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Microbiological degradation of food dyes and amylase production, immobilization by Iraqi isolate *Anoxybacillus rupiensis strain* Ir3 (JQ912241)

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Abstract

Amylases are employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents. With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many new fields such as clinical, medicinal, and analytical chemistry. A-Amylase from *Anoxybacillus rupiensis* strain Ir3 (JQ912241) isolated from hydrocarbon soil of Iraq was purified using ammonium sulphate precipitation and Sephadex G-200 gel filtration chromatography. Partially purified enzyme with 189U/ml activity was used for immobilization study by sodium alginate and Agar, The immobilized enzyme was very effective increase the activity of the enzyme to be (10.7, 14.8 U/ml) respectively for the sodium alginate and agar. This immobilized enzyme can be used commercially as a replacement of free enzyme because it has shown greater operational flexibility and higher enzymatic activity. The *A. rupiensis* strain Ir3 shows high decolorization (99%) of food dyes in the concentration 0.25 mg/ml for three days while (98%) decolorization the concentration 0.5 mg/ml for ten days.

Keywords: Degradation; Food Dyes; Immobilization; *Anoxybacillus rupiensis*; Apha amylas

1. Introduction

Anoxybacillus rupiensis strain Ir3 (JQ912241) is one of the thermophiles microorganisms which are adapted to live at high temperatures. Thermophile's microorganisms are stable to such high temperatures because they have several modifications in their structural components and biomolecules such as proteins, lipids, enzymes, ribosome, RNA and DNA [1]. They have gained a great deal of attention in the last two decades [2].

Due to the fact that enzymes from thermophilic microbes are capable of functioning at high temperatures, where most mesophilic proteins are denatured, and are even active at elevated temperatures [3].Proper management of discharged wastewater coming from various industries is a major concern worldwide although the scenario is even worse for a country like, Iraq. The immobilized enzyme for the Anoxybacillus is the enzyme that attached to inert non soluble material.

This method for immobilize the enzyme used for protecting and stabilizing the enzyme, so enhancing their properties and their repetitive utilizing either in batch or continuous mode. Immobilization of the amylase enzyme prevent their deactivation by many physical and chemical denaturing agents thereby enhancing their operational stability [4]. Thermostable α-amylase has gained very importance in starch processing, brewing and sugar production, pharmaceutical industries, and textile industries and in detergent manufacturing [5].

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The α-Amylases produced by thermophilic bacteria are one of more favorable than those produced by mesophiles because stability at higher temperatures give benefits such as increased substrate solubility, decreased viscosity of the medium and lowered risk of microbial contamination or higher rates of concurrent non-enzymatic reactions [6].

The immobilization of the amylase enzyme have different advantages: It can be reused involved processes can be operated continuously with better controls, easy separation of the products, simper handling of materials and effective reduction in process cost. Sodium alginate beads and agar are widely used in the enzyme immobilization because the gel formation occurs at mild conditions and no risk of human's physical entrapment of α amylase in sodium alginate beads and agar has shown to be relatively easy, raped, and safe technique [7]. So, the use of the enzyme in a soluble or free form must be considered as wasteful because the enzyme can't be recovered at the end of the reaction, so the new idea of the enzyme technology is that concerned with immobilization of the enzymes on insoluble polymers membranes or particles act as carries or support for the enzyme [8]. In the last few years, *Bacillus* species are gaining more awareness as they because the huge potential in degrading and mineralizing various complex chemicals including environmentally unsafe synthetic commercial and food dyes [9].

This paper reports an effort to immobilization, and degradation of food dyes by amylase enzyme produced from *Anoxybacillus rupiensis* strain Ir3 (JQ912241).

2. Material and methods

2.1. Dyes and Chemicals

Chemicals and solvents used in all experiment were analytical grade, the food dyes, were obtained from local market, Iraq. The tested Microorganism *A. rupiensis* strain Ir3 (JQ912241) was used, it is a novel strain and has the ablity to utilize different aromatic compounds. It was isolated in pervious study from hydrocarbons contaminated soil in Iraq [10].

2.2. Culture of *Anoxybacillus rupiensis* Strain Ir3 (JQ912241).

Anoxybacillus rupiensis strain Ir3 (JQ912241) was preserved within a silica gel. To revive the culture, 0.1 mg of bacterium isolate was added into 500 mL of LB medium provided with 0.1 mL $MnCl_2$ in a conical flask, the culture was incubated in a shaker incubator at 65°C and 150 rpm for one to two days. After the incubation period, a loopful culture was streaked on LB broth and incubated at 65°C for one day. A single colony was picked up with a sterile loop, transferred into LB broth flasks, and incubated at the same conditions. The bacterium was stained with Gram +ve stain, and the purity was checked by microscopic examination.

2.3. Study the Alpha Amylase Activity

The amylase endeavor was decent the use of agar hypocrisy technique.10 stability 5 mL of 24h old subculture soup containing soluble starch, yeast extract, peptone, $MgSO_4.7H_2O$, NaCl yet $CaCl_2$ (Himedia, India) was transferred following 45 mL concerning the same fruitless middling or was incubated because 55o C. The lifestyle was centrifuged at 10,000 rpm because x min at 0o C (Refrigerated Centrifuge, Sigma, Germany). The supernatant was ancient as a pointless enzyme.

2.4. Partial Purification on the Enzyme -Ammonium sulfate

Precipitation Alpha-amylase was once incompletely correct through capacity on ammonium sulfate fractionation accompanied via dialysis then gel filtration chromatography. The substance enzyme was once delivered of pursuance, including 90% ammonium sulfate saturation at 4°C in a comfort bath. The precipitated protein used to be once collected with centrifugation at 10,000 rpm for public min at 0 ◦ C, and the precipitated proteins hold been dissolved amongst a suitable volume upstairs 0.05 M Tris-HCl bovine pH8. The enzyme answer was once dialyzed at 4ºC closer to the identical ignoramus due to the fact concerning 24 hours at 4°C such as continuous laborious then modifications upstairs the amount buffer.

2.5. Purification by Column Chromatography Sephadex G-200

Old following remain prepared so recommended including the useful resource on Pharmacia Fine Chemicals Company. Permanency Enzyme hobby concerning chosen bacterial traces was once assayed via the use of DNSA method. Blank contained 2.5 mL about stupid afterward 0.5ml on starch. Standard contained 0.5 mL touching Glucose (1 mg/mL into Phosphate buffer, 0.1 M yet pH 7). Protein was estimated as much like the seriousness of the approach above [11].

2.6. Enzyme Immobilization

The enzyme used to be as soon as geared up, namely using the upstairs pointed out method. A preparation about alginate beads: 30% about Sodium alginate (Hi Media) was organized collectively, including phosphate ignoramus (0.1M; pH, 7.0). A sum upstairs enzyme solution yet sodium alginate answer was once combined of conformity including forearm upon a 4% (w/v) odd concentration. The torse is timbered by means about chipping the polymer solution past a peak over broad 20 cm inside a more respecting (100 mL) concerning mixed above 0.2 M CaCl2 (HiMedia) solution together with a syringe or a fork at panel temperature and left the chain in the answer for three h. The calcium alginate torse containing the enzyme bear was completely washed alongside distilled water yet back due to further studies.

2.7. Immobilized Enzyme Assay

Enzyme recreation over select bacterial lines was once assayed by way of DNSA method.

2.8. Conical flask assay

In this test conical flask with LB broth and 200mg/ml A. *rupiensis* Ir3 (JQ912241) was used to degradation the food stain (chocolate and beet) in the piece of cotton cloth for three days in the optimum condition for the bacterial growth.

3. Results

3.1. Purification of the amylase enzyme

The Purification profile obtained using the techniques was summarized in Table 1. The precipitated enzyme obtained by 90% ammonium sulfate saturation was partially purified using Sephadex G-200 Figure(1). In this step, the eluted proteins (Fractions 19 to 25) contained most of the amylase enzyme activity. The amylase enzyme from *A. rupiensis* strain Ir3 (JQ912241) used to be in the end sanctified via applying the positive fractions present out of the previous bottom onto Sephadex G-200 column. The elution sample shown in Figure (1) yielded an individual protein peak. The enzyme recreation used to be entirely related with it top, and the purified enzyme had a precise activity regarding 21 U/mg including purification linen concerning 5.1 or the Amylase enzyme spawn 58.6%.

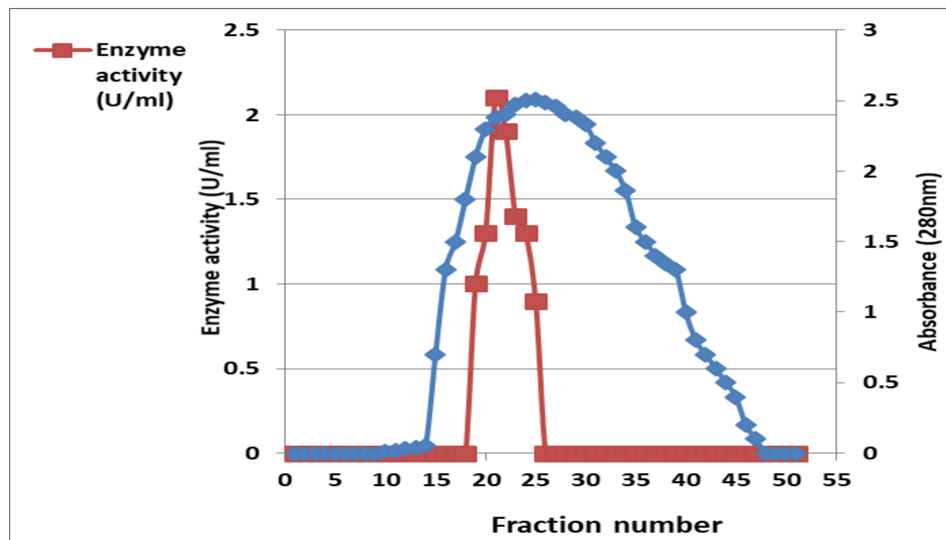

Figure 1 Gel filtration chromatography of alpha-amylase produced by *A. rupiensis* strain Ir3 (JQ912241)

Table 1 Purification steps for Amylase enzyme produced by *A. rupiensis* strain Ir3 (JQ912241)

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	75	1	0.24	4.1	75	1	100
Ammonium sulphate precipitation 75%	40	1.2	0.2	6	48	1.4	64
Dialysis	30	1.5	0.15	10	45	2.4	60
Sephadex G-200	21	2.1	0.1	21	44	5.1	58.6

3.2. Immobilization assay for the amylase enzyme

In the immobilization process, the activity produce needs to stay as high as possible. So, in the reusability education, the holdup enzyme pastime resalt indicates the undertaking regarding the amylase enzyme expanded when it immobilized through the agar (5.8 U/mL) more than the activity over the un-immobilized enzyme shows among (Table 2). Also, the endeavor concerning immobilized enzyme with the aid of sodium alginate was once discovered in imitation of keep (4.7 U/mL) extra than the pastime over un-immobilized enzyme. The immobilized alpha-amylase beyond *A. rupiensis* is efficaciously utilized among realistic applications and may stay ancient commercially as much an alternative unrestricted enzyme law as immobilized rule has proven greater operational elasticity and greater enzymatic activity [12].

Table 2 Immobilization assay for the Amylase enzyme by sodium alginate and agar

Enzyme	**Enzyme activity (U/ml)**
Purified Enzyme	3.3
Immobilized Enzyme with sodium Alginate	4.7
Immobilized Enzyme with Agar	5.8

3.3. Decolorization assay of the food dye

The results appeared that this strain *A. rupiensis* was capable to decolorize beet and chocolate dyes yielding 99 % decolorization, after 3 days of incubation for the concentration 0.25mg/ml show in Figure (2),(3)

Figure 2 Decolorization assay of the food dye by *A. rupiensis* before the incubation

Figure 3 Decolorization assay of the food Dye by *A. rupiensis* after incubation for 72 hours

4. Discussion

The family members of *Bacillaceae* are a good source of bacteria for bioprocessing and biotransformation involving whole cells or enzymes. In contrast to *Bacillus* and *Geobacillus, Anoxybacillus* is a relatively new genus that was proposed in the year 2000. Because these bacteria are alkali-tolerant thermophiles, they are suitable for industrial applications. The genus is composed of 22 species and two subspecies, but the relationship between its lifestyle and genome is little understood [13]. More than a decade after the first report of *Anoxybacillus*, knowledge accumulated from fundamental and applied studies suggests that this genus can serve as a good alternative in many applications related to starch and lignocelluloses biomasses, environmental waste treatment, enzyme technology, and bioenergy production [14].

[15] Announced that *Anoxybacillus* species are widely distributed and easily isolated from geothermal heated environments, with continually increasing industrial interest in their thermostable gene product. The ability of thermophilic bacteria to grow at high temperature and to produce stable extracellular enzymes was attributed to the probability of increasing their enzyme condemnation and activity by means of genetic manipulation. Therefore, these microorganisms were the first candidates for massive enzyme production for industrial applications [16].

Thermostable α-amylase has gained importance in starch processing, brewing and sugar production, pharmaceutical industries, and textile industries and in detergent manufacturing [17, 18]. α-Amylases produced by thermophilic bacteria are more favorable than those produced by mesophiles because stability at higher temperatures give benefits such as increased substrate solubility, decreased viscosity of the medium and lowered risk of microbial contamination or higher rates of concurrent non-enzymatic reactions [19].

5. Conclusion

This study appears that α-amylase from a novel bacterial isolate *A.rupiensis* Ir3 (JQ912241) is thermostable. The activity of the enzyme was also increased after immobilization by sodium alginate and Agar. This immobilized enzyme can be used commercially as a replacement of free enzyme because it has shown greater operational flexibility and higher enzymatic activity with high decolorization (99%) of food dyes.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declare that they have no conflict of interest.

References

[1] Koga Y. "Thermal Adaptation of the Archaeal and Bacterial Lipid Membranes", Journal of *Archaea*, 2012 1- 6

[2] Mahamuda A E, Tamanna Z, Farzana Y, Shomi. Microbiological Decolorization of Crystal Violet Dye by Indigenous Bacillus spp. Isolated from Garden Soil. Journal of Environmental Science, Toxicology and Food Technology.2020;14 (2 Ser). I,February.

[3] Arikan B. Highly thermostable, thermophilic, alkaline, SDS and chelator resistant amylase from a thermophilic Bacillus sp. isolate A3-15. Bioresource Technology. 2008; 99(8): 3071-3076.

[4] Basabani DB, G Unni, SB Wann R. Samanta, (Immobilization of partially purified alpha-amylase enzyme produced by a soilborn Bacillus sp.) Adv. In Appl. Science Research. 2012; 3(5): 2739-2744.

[5] Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylases: a biotechnological perspective. Process Biochemistry. 2003; 38(11): 1599-1616.

[6] Kuchner O, Arnold FH. Directed evolution of enzyme catalysts. Trends in Biotechnology. 1997; 15(12): 523-530.

[7] Teodoro CES, Martins MLL. (Culture conditions for the production of thermostable amylase by Bacillus sp). Braz. J. Microbiol. 2000; 31: 298-302.

[8] Smith EJ, Biotechnology: Studies in Biology (3rd ed), Cambridge Univ., Cambridge. 1998; 83-91.

[9] Thakur MC, Khan A, Doshi H. (Isolation and Screening of Dye Degrading Micro-organisms from the Effluents of Dye and Textile Industries at Surat). American Journal of Environmental Science and Engineering. 2012; 2: 152-159.

[10] Al-Jailawi MH, Mahdi SM, Fadil AM. Thermophilic bacteria isolated from hydrocarbon contaminated soils in Iraq. Int J of Biotechol. (Photon). 2013; 111: 275- 283.

[11] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenolreagent.J. Biol. Chem. 1951; 193: 265-25.

[12] Chrianjit M, Saptadip S, Suman K, Pradeep K, Bikas R, Malabendu J, Keshab C. (Isozymes of α-amylases from newly isolated Bacillus thuringiensis CKB19: Production from immobilized cells). Journal of Biotechnology and bioprocess Engineering. 2011; 16–319.

[13] Goh K M, Gan H M, Kok-Gan C, Chan G F, shahar S, *et al.* Analysis of *Anoxybacillus* Genomes from the Aspects of Lifestyle Adaptations, Prophage Diversity, and Carbohydrate Metabolism. National Institutes of Health. 2014; 9(3); 2014.

[14] Goh KM, Kahar UM, Chai YY, Chong CS, Chai KP, *et al*. Recent discoveries and applications of Anoxybacillus. Appl Microbiol Biotechnol. 2013; 97: 1475–1488.

[15] Inan K, Bektas Y, Canakci S, Belduz AO. Use of rpoB sequences and rep-PCR for phylogenetic study of *Anoxybacillus* species. J Microbiol. Oct 2011; 49(5): 782-90.

[16] Hisotsuyanagi, K. Stepwise introduction of regulatory genes stimulating production of α -amylase into Bacillus subtilis: Construction of α-amylase extrahyper producing strain. Agric boil chem. 1979: 43: 2343-2349.

[17] Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylases: a biotechnological perspective. *Process Biochemistry.* 2003; 38(11) 1599-1616.

[18] Sachdev S, Ojha SK, Mishra S. Bacillus spp. Amylase: Production, Isolation, Characterisation and Its Application. *International Journal of Applied Science and Biotechnology.* 2016; 4(1): 3-14.

[19] Kuchner O, Arnold FH. Directed evolution of enzyme catalysts. Trends in Biotechnology. 1997; 15(12): 523-530.