Cilt 12, Sayı 2, Sayfa 99-103, 2023 Volume 12, Issue 2, Page 99-103, 2023
Araştırma Makalesi https://doi.org/10.46810/tdfd.1233931 Research Article

BİNGÖL ÜNİVERSİTESİ

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Investigation of Arginine Deiminase Activity in *Bacillus cereus* and *Ralstonia eutropha* Under Minimal Conditions

Cennet Canan KARADERİ[1*], Hüseyin KAHRAMAN[2]

[1] İnönü Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Bölümü, Malatya, Türkiye
[2] İnönü Üniversitesi, Fen Edebiyat Fakültesi , Biyoloji Bölümü, Malatya, Türkiye
Cennet Canan KARADERİ ORCID No: 0000-0002-4841-4435
Hüseyin KAHRAMAN ORCID No: 0000-0001-6235-5497

**Corresponding author: ckaraderi@gmail.com*

(Received: 18.01.2023, Accepted: 12.06.2023, Online Publication: 22.06.2023)

Keywords
Bacillus cereus, *Ralstonia eutropha*, Arginine deiminase

Abstract: Prokaryotes form an important part of our lives. These microorganisms, which we can not see with the naked eye, are used in medicine, biotechnology, microbiology and many other fields. They can produce many anti-cancer enzymes (Arginine deiminase, Asparaginase, Methionine gamma lyase etc.). In this study, an important bacterial anti-tumor enzyme of *B. cereus* and *R. eutropha* in the presence of different minimal sources (Dextrose, Fructose, Glucose, Xylose, Maltose, Nutrient Broth, Rhamnose, Ribose, Sucrose) under static and shaking (150 rpm) conditions.

Arginine deiminase activity was searched. In accordance with results of our study, these microorganisms showed the highest ADI activity in the disaccharide medium, maltose and sucrose, and in the aldose group, in the ribose medium. It has been shown that this enzyme, which is used in cancer treatment, can be produced more cheaply and easily in minimal environments.

Minimal Koşullar Altında *Bacillus cereus* ve *Ralstonia eutropha*' da Arjinin deiminaz (ADI) Aktivitesinin Araştırılması

Anahtar Kelimeler
Bacillus cereus, *Ralstonia eutropha*, Arjinin deiminaz

Öz: Prokaryotlar, hayatımızın önemli bir parçasını oluşturmaktadır. Çıplak göz ile göremediğimiz bu mikroorganizmalar tıp, biyoteknoloji, mikrobiyoloji ve daha pek çok alanda kullanılmaktadır. Pek çok anti kanser enzimlerini (Arjinin deiminaz, Asparaginaz, Metiyonin gama liyaz vb) üretebilmektedirler. Bu çalışmada *B. cereus* ve *R. eutropha*' nın statik ve çalkalamalı (150 rpm) koşullarda, farklı minimal kaynakları (Dekstroz, Fruktoz, Glukoz, Ksiloz, Maltoz, Nutrient Broth Ramnoz, Riboz, Sükroz) varlığında önemli bir bakteriyel anti-tümör enzimi olan ADI aktivitesi araştırılmıştır. Çalışmanın sonuçlarına göre bu mikroorganizmalar, disakkarit ortamlarında maltoz ve sükroz, aldoz grubunda ise riboz ortamında en yüksek ADI aktivitesi göstermiştir. Kanser tedavisinde kullanılan bu enzimin minimal ortamlarda, daha ucuz, kolay bir şekilde üretilebileceği gösterilmiştir.

1. INTRODUCTION

Cancer is an important metabolic syndrome and causes serious deaths. It is associated with high mortality rates though important progresses have been made in early discovery and treatment. Cancer is an important metabolic syndrome and causes serious deaths [1]. It is associated with high mortality rates though important progresses have been made in early discovery and treatment. Hence many methods such as chemotherapy and radiotherapy are being developed to detect cancer early and to stop its progression [2].

Cancer cells; They are deadly cells that lose their functional properties, mutate due to external factors, and can multiply rapidly. These cells require large amounts of proteins to reproduce. Tumor cells can't perform their functions and reproduce because they can't perform protein synthesis without amino acids [3]. Therefore, in the medical world, a single amino acid starvation is seen as an effective strategy in cancer treatment [4]. Recent

scientific research has targeted amino acid metabolic enzymes that reduce specific enzyme metabolism required for cancer cell proliferation. With the degradation of amino acids, both DNA replication in cancer cells is prevented and the progression of cancer is stopped [3]. Enzymes are used as chemotherapeutic agents because of their high affinity and specificity towards their substrates and their rapid reaction [5].

Arginine deiminase (EC: 3.5.3.6), a bacterial arginine-catabolizing enzyme, has because of its antitumor activity in arginine auxotrophic cancers, particularly hepatocellular carcinomas and melanomas through consuming plasma arginine and causing cell deficiency [6]. Arginine deiminase catalyzes the hydrolysis of arginine to citrulline and ammonia by deamination of the guanidino group. In widespread, the hydrolysis of arginine with ADI is thought about the first step of the Arginine deiminase system consisting of two reactions: conversion of citrulline to ornithine and reduction of carboamyl phosphate to ammonia and CO_2 by carbamyl phosphate and carbamate kinase catalyzed by ornithine transcarbamylase [7]. Arginine deiminase is a medicinal protein for cancer therapy of arginine auxotrophic tumors [8]. Curiously, several researches demonstrated that cancer cell migration has been influenced by the exhausting of arginine. Though motility is a normal physiological process associated with injury healing, embryonic development and immune responses, it is a process used by cancer cells to metastasize to other organs [2]. L-Arginine homeostasis depends on catabolism and transport efficiency of L-arginine through cell membranes. Approximately 80% of L-arginine comes from recycled amino acids released by protein breakdown. The significative impact on the performance of endogenous L-arginine synthesis is the availability of cytokines produced by the enterocytes. It is the pioneer of many substances substantial for the organism [9].

Arginine deiminase, a metabolizing enzyme extracted from *Mycoplasma*, catalyzes arginine to its pioneer citrulline [10]. Besides, *Lactobacillus plantarum* is a Gram-positive widespread probiotic beneficial bacterium, and it can produce arginine deiminase enzyme in the presence of arginine [11]. ADI activity is reported in many lactic acid bacteria including *Leuconostoc*, *Oenococcus*, *Streptococcus* and *Weissella* [12]. Production of ADI enzyme is used as routinely to identify many species of *Enterococcus* like *E. faecium* and *E. faecalis* but *E. pseudoavium*, *E. raffinosus* and *E. avium* can not produce this enzyme [13].

Bacillus cereus; It is a facultative aerobic Gr (+) bacterium that is plenty in air, soil and water. It's strains are psychrotrophic and mesophilic. Morever psychrotrophic strains are found in frozen foods and in some cases fresh nourishments. Mesophilic ones can grow at 37 °C and alive at temperatures below 10 °C. One of the most important features of these bacteria is that they can form endospores and biofilms under stress conditions (low temperature, pH, drought, diverse radiations etc.) [14].

Ralstonia eutropha (also *Alcaligenes eutrophus* or *Cupriavidus necator*) is rod-shaped microorganism with wideness of 0.5-1.0 µm and a long of 1.8-2.6 µm. It can use unlike organic synthesis as carbon sources. Optimum reproduction temperatures are about 30 ºC. It can degrade nitrates to nitrogen gas. Besides, it is one of the non-pathogenic proteobacteria belonging to the Gr (-) and facultative chemoliotrophic β- class [14]. It is model organism for PHB (Polyhydroxybutyrate) production and commercially can be used for dissimilar goals [15]. When growing heterotrophically, it can use an various organic compounds as the sources of carbon and energy [16]. This study was carried out to investigate the production of Arginine deiminase enzyme in static and shaking (150 rpm) environments in the presence of minimal carbon sources of Gram (-) and Gram (+) some microorganisms.

2. MATERIAL AND METHOD

2.1.Microorganism and Growth conditions

In this study; *Bacillus cereus* (ATCC 10876) and *Ralstonia eutropha* (ATCC17699) were used. They were produced in an oven at 37 ºC for 24 hours by passing them into Nutrient Agar media with loops at intervals of 20 days. The next day, the plates were removed from the oven and stored at +4 °C. Experimental stages were continued by producing bacteria in Nutrient Broth medium by using solid-to-liquid sowing method from these solid nutrient cultures that we prepared in the first stage of the experiment. In addition, Microorganisms were grown in static and shaking (150 rpm) conditions [17].

2.2.Carbon sources

1% Dextrose, 1% Glucose, 1% Fructose, 1% Maltose, 1% Nutrient Broth, 1% Rhamnose, 1% Ribose, 1% Sucrose, 1% Xylose.

2.3. Chemials

Tris HCl, $CaCl_2$, Dithiothreitol, Bovine Serum Albumin, Benzoyl Arginine Ethyl Ester, Perchloric acid, Iron Ammonium Sulphate Hexahydrate, Ammonium Iron (III) Sulphate Dodecahydrate, Phosphoric acid, H_2SO_4, Butanedione Monoxime, Nutrient Agar

2.4. Method

100 µl of bacterial cultures were inoculated into NB (Nutrient Broth), PBS and PBS, and nutrient media containing different carbon sources (1% Dextrose, 1% Glucose, 1% Fructose, 1% Xylose, 1% Maltose, 1% NB, 1% Rhamnose, 1% Ribose, 1% Sucrose) 200 µL Tris HCl (pH=7.2) + 100 µL $CaCl_2$ + 100 µL Dithiothreitol solution + 200 µL culture samples + 200 µL Bovine Serum Albumin solution was added.

It was kept on incubator at 55 ºC for min. 100 µL of Benzoyl Arginine Ethyl Ester solution was added. It was kept statically on incubator at 55 ºC for 30 minutes. 100

µL of Perchloric acid was added to the samples and the blank. Centrifugation (13.500 rpm) was performed for 5 minutes at room temperature.

400 µL of the prepared mixture was put into new glass tubes. 100 µL of Redox solution (Iron Ammonium Sulphate Hexahydrate + Ammonium Iron (III) Sulphate Dodecahydrate) was added. It was vortexed for 1 second and kept in boiling water for 10 minutes. This solution, which was kept in boiling water, was then cooled in tap water.

500 µL of the acid mixture (3 units of Phosphoric acid + 2 units of dH_2O and 1 unit of H_2SO_4) was added. 200 µL of Butanedione Monoxime solution was added to it.

The final mixture was vortexed, kept in boiling water for 20 minutes and then cooled in tap water.

The samples were measured at 490 nm against the blank (other mixtures except the enzyme solution and Bovine serum albumin solution) [18]. Arginine deiminase activitiy (U/mL) were found with the Citrulline Standard graph of the obtained data.

2.5. Preparation of Arginine deiminase standard graph

By taking different concentrations (5, 10, 20, 25 µg/mL) from commercially available pure citrulline and adding 200 µL Butanedione Monoxime solution + 500 µL Acid mixture (3 units of Phosphoric acid + 2 units of dH_2O and 1 units of H_2SO_4) on them. It was kept in boiling water for 20 minutes,

Cooled in tap water,
The prepared solutions were measured at a wavelength of 490 nm and a standard graph was obtained with the values obtained.
The amount of Arginine deiminase production of bacteria was compared with the standard graph and the amount was calculated as µg/mL [18].

3. RESULTS

In a static, stable environment, the microorganism has more oxygen and nutrients, but as the ambient conditions change, when agitation conditions such as 100, 150, 200 rpm are passed, the amount of oxygen and nutrients in the environment begins to decrease. Therefore, in this study, static and shaking culture media were preferred comparatively in order to understand whether microorganisms would react in these environments.
Nutrient Broth is a nutrient medium with a rich content (peptone, yeast extract, salt, water) in which microorganisms can grow. *Bacillus cereus* and *Ralstonia eutropha* showed high Arginine deiminase activity in this medium. *B. cereus* 14.799 (U/mL) under static conditions, *R. eutropha* 17.169; Under shaking conditions (150 rpm), *B. cereus* showed 9.08 (U/mL), *R. eutropha* 7.984 (U/mL).

Looking at minimal environments; The highest ADI activity in *B. cereus* under static conditions was 4.917 U/ml in Ribose medium and 4.265 U/mL in Dextrose medium in the aldose group; showed 3.747 U/mL in maltose medium among disaccharides. *B. cereus* showed ADI activity of 3.895 U/mL in fructose medium. It showed the lowest ADI activity in glucose medium at 2.221 U/mL. In agitated conditions (150 rpm), the highest ADI activity was 4.695 U/mL in Sucrose medium in disaccharides; In the aldose group, it showed 3.302 U/mL in Ribose medium. In agitated conditions, the lowest ADI activity was 2.813 U/mL in dextrose medium (Fig.1).

In *R. eutropha*, the highest ADI activity under static conditions was 3.776 U/mL in Ribose medium in the aldose group; 5.658 U/mL in the Fructose medium in the ketose group; showed 3.761 U/mL in Maltose medium among disaccharides. It showed the lowest ADI activity at 2.132 U/mL in rhamnose medium. In agitated conditions (150 rpm), the highest ADI activity was 4.813 U/mL in sucrose medium in disaccharides; showed 3.436 U/mL in Ribose medium in the aldose group. It showed 3.924 U/mL ADI activity in fructose medium, which is in the ketose group. In agitated conditions, the lowest ADI activity was 2.102 U/mL in rhamnose medium (Fig. 2).

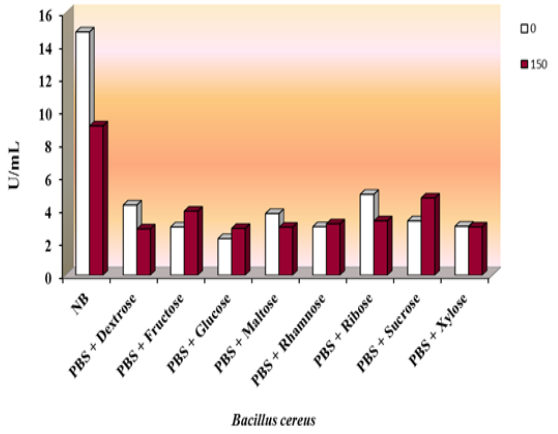

Figure 1. Arginine deiminase activity (U/mL) at 0 and 150 rpm under minimal conditions in *Bacillus cereus*

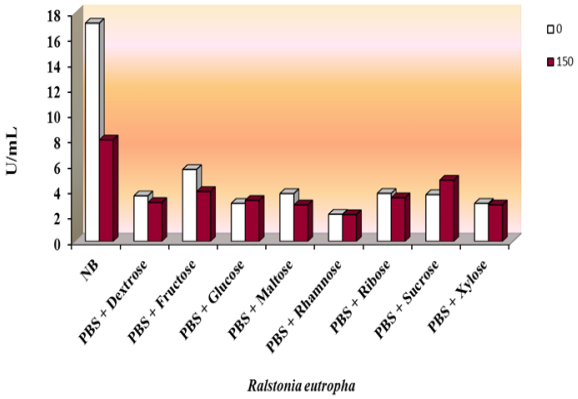

Figure 2. Arginine deiminase activity (U/mL) at 0 and 150 rpm under minimal conditions in *Ralstonia eutropha*

4. DISCUSSION AND CONCLUSION

Biologically structured molecules (for example, enzymes, proteins) provide the continuity of life by performing biochemical reactions in the living system. It is also used as therapeutic agents in cancer, coronary heart diseases, neurological disorders and many other diseases that cause fatal diseases. [19]. Arginine deiminase, is an important anti-cancer enzyme produced by many microorganisms. It is catalyzed by many microorganisms such as *Mycoplasma arginini*, *Pseudomonas aeruginosa* and some species of *Enterococcus* [13]. In the experiments of Sharma et al., *Pseudomonas aeruginosa* PS2 strain showed 3.12 IU/mL ADI activity in minimal medium + 0.5 % galactose medium [19]. In our study, in static conditions, the highest ADI activity was found in *B. cereus* PBS + 1 % Ribose medium at 4,917 U/mL (Fig.1); in agitated conditions, *R. eutropha* showed 4,813 U/mL in PBS + 1 % Sucrose medium (Fig. 2).

In the research of Dhankhar et al., *Pseudomonas furukawaii* RS3 strain showed the highest ADI activity at 0.222 µmol/mL in Super Broth medium + 1.5 % Fructose medium [20]. In our study, *B. cereus* was 3.895 U/mL (Fig. 1); *R. eutropha* showed 5,658 U/mL in PBS + 1% Fructose medium (Fig. 2). When we look at the results; *B. cereus* and R. *eutropha* showed the highest ADI activity in the disaccharide media, maltose and sucrose, and ribose in the aldose group.

Mahdy et al. (2014) conducted a study on ADI activity by adding different carbon sources to the Mineral Salt Broth medium under different conditions (1% Glucose, 1% Fructose, 1% Maltose, 1% Sucrose, 1% Arabinose, 1% Rhamnose, 1% Xylose, 1% Sorbitol and 1% Mannitol) with *Enterococcus faecium* M1 strain. *Enterococcus faecium* M1 showed the highest ADI activity in 1% Sucrose medium (3.9 U/mg) [21]. In our study; *R. eutropha* showed the highest ADI activity at 3.673 U/mL in PBS + 1% Sucrose medium under static conditions. *R. eutropha* showed the highest ADI activity at 4.813 U/mL under shaking conditions (Fig.2).

Ibrahim et al. (2019) conducted a study on ADI activity by adding 0.5% (glucose, galactose, lactose, maltose) different carbon sources to the minimal nutrient medium under different conditions with *Pseudomonas aeruginosa*. It showed the highest ADI activity in 0.5% maltose medium (1.2 U/mg) [22]. In our study; In PBS + 1% Maltose medium, *R. eutropha* showed the highest ADI activity of 3.761 U/mL under static conditions (Fig.2). In shaking conditions, *B. cereus* showed the highest ADI activity at 2.917 U/mL (Fig.1).

Khaleed Shaikh and Khobragade (2019); The Screening made a study on ADI activity by adding 0.2% (fructose, galactose, glucose, maltose, sucrose) different carbon sources to the medium with 11 bacterial isolates. These bacterial isolates showed the highest ADI activity in 0.2 % glucose medium (0.3-0.35 U/mL) [23]. In our study; *R. eutropha* showed the highest ADI activity at 2.991 U/mL in PBS + 1% Glucose medium under static conditions. *R. eutropha* showed the highest ADI activity at 3.228 U/mL under shaking conditions (Fig.2).

Unissa et al. (2015) investigated ADI activity by adding various carbon sources (glucose, glycerol, maltose, mannitol, sucrose) to a nutrient medium containing 2% sea water with *Vibrio alginolyticus* 1374 strain. It showed the highest ADI activity in the presence of 2% sea water + 2% Maltose (192 IU/mL) [24].

With this study, it has been shown that Arginine deiminase, an important anti-cancer enzyme, can be produced with Gram (+) (*B. cereus*) and Gram (-) (*R. eutropha*) bacteria in less costly minimal environments. In addition, it is thought that this study will contribute to the literature.

Acknowledgement

This research study was supported by a Grant (APYB 2020/2145) from Research Fund Unit of Inonu University, Turkey.

REFERENCES

[1] Iqbal J., Abbasi B.A., Mahmood T., Kanwal S., Ali B., Shah S.A., Khalil A.T. Plant-derived anticancer agents: A green anticancer approach, Asian Pac J Trop Biomed. 2017; 7(12): 1129–1150.

[2] Al-Koussa H., El Mais N., Maalouf H., Abi-Habib R., El-Sibai M. Arginine deprivation: a potential therapeutic for cancer cell metastasis? A review, Cancer Cell Inter. 2020; 20(150): 1-7.

[3] Prajapati B., Supriya N. R. Review on anticancer enzymes and their targeted amino acids, World J Pharm Res, 2017; 6(12), 268-284.

[4] Zolfaghar M., Amoozegar M. A., Khajeh K., Babavalian H., Tebyanian H. Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran, Mol Biol Rep, 2019; 46: 3275–3286.

[5] Özalp Erenler Ş. L-asparaginaz geninin (ansB) farklı gram-negatif bakterilere klonlanması, izolasyonu ve ekspresyonu (Doktora tezi). 2007; İnönü Üniversitesi, Fen Bilimleri Enstitüsü, Malatya

[6] Zarei M., Nezafat N., Rahbar M.R., Negahdaripour M., Sabetian S., Morowvat M.H., Ghasemi Y. Decreasing the immunogenicity of arginine deiminase enzyme via structure-based computational anal ysis, J Biomol Structure and Dynamics. 2019; 37(2): 523-536.

[7] Kahraman H., Karaderi C.C., The Importance of Arginine deiminase, Arch Chem and Chem Eng. 2019; 1(1): 1-3.

[8] Cheng F., Yang J., Schwaneberg U., Zhu L. Rational surface engineering of an arginine deiminase (an antitumor enzyme) for increased PEGylation efficiency, Biotechnol Bioeng. 2019;116: 2156–2166.

[9] Szefel J., Danielak A., Kruszewski W.J. Metabolic pathways of L-arginine and therapeutic consequences in tumors, Adv in Med Sci. 2019; 64:104-110.
[10] Zou S., Wang X., Liu P., Ke C., Xu S. Arginine metabolism and deprivation in cancer therapy, Biomed Pharm. 2019; 118:1-11.
[11] Zaki Mahdi N. Investigation of the lethal effect of purified Arginine deiminase purified from *Lactobacillus plantarum* p5 on murine mammary adenocarcinoma and vero cell lines, Archives of Razi Institu, 2022; 77(1): 223-229.
[12] Kaur B., Kaur R. Application of Response Surface Methodology for Optimizing Arginine deiminase Production Medium for *Enterococcus faecium* sp. GR7, The Sci World J. 2013; 1-12.
[13] Mahdy N.Z., Al-Tahan S.S., Yaseen N.Y., Optimization of Arginine deiminase production from a local higher productive isolate *Enterococcus faecium* M1, Iraqi J Cancer and Med Genet, 2014; 7(1):36-43.
[14] Karaderi C.C., Kahraman H. Trehalose Production at Different Mediums in *Bacillus cereus* and *Ralstonia eutropha*, Acta Sci Agricul. 2021; 5(10): 2-4.
[15] Juengert J.R., Borisova M., Mayer C., Wolz C., Brigham C.J., Sinskey A.J., Jendrossek D. Absence of ppGpp leads to increased mobilization of intermediately accumulated Poly (3-Hydroxybutyrate) in *Ralstonia eutropha* H16, Appl Environ Microbiol. 2017; 83(13): 1-17.
[16] Arenas-López, C., Locker, J., Orol, D., Walter, F., Busche, T., Kalinowski, J., Minton, N.P., Kovács, K., Winzer, K., The genetic basis of 3-hydroxypropanoate metabolism in *Cupriavidus necator* H16, Biotechnol for Biofuels. 2019; 12(150): 1-16.
[17] Karaderi, C.C., Bakterilerin Farklı Karbon Kaynaklarının Varlığında Arjinin deiminaz, Trehalaz ve Poli Hidroksi Bütirat Üretiminin Araştırılması (Doktora Tezi), İnönü Üniversitesi, Malatya. 2022.
[18] Takahara H., Okamoto H., Sugawara K. Enzymatic assay of peptidyl Arginine deiminase, J Biochem. 1986; 99:1417- 1424.
[19] Sharma A., Bala K., Husain I. Optimization of Arginine Deaminase production from Indigenous bacterium *Pseudomonas aeruginosa* PS2, Int J Curr Microbiol Appl Sci. 2017; 6(11): 3621-3632.
[20] Dhankhar R., Kumar A., Kumar S., Chhabra D., Shukla P., Gulati P. Multilevel algorithms and evolutionary hybrid tools for enhanced production of arginine deiminase from *Pseudomonas furukawaii* RS3, Bioresour Technol. 2019; 290: 1-9.
[21] Mahdy N.Z., Al-Tahan S.S., Yaseen N.Y. Optimization of arginine deiminase production from a local higher productive isolate *Enterococcus faecium* M1, Iraqi J Cancer Med Genet, 2014; 7(1): 36-43.
[22] Ibrahim R.A., Hussein A.A., Abdulwahed S. Optimizing arginine deiminase production from *Pseudomonas aeruginosa* clinical isolate, J Pharm Sci Res, 2019; 11(2): 656-660.
[23] Khaleed Shaikh A.N., Khobragade R.M. Production and partial purification of arginine deiminase isolated from bacteria, J Emerg Technol Innov Res, 2019; 6(3): 241-248.
[24] Unissa R., Sudhakar M., Kumar Reddy A.S. Condition optimization and production of extracellular l-Arginine deiminase from *Vibrio alginolyticus* 1374, Curr Biotechnol, 2015; 4: 254-260