeyer flask containing 50 ml MMS medium supplemented with agar (0.3 % w/v). In order to check the effect of fermentation period the flasks were incubated on a rotary shaker at 170 rpm and 37 °C for 60 h. The effect of incubation period was analysed by withdrawing 2 ml of culture medium aseptically at different intervals. To study the effect of initial pH, the culture inoculated media flasks were individually incubated at different pH (pH 5-11). To check the effect of temperature, the flasks were incubated at different temperature such as 20, 25, 30, 37 and 40 °C. Similarly the effect of ionic strengths (0-5 % NaCl, w/v) was also investigated.

2. 3. 2. Effect of carbon and nitrogen sources on agarase production

To study the effect of different nitrogen sources on agarase production, NH_4NO_3 in the medium was replaced with different organic nitrogen sources (0.1 %, w/v) such as peptone, yeast extract, beef extract, and inorganic nitrogen sources (1 %, w/v) such as KNO_3, NH_4Cl, $NaNO_3$ or $(NH_4)_2SO_4$ and fermentation was carried out as described earlier. The influence of carbon source on the agarase production was analysed by growing *P. aeruginosa* ZSL-2 in the MMS medium supplemented with various carbon sources (0.2 % to 0.5 %, w/v) such as glucose, galactose, fructose, lactose, sucrose, mannose, maltose and agar alone or the said carbon sources were co-supplemented with agar.

2. 4. Optimization of agarase production by orthogonal array testing strategy (OATS)

Based on the one-factor-at-a-time, an L_9 (3^4) orthogonal array method was used to determine optimal medium conditions for caarrageenase production. This enables to determine which process variables affect the response. The level-setting values of the factors used in the orthogonal array design are shown in Table 3. The four variables, agar, NH_4NO_3, temperature and pH with three concentration levels were used. The experiments were carried out in 250 ml Erlenmeyer flasks containing 50 ml MMS medium. The agarase activity of each flasks was assayed after 24h of incubation and analysed statistically by ANOVA.

2. 5. LC-ESI-MS analysis of agar oligosaccharides

The hydrolysed product of agar by *P. aeruginosa* ZSL-2 supplemented in the growth medium were analyzed by LC- ESI-MS as reported by Baswaraj et al., [20]. Briefly, a 1-ml of the culture medium was withdrawn, cooled to 4 °C and was centrifuged for 15 min at 14000 rpm. A 500 µl of clarified culture supernatant was extracted by 80 % methanol (-20 °C), and evaporated to dryness by a vacuum manifold. The residue was then re-dissolved in 100 µl of 80 % methanol and was filtered through a 5 kDa centricon filter and the filtrate obtained was transferred to an autosampler vial. Ten microliters of culture supernatant

filtered through 0.22 µ syringe filter was injected through auto sampler into a 150 mm x 4 mm i.d, 5µ phenomenex C-18 RP-column (thermostated at 40 °C) at flow rate 1ml min^{-1}. LC is synchronized with mass Q TRAP 4000 MS-MS (Applied Biosystems MDS-SCIX). LC-ESI-MS data acquisition was carried out at 22 min. EMS spectra were generated from the TIC. Spectra were recorded in negative mode between m/z 50 and 1200. Identification was carried out with AB's Analyst 1.2.2 software.

3. RESULTS AND DISCUSSION

3. 1. Optimization of culture parameters for the production of extracellular agarases from

P. aeruginosa ZSL-2 by "one-factor-at-a-time" (OFAT)

A typical growth profile and agarase production by *P. aeruginosa* ZSL-2 (Fig. 1) reveals that the agarase production in extracellular medium was observed after 12 h of incubation, which increased with an increase in the incubation period. Maximum agarase activity (0.166 U/ml) was observed in late log phase at 24 h incubation, thereafter the agarase production decreased. The agarase production in *P. aeruginosa* ZSL-2 was maximum (0.179 U/ml) when the bacterium was grown at 30 °C (Fig. 2). The agar utilising bacterium *T. agarivorans* is reported to grow over a temperature range of 20 to 35 °C, with an optimum temperature of 25 °C [21] and growth of *P. agarivorans* was observed over a range of 20 to 30 °C, with an optimum at 20 to 25 °C [22].

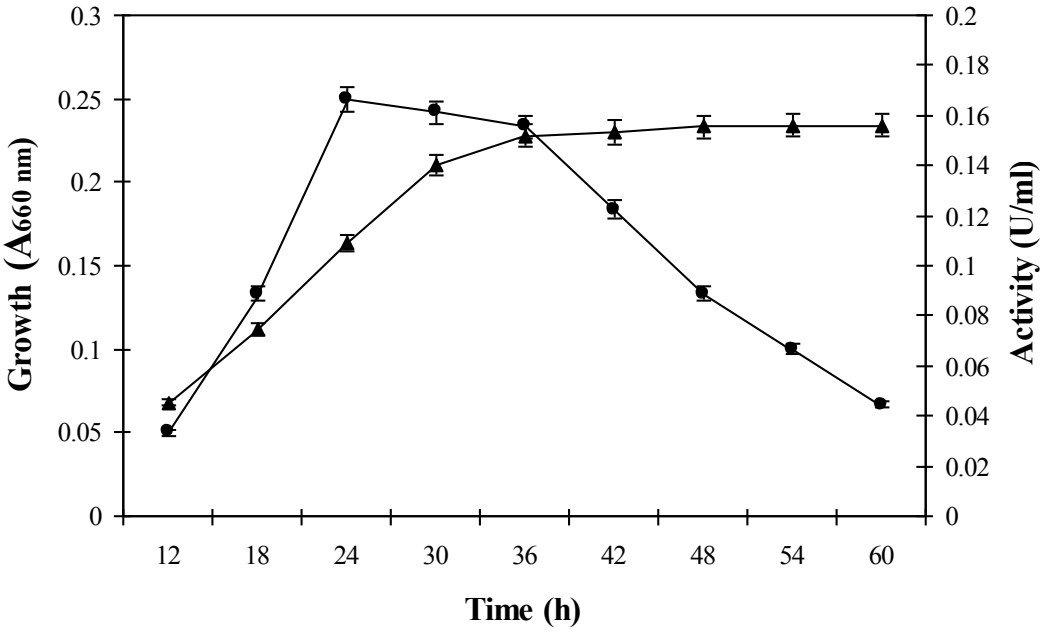

Fig. 1. Effect of incubation time on growth (▲) and production (●) of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2.

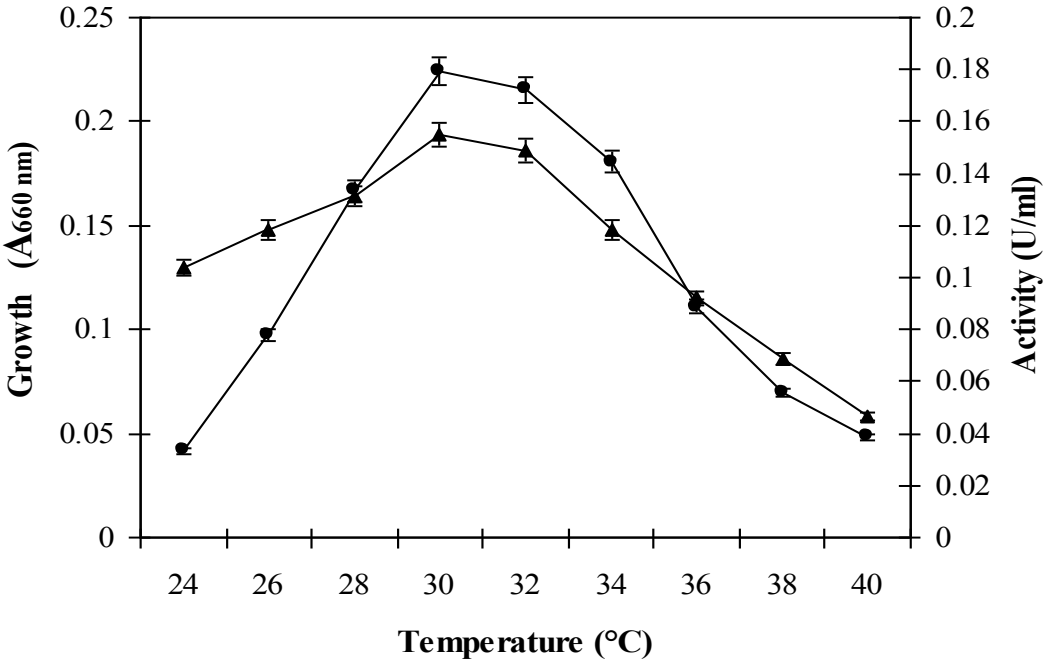

Fig. 2. Effect of temperature on growth (▲) and production (●) of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2.

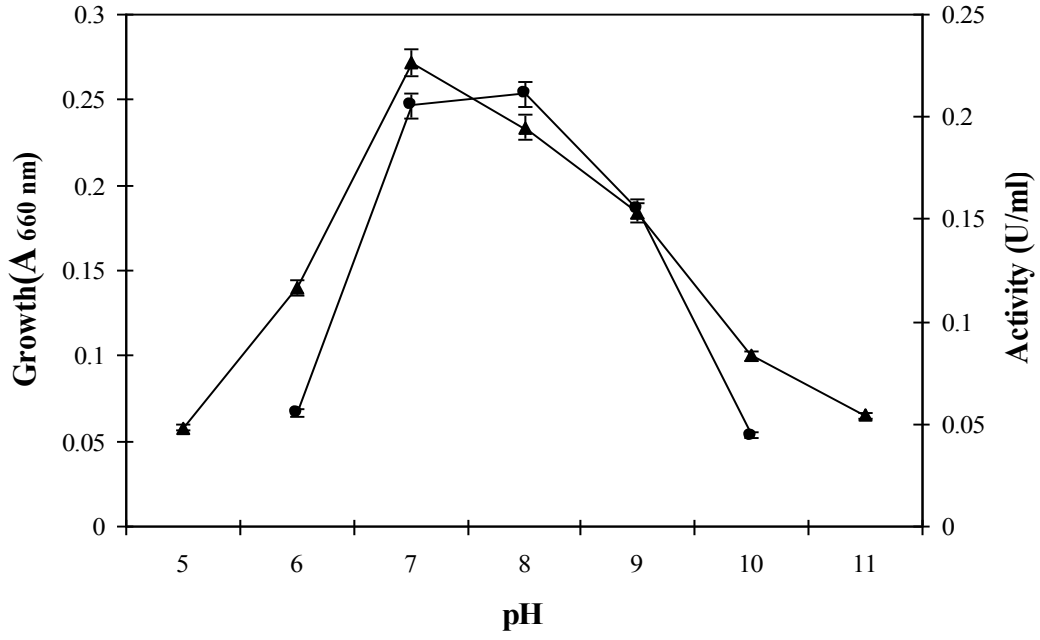

Fig. 3. Effect of pH on growth (▲) and production (●) of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2.

However; agarase was produced in the culture medium at pH range of 5.0-11.0 and maximum agarase production (0.211 U/ml) was observed at pH 8.0. (Fig. 3). Most agarolytic bacteria those are reported to date grew at pH range between 6.5 and 7.8. However, the *Agarivorans sp.* HZ105 is reported to grew at the broad pH range of 6.0-11.0, and optimally at about pH 8.0 [13]. The *Alteromonas* strain 2-40 is reported to grow at pH range between 5.0 and 9.2 and optimally at pH 7.5 [23], The *Pseudomonas aeruginosa* AG LSL-11 is reported to grow in the pH range 5.0-11.0 and optimally at pH 8.0 [10]. *Acinetobacter sp.,* AG LSL-1 grew at pH range from pH 4.0 to 9.0, and optimally at pH 7.0 [24]. However, it was reported *P. agarivorans* as a slightly alkali tolerant with pH growth range of 6 to 9 and with an optimum pH of 8 to 9 [22]. As we know, the natural seawater is of a weak alkaline pH, thus it is reasonable that the marine isolates show optimized growth at slightly alkaine pHs and agarases derived from marine bacteria show maximum agarse production in those conditions. The effect of sodium chloride (0-5 %, w/v) on agarase production revealed that the production of agarase by *P. aeruginosa* ZSL-2 was found to be slightly increased with increase in concentration of NaCl up to 1 % (w/v), beyond which there was sharp decline in the secretion. At 4 % NaCl, the considerable agarase production was not observed though the bacterium was able to grow (Fig 4).

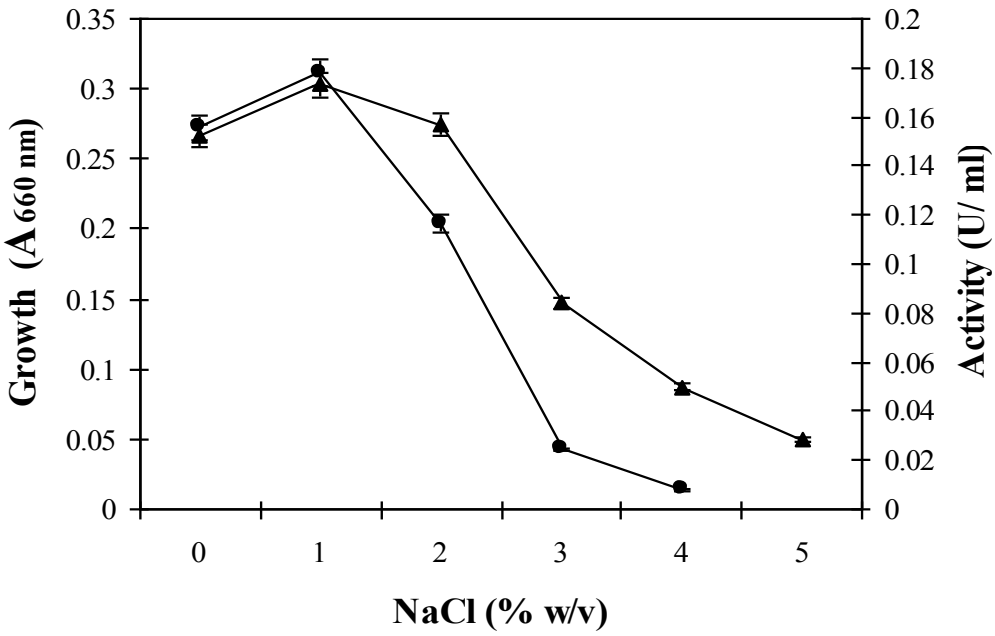

Fig. 4. Effect of NaCl on growth (▲) and production (●) of extracellular agarase *by Pseudomonas aeruginosa* ZSL-2.

It is commonly assumed that the marine bacteria, since they live in the sea, most of them are salt-tolerant. Most agarolytic bacteria, viz., *Pseudomonas atlantica*, [25], *Pseudomonas* AP5333, [26], *Pseudoalteromonas antartica* strain N-1 [27], *Alteromonas* strain 2-40 [23], *Alteromonas* sp. strain C-1 [28], *Vibrio* sp strain JT0107 [29], those are isolated from marine source are reported to have a specific requirement of sodium chloride (2.5 %) for their growth. Agarolytic bacterium *Bacillus cereus* ASK 202 is reported to grow well even at 5 % NaCl concentration [30]. The *Pseudomonas aeruginosa* ZSL-2 grows better

at 1 % NaCl concentration and unlike other agarolytic marine *Pseudomonads*, *Pseudomonas aeruginosa* ZSL-2 do not demand for sodium chloride as a specific requirement, moreover, even in absence of which, the bacterium grows well and produces extracellular agarases. Similar observations are reported for *P. aeruginosa* AG LSL 11 [10].

An understanding of the influence of nitrogen source on the growth of *P. aeruginosa* ZSL-2 was derived by growing the later in the fermentation medium containing different nitrogen sources such as, KNO_3, NH_4Cl, $NaNO_3$ or $(NH_4)_2SO_4$ and supplemented with agar (0.3 %, w/v) as sole carbon source. The growth of the bacterium was found to be optimum in the medium supplemented with $NaNO_3$ or NH_4NO_3 as nitrogen source and agar as the carbon source. The production of agarase (0.354 U/ml) was found to maximum in the medium, which had received NH_4NO_3 as nitrogen source. The production of agarase was found to be less in the fermentation medium supplemented organic nitrogen sources, however peptone and beef extract served as the good sources than the other organic nitrogen, but less agarase production was observed (Table 1).

Table 1. Effect of nitrogen sources on the growth and production of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2.

Nitrogen source *	Growth (A_{660nm})	Agarase activity ($U.ml^{-1}$)
NH_4NO_3	0.441	0.354
KNO_3	0.372	0.234
NH_4Cl	0.177	0.0743
$NaNO_3$	0.409	0.296
$(NH_4)_2SO_4$	0.201	0.173
Peptone	2.084	0.112
Yeast extract	1.011	0.09836
Beef extract	2.104	0.082

* Fermentation conditions: MMS media containing one of the nitrogen sources, supplemented with agar at pH 8, temperature 30 °C after 24h of incubation

The observed results indicated that NH_4NO_3 as a sole nitrogen source supported the optimum growth and maximum enzyme production, thus nitrogen was found to be an essential element for the production of the agarolytic enzyme. This effect had been reported with a similar observation using yeast extract as a nitrogen source [10,14].

Agarase production was observed in the fermentation media supplemented with agar (Table 2). The bacteria grew well utilizing other simple sugars, while agarase production was not observed. On the other hand agarase production was observed when simple sugars were co-supplemented to agar containing fermentation media.

The results revealed that agarase production was inducible only in presence of agar when the simple sugars alone were included in the medium, although good growth was

observed, agarase production was not occurred in the medium. Co-supplementation of other simple sugars to agar found to reduce the production slightly though luxuriant growth by utilizing simple sugars was observed.

Table 2. Effect of carbon sources on growth and production of extracellular agarase by *Pseudomonas aeruginosa* ZSL-2.

Carbon source	Growth (A_{660})	Agarase production (U/ ml)	Co-Supplimentation of sugars	Growth (A_{660})	Agarase production (U/ ml)
Agar	0.438	0.37	Agar	0.465	0.374
Glucose	1.123	ND	Agar + Glucose	0.578	0.354
Galactose	1.118	ND	Agar +Galactose	0.435	0.388
Fructose	1.260	ND	Agar +Fructose	0.448	0.420
Sucrose	1.048	ND	Agar +Sucrose	0.236	0.328
Mannose	1.218	ND	Agar +Mannose	0.304	0.303
Lactose	1.130	ND	Agar +Lactose	0.911	0.330
Maltose	1.003	ND	Agar +Maltose	0.873	0.268

ND-Not detected

This explains that the agarase production is inducible by agar/ agarose and production was reduced when co-supplemented with other simple carbohydrates, However catabolite repression was not observed.

Catabolite repression is a very common phenomenon observed in microorganisms, which regulate the production of enzymes of catabolic pathways, which in turn determines the extent of utilization of a compound by the microorganisms. Catabolite repression for agarase production was observed in *Pseudomonas aeuroginosa* AG LSL-11, in which agarase production was repressed when other carbon sources co-supplemented to agar [10]. Similar observations were reported in *Alteromonas sp.* (049/1) and *Cytophaga saccharophila* (024), in which agarase production was repressed when glucose was co-supplemented to agar [6]. In contrast, the co-supplemented simple sugars did not repress the agarase production in *Pseudomonas aeuroginosa* ZSL-2 and further fructose was found to enhance agarase production. This may be due to rapid utilization of simple sugars by the bacterium resulting in an increase in cell mass by vigorous growth.

The results with the one-factor-at-a-time method revealed that optimal conditions for the production of agarase were 24 h of fermentation period, 30 ^{o}C incubation temperature at pH 8.0 with NH_4NO_3 as nitrogen source and agar as carbon source in MMS media. Among these four factors, temperature, pH, NH_4NO_3 and agar with their relevant levels are selected for further optimization of agarase production by using orthogonal experimental design L_9 (3^4). The level-setting values of the factors used in the orthogonal array design are shown in Table 3.

Table 3. Factors and their levels employed in the L_9 (3^4) in OATS for production of agarase by SmF of *P. aeruginosa* ZSL-2.

Factors / Level	**A** Temp (^oC)	**B** pH	**C** Nitrogen ($g.L^{-1}$)	**D** Carbon ($g.L^{-1}$)
level 1	28	7.5	1	1
level 2	30	8.0	2	2
level 3	32	8.5	3	3
	Nitrogen – NH_4NO_3		Carbon - Agar	

3. 2. Experimental results of L_9 (3^4) orthogonal matrix method

The optimal conditions for the culture parameters were obtained by using orthogonal design L_9 (3^4) based on one factor at a time method. The effects of these factors on agarase production were analysed statistically as reported earlier [31] and results are shown in Table 4.

Table 4. L_9 (3^4) Orthogonal array testing strategy (OATS) method for optimization of fermentation parameter for agarase production by SmF of *P. aeruginosa* ZSL-2.

S. No.	**A**	**B**	**C**	**D**	Agarase activity U/ml	
1	1(28 °C)	1(pH 7.5)	1	1	0.122	± 0.003
2	1(28 °C)	2(pH 8.0)	2	2	0.311	± 0.009
3	1(28 °C)	3(pH 8.5)	3	3	0.377	± 0.011
4	2(30 °C)	1(pH 7.5)	2	3	0.444	± 0.013
5	2(30 °C)	2(pH 8.0)	3	1	0.1	± 0.003
6	2(30 °C)	3(pH 8.5)	1	2	0.3	± 0.009
7	3(32 °C)	1(pH 7.5)	3	2	0.188	± 0.005
8	3(32 °C)	2(pH 8.0)	1	3	0.177	± 0.005
9	3(32 °C)	3(pH 8.5)	2	1	0.366	± 0.011
[a] K 1	0.811	0.755	0.6	0.588		
K 2	0.844	0.588	1.122	0.8		
K 3	0.733	1.044	0.666	1		
[b] k 1	0.270	0.251	0.2	0.196		
k 2	**0.281**	0.196	**0.374**	0.266		

k 3	0.244	**0.348**	0.222	**0.333**
[c] Δ	0.037	0.152	0.174	0.137
R	4	2	1	3
Opt	**A_2**	**B_3**	**C_2**	**D_3**

The assignments of column A, B, C, and D were performed by orthogonal array consisted of nine experiments corresponding to the nine rows and four columns.
[a] $k_i = \sum k$ of all experiment at the same factor level.
[b] Average of k_i.
[c] Δ = max{average of k_i} - min{average of k_i }.
Values are mean of triple determinations with standard deviation (±).
Opt: optimization

In the present study, L_9 (3^4) design was applied to screen the significant factors according to preliminary experiments. Four factors; agar, NH_4NO_3, temperature and pH and their relevant levels were selected for this study. The effect of these factors on agarase production were evaluated and analysed. Optimal conditions for the agarase production were; temperature of 30 °C, pH 8.5, nitrogen source NH_4NO_3 2g L^{-1} and carbon source agar 3g L^{-1}. The order of effects of four factors on agarase production was NH_4NO_3 > pH > agar > temperature. According to the factor (Table 5), temperature had significant effect on the agarase production ($p < 0.10$). These conditions were later tested to ascertain the reliability of these results. The agarase activity of 0.439 U/mL was produced which was higher than results in the orthogonal experiment.

Table 5. ANOVA of L_9 (3^4) Orthogonal array testing strategy (OATS) for optimization of fermentation parameters for production by SmF of *P. aeruginosa* ZSL-2.

	Sum of squares	df	Mean of square	F value	Critical value	Significance
Temperature	7.224	2	3.612	3.978	$F_{0.10}(2,8) = 3.113$	*
pH	0.0118	2	0.0059	0.0064		
Nitrogen	0.0179	2	0.0089	0.0098		
Carbon	0.0093	2	0.0046	0.0051		
Error	7.2630	8	0.9078			

df – Degree of freedom F value - $F_{0.10}$ * Significant terms.

The orthogonal array design technique that has been successfully applied to improve the culture conditions for fermentation process and provides the relationships among various factors, and the order of significant factors for the optimum results [31-34]. In L_9 orthogonal array method only 9 experiments were performed whereas, if factorial experimental design

(Planket Burner design and central composite design) was used, at least 81 experiments in triplicate would be necessary to achieve the same results.

Very few reports have described the optimization of agarase production using one factor at time method [10,12,14] reported optimisation of agarase production by statistical strategy using combination of Planket Burner design and central composite design (CCD). One-factor-at-a-time technique requires a considerable amount of time work. An alternative statistical strategy is to use factorial experimental design, orthogonal and response surface methodology (RSM), which involves a minimum number of experiments and covers large number of factors. These methods are also been employed to improve the production of microbial agarase in submerged fermentation.

3. 3. LC-ESI-MS analysis of agar hydrolysed products

The mass spectra of the peaks indicated clearly that agar was hydrolysed initially by the action of extracellular agarases secreted by *P.aeruginosa* ZSL-2. The results of the LC-ESI-MS analysis of the products revealed the presence of oligosaccharides ranging from mono- to octa- agar oligosaccharides. The products identified were anhydrogalactose (DA), Galactose (G), agarobiose, agarotetrose and agaroctaose. Signals observed at m/z 162.7 and m/z 179.9 were corresponds to the anhydrogalactose and galactose respectively. The signals observed at m/z 325, 631.1 and 937.4 were corresponds to agrobiose, tetraose and octaose respectively (Table 6).

Table 6. LC-ESI-MS spectrometry analysis of agarase hydrolyzed products of Agar in culture supernatant by *P.aeruginosa* ZSL-2 grown for 24 h.

Agar hydrolysed products	m/z
DA	162.7
G	179.9
(DA-G)	325
$(DA-G)_2$	631.1
$(DA-G)_3$	937.4

DA- anhydrogalactose, G-Galactose, (DA-G) -Biose, $(DA-G)_2$-Tetraose and $(DA-G)_3$-Hexaose

Agarases have been used for agar derived oligosaccharides production. Comparing to the traditional acid hydrolysis method, the enzyme hydrolysis methods have lot of remarkable advantages, such as tender reaction conditions, excellent efficiency, controllable products, simple facilities, low energy cost and little environment pollution.

Most of the reported agarases are endo-type enzymes that hydrolyse agar and agarose to give in order, neoagarohexose ≥ neoagarotetraose ≥ neoagarobiose as the main hydrolysis products, but majority of them do not cleave neoagarotetraose [35]. Oligosaccharides have been prepared using agar by crude agarase from *Vibrio* QJH-12 isolated from the South China Sea coast [36]. Two α-agarases derived from marine bacteria *Alteromonas agarlyticus* GJ1B and *Thalassomonas* sp. JAMB-A33, have been reported to produce agarotetraose as the

main product. Other β-agarases have been reported to produce neoagarobiose (NA2) [25,37,38] neoagarotetraose (NA4) [27,28] and neoagarohexaose (NA6) [39] as the predominant products. β- Agarase from *Acinetobacter* sp. AG LSL-1 [40] has been reported to hydrolyse agarose into NA4, and NA6 and to NA2 as the only final product.

4. CONCLUSION

The agarases secreted by *Pseudomonas aeruginosa* ZSL-2 acted as endo enzymes and were capable of hydrolysing mainly agar into octaose, tetraose and biose which provides helpful tool to prepare agar oligosaccharides which have potential applications in food, pharmaceutical, and cosmetic industries.

Acknowledgements

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