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RESEARCH ARTICLE

Investigation of the relationship between bioluminescence and the production of α-amylase of the first bioluminescent *Vibrio gigantis* strains from Izmir Bay

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ABSTRACT

As an alternative to chemicals used in the industry, the use of enzymes is gradually increasing, with their high reaction specificity and their ability to show minimal by-product formation. In the detergent industry among the industrial areas where enzyme use is widespread; due to high washing temperatures, loss of activity of the detergent and high energy consumption, cold active enzymes that exhibit high catalytic activity at low temperatures and have the potential to save energy are noteworthy. As one of these enzymes, α-amylase is intensely produced by marine bioluminescent microorganisms that show optimum microbial activity at 20°C. However, since the enzyme production differs among microorganisms, selection of the most suitable microorganism to be used in production is very important. In this study, based on the idea that bioluminescence will benefit by facilitating the selection of microorganisms that will come to the fore for α-amylase production, the relationship between bioluminescence and the production of extracellular α-amylase enzyme of *Vibrio gigantis* strains, which were obtained from the sources of our country, were isolated from Izmir Bay and were determined to have a high rate of α-amylase production, and which was the first record in terms of bioluminescent properties, was investigated. Among 20 *V. gigantis* strains, 2 different microorganisms, which are thought to be more advantageous in terms of enzyme production and bioluminescence, were selected and the extracellular protein and α-amylase production amounts of these organisms as well as the amount of bioluminescence were measured. By evaluating the data obtained as a result of the studies carried out, further studies were carried out with 2 strains, S2W42 and FU-9 gill, which exhibit both low and high enzyme activity. Also, an inverse relationship was observed between α-amylase enzyme activity and bioluminescence. It has been determined that both microorganisms used are effective in α-amylase production and can be used as model organisms in cold active enzyme production. For this reason, it is thought that our study will shed light on comprehensive studies to be carried out in the relevant field.

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Introduction

Bioluminescence; is an interesting chemical reaction in which visible light is produced as a result of an enzyme-catalyzed oxidation reaction emitted by light-forming organisms (Ramesh & Mohanraju, 2015; Brodl et al., 2018). Although the benefit of bioluminescence, which has evolved independently dozens of times, cannot be determined for some species, in many cases; it is known that providing visual communication for behaviors such as attracting prey or intimidating predators provides a significant advantage to organisms in ecological terms (Fleiss & Sarkisyan, 2019). At the same time, it has also been reported that marine organism use bioluminescence for physiological events such as food recycling, concealment, mating and defense (Sharifian et al., 2018).

This feature, which attracts great attention in the world of science; is found in many phyla from fish to bacteria, bioluminescent organisms that persist in terrestrial, freshwater and marine ecosystems, and is observed in a large number of species (Nunes-Halldorson & Duran, 2003; Tanet et al., 2020). Such that; although the ability to emit light in the dark is observed in 800 genera containing approximately 10,000 species, it is thought that this number may actually be higher (Fleiss & Sarkisyan, 2019). Bioluminescent bacteria, which constitute an important group of these organisms, can survive in relation to marine animals by forming a symbiotic relationship with a host animal as well as being able to live in free state (Burtseva et al., 2020). In symbiotic forms, they are usually colonized in the light organs and intestines because they have more favorable growth conditions than open oceans and in the light of available information, most of the known bacterial luminous species show heterotrophic, copiotrophic and facultative anaerobic characteristics (Tanet et al., 2020).

Bioluminescence in bacteria occurs as a result of the reaction between molecular oxygen, a coenzyme (a mononucleotide flavin in a reduced state, $FMNH_2$) and a long chain aldehyde catalyzed by the luciferase enzyme. While it is seen that the products formed as a result of the reaction are FMN (in oxidized state), water and carboxylic acid, a photon is emitted during the reaction at the same time. While the oxidized flavin (FMN) and the carboxylic acid formed in the reaction are recycled by parallel reactions, the light production occurs continuously by living cells (Erzinger et al., 2018).

Looking at the literature; although interest in bioluminescent bacteria and light regulation has continued for a long time, the majority of studies have been limited to two model organisms, *Aliivibrio fischeri* and *Vibrio campbellii* (previously identified as *Beneckea* or *V. harveyi*). However; although bioluminescent bacteria cannot be limited to these two species, 25 bacterial species belonging to 5 genera have been discovered in the families of *Shewanellaceae* (*Shewanella*), *Enterobacteriaceae* (*Photorhabdus*) and *Vibrionaceae* (*Aliivibrio*, *Photobacterium* and *Vibrio*), which are three families of Gammaproteobacteria (Tanet et al., 2019).

Genetic, physiological and biochemical studies are carried out within the scope of bioluminescence, which has a wide application potential in different fields, and the progress achieved as a result of these studies sheds light on studies in ecological, medical and industrial fields (Nunes-Halldorson & Duran, 2003). Considering the industry as one of these fields; enzymes, which constitute an important alternative to chemicals used in many processes, are seen as substances with minimal risk potential in terms of environmental health with their reaction specificities and minimal by-product generation properties (Özcan & Çorbacı, 2018).

Industrial enzyme purification applications are generally focused on lipases, proteases and amylases (Gopinath et al., 2017). Amylases, which have a 30% share of the enzymes produced in the world today; it attracts attention with its potential applications in the textile, pharmaceutical, fermentation, detergent, food and paper industries (Özcan & Çorbacı, 2018). Amylases, which are starch-degrading enzymes, act on α-1-4 glycosidic bonds and are also called glycoside hydrolases, are widely distributed in living systems (Gopinath et al., 2017; Özcan & Çorbacı, 2018). In particular, enzymes known as cold active enzymes come to the fore with their high catalytic activity at low and medium temperatures, the need for low amounts of enzyme for efficient catalysis, and their economic advantage as a result of their energy-saving applications (Al-Maqtari et al., 2019).

In general terms, biologically active enzymes are obtained by animals, plants and microorganisms. But among these groups; it brings to the fore microorganisms that have the characteristics of being stable under extreme conditions due to their easy and high isolation, low cost and short time-consuming production and less harmfulness. In addition to these advantages, the production and expression of recombinant enzymes are easier in cases where the host cell is a microorganism (Gopinath et al., 2017). In this context; because of the aforementioned advantages, especially amylase-producing microorganisms adapted to low temperature and alkaline conditions are becoming popular for commercially important applications, especially in the detergent industries. For example, with respect to cold active amylase determined to be compatible with detergents, α-amylase was isolated from *Zunongwangia profunda*, a marine bacterium, and was found to be compatible with detergents. And then, it was determined

that the recombinant enzyme showed activity even at very low temperatures such as 0-5°C with the expression of the relevant gene in *Escherichia coli* (Al-Ghanayem & Joseph, 2020).

In the light of all this information, it is thought that bioluminescence, which has wide application areas as mentioned above, will have an effective contribution. In this study, it is aimed to reveal the relationship between extracellular α-amylase enzyme production and bioluminescence of *V. gigantis* strains, which were obtained from the sources of our country and were found to produce a high rate of extracellular α-amylase enzyme and at the same time recorded bioluminescence by us for the first time. With the data obtained as a result of our study, it was determined whether it is appropriate to use bioluminescence in pre-screening stages to select the strain that produces the highest amount of α-amylase enzyme from bioluminescent marine organisms, which are target organisms for the production of commercially important cold active α-amylase enzyme. As a result, it is thought that this study will facilitate strain selection in this context and shed light on further studies that can produce cold active α-amylase enzyme on an industrial scale.

Material and Methods

Activation of V. gigantis Strains

Twenty bioluminescent *V. gigantis* strains isolated from different seawater, sediment, and fish samples and collected from regions at discrete depths in the Gulf of Izmir were used for this study (Table 1). The bioluminescent bacteria identified by phenotypic and molecular methods (Ersoy Omeroglu, 2011; Ersoy Omeroglu & Karaboz, 2012; Ersoy Omeroglu et al., 2014) were streaked onto a Seawater Complete Agar (SWC) medium to obtain a single colony. After checking the purity of the strains, they were grown in a liquid SWC medium (Liu et al., 2003). Following the inoculation procedures, the strains were incubated at 20°C for 17 hours (Ersoy Omeroglu & Karaboz, 2012). For the activation and bioluminescence controls (Figure 1), the microorganisms showing the most emission were determined by comparing the biological luminescence observed on the plates belonging to the strains.

Table 1. Some properties of bioluminescent *V. gigantis* strains used in the present study (Ersoy Omeroglu, 2011; Ersoy Omeroglu & Karaboz, 2012; Ersoy Omeroglu et al., 2014).

Strain No	Strain	Isolation Date	Accession Number	Source	Coordinate	Depth (m)
1	SW15	30 March 2007	JF412215	Seawater	38°29'03" N – 26°47'05"E	0-15
2	SWPort	30 March 2007	JF412216	Seawater	38°27'22" N – 27°09'65"E	
4	Selü25	30 March 2007	JF412217	Sediment	38°23'50" N – 26°39'00"E	
6	S2W42	22 January 2008	JF412218	Seawater	38°24'58" N – 26°56'88"E	
7	S2W9	22 January 2008	JF412219	Seawater	38°34'99" N – 26°39'00"E	
8	S3W46	17 April 2008	JF412220	Seawater	38°26'70" N – 27°06'10"E	
9	S3W28	17 April 2008	JF412221	Seawater	38°23'50" N – 26°55'00"E	
10	S3W2	17 April 2008	JF412222	Seawater	38°40'90" N – 26°34'90"E	
12	Se2Lü48	22 January 2008	JF412223	Sediment	38°24'75" N – 26°58'90"E	
15	Se3Lü25	22 January 2008	JF412224	Sediment	38°23'50" N – 26°39'00"E	
19	FU-10 internal	30 March 2007	JF412225	*Mullus barbatus* (internal area)	Between 38°37'00"N – 26°42'20"E and 38°37'45"N – 26°43'30"E	67
20	FU-9 gill	30 March 2007	JF412226	*Diplodus annularis* (gill)		
31	E-14 gill	17 April 2008	JF412227	*Lepidotrigla cavillone* (gill)	Between 38°34'40"N – 26°46'10"E and 38°33'45"N-26°46'55"E	42-44
32	E-16 surface	17 April 2008	JF412228	*Boops boops* (surface)		
33	E-15 surface	17 April 2008	JF412229	*D. annularis* (surface)		
34	E-10 gill	17 April 2008	JF412230	*Citharus linguatula* (gill)		
36	E-11 internal	17 April 2008	JF412231	*Arnoglossus laterna* (internal area)		
44	H-3 surface	06 August 2008	JF412232	*Merluccius merluccius* (surface)		
49	H-2 gut	06 August 2008	JF412233	*D. annularis* (intestine contents)		
50	H-16 gill	06 August 2008	JF412234	*B. boops* (gill)		

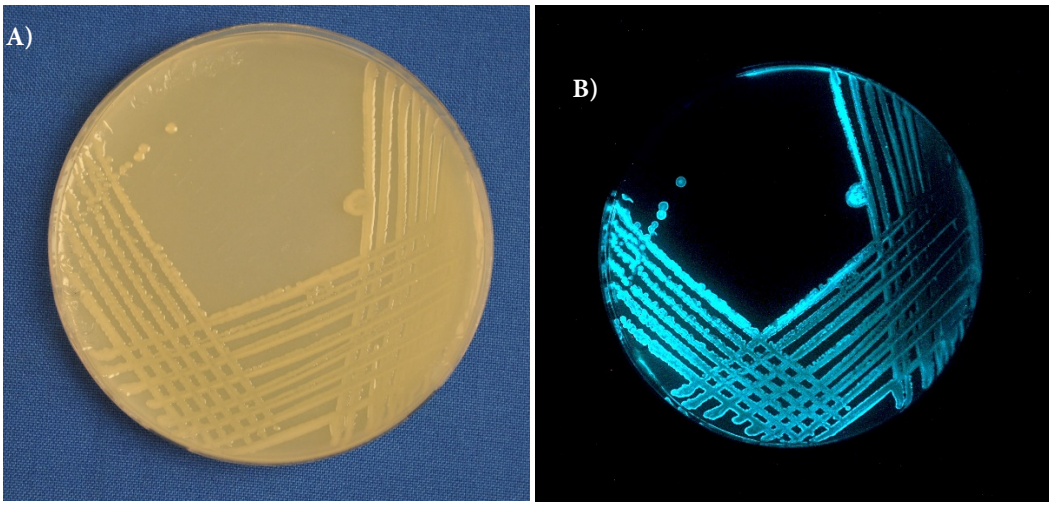

Figure 1. Streak plate images of *V. gigantis* SW15 strain. A) In the light; B) In the dark

Determination of Extracellular α-Amylase Production

Activated *V. gigantis* strains were inoculated into Starch Agar (SA) medium to determine their extracellular enzyme production and incubated for 17 hours at 20°C. At the end of the incubation period, gram iodide was dropped on the plates and left for 10 minutes incubation in order to observe the zone diameters. After the incubation period, the light-colored zone diameters observed around the colony and considered as positive results were measured (Özcan & Çorbacı, 2018).

By evaluating the amount of biological luminescence observed in the petri dishes during the activation phase and the zone diameters obtained in the enzyme production trials, the microorganisms with the highest bioluminescence and zone diameter formation were determined. The strain number 6 *V. gigantis* (S2W42) isolated from the marine environment and the strain number 20 *V. gigantis* (FU-9 gill) isolated from the gill of *D. annularis*, which were thought to have an advantage in terms of the hypothesis of the study, were selected and used in the other stages of the study. At the same time, while these two organisms were selected; their isolation from different regions has also been taken into account in terms of providing diversity.

Extracellular Protein Quantification by Bradford Method

Protein quantification was performed using the Bradford method for *V. gigantis* (S2W42) and *V. gigantis* (FU-9 gill). In order to obtain extracellular proteins, the nutrient medium was centrifuged at 10,000 rpm for 5 minutes and the supernatant obtained was used as a protein sample in the next steps. The standard was set containing 15 different concentrations of Bovine Serum Albumin (BSA), ranging from 0.02 to 2 mg/mL. In order to prevent all organic contamination that may cause erroneous results in enzyme amount measurements, all glass materials that may be required in the experiment were acidified and cleaned with distilled water before application. It was then dried in a Pasteur oven. After the determined concentrations and samples were prepared and reagent was added to them, color variations were observed at the end of incubation. After the incubation period, extracellular protein was determined using the spectrophotometric method (Yaşa & Çadırcı, 2007). In this study, standards and samples have been studied in duplicate.

Detection of Reducing Sugar

Dinitrosalicic acid (DNS) was used as a color reagent to detect the reducing sugar that will be formed by hydrolysis as a result of the interaction of starch with amylase. All glass materials used were acidified, cleaned with distilled water, dried in the Pasteur oven and used. Thus, the possibility of protein contamination was prevented. The organisms to be used were inoculated into Starch Broth (SB) medium and incubated at 20°C for 17 hours. Maltose standard solution (4 mg/mL stock solution), phosphate buffer, starch solution and DNS as reagent were prepared to be used in assay. From 4 mg/mL stock maltose solution, 7 standards were determined from 0.025 to 0.9 mg/mL. The determined concentrations and samples were prepared, vortexed and then incubated at 25°C for 5 minutes. At the end of the incubation, the DNS reagent was added to the tubes, mixed with vortex, kept in boiling water for 10 minutes and then allowed to cool. After the tubes were cooled, they were taken into polystyrene cuvettes and spectrophotometrically measured at 546 nm wavelength (Yaşa & Çadırcı, 2007).

Bioluminometric Measurements

Bacterial inoculations were made by preparing SB and Nutrient Broth (NB) with 70% natural seawater to be used in measurements with a white 96-well plates. The strains were incubated at 20°C for 17 hours in order to obtain bacteria in the logarithmic phase. Following the incubation period, transfer of the medium prepared to the wells determined on the microplate and microorganism inoculations were performed. To each well, 180 µL of the medium was added and then 20 µL of bacterial suspension with a turbidity equivalent to 1.0 McFarland (Osawa et al., 1997; Musa et al., 2008; Ersoy Omeroglu et al., 2014) was transferred into each well. *V. gigantis* (S2W42) and *V. gigantis* (FU-9 gill) inoculated to SB media and in addition, both strains were inoculated in the same way in NB media containing 70% natural seawater. These groups formed PK1 and PK2, respectively. Non-inoculated SB and NB media were also included in the measurements and designed as negative controls and evaluated as NK1 and NK2, respectively. 17 hours and 41 hours after the completion of the inoculation in the determined wells in white 96-well plates, two measurements were made with a bioluminometer, the unit of which is Relative Light Unit (RLU) (Tanet et al., 2019). In order to increase the reliability of the measurement results, the inoculations were made on a microplate in 3 repetitions and the average value was calculated in the measurement results.

Results

Detection of Extracellular α-Amylase Production

The measurement results of the zone diameters resulting from the enzyme activities of bioluminescent *V. gigantis* strains were given in Table 2. When the formed zone diameters are evaluated; it is seen that SWPort, S3W46 and S3W28 strains obtained from different locations from sea water stand out as the species with the highest activity. The lowest activity result, on the other hand, was obtained from surface swabs (E-15 surface) of *D. annularis* as a result of sampling in the spring season. However, it was determined that extracellular α-amylase enzyme production was not performed in two of the 20 strains studied (FU-10 internal and E-11 internal) because no zone formation was observed. Considering their characteristics such as the amount of bioluminescence (Table 2), growth rate and enzyme activity, 2 strains were determined at this stage and studies were continued with these 2 strains. In the last step of the selection, 2 strains with both low and high enzyme activity were decided and further studies were carried out with S2W42 and FU-9 gill.

Table 2. Observed zone diameters based on extracellular α-amylase production and the amount of bioluminescence by *V. gigantis* strains

Strain No	Strain	Zone Diameter (mm)	Bioluminescence
1	SW15	5.0	+[c]
2	SWPort	8.0	++
4	Selü25	5.0	++[b]
6	S2W42	5.0	+++[a]
7	S2W9	7.0	+
8	S3W46	8.0	++
9	S3W28	8.0	+
10	S3W2	4.0	+++
12	Se2Lü48	5.0	+
15	Se3Lü25	5.0	++
19	FU-10 internal	*	+++
20	FU-9 gill	7.0	+++
31	E-14 gill	5.0	+++
32	E-16 surface	6.0	+
33	E-15 surface	2.0	+++
34	E-10 gill	6.0	+
36	E-11 internal	*	+
44	H-3 surface	5.0	++
49	H-2 gut	6.0	++
50	H-16 gill	6.0	++

Note: *: The zone is not detected. [a]: Good bioluminescence; [b]: Average bioluminescence; [c]: Poor bioluminescence.

Extracellular Protein Quantification

As a result of the spectrophotometric measurements performed as specified in the method, a standard graphic was created and its reliability was checked based on the R^2 value. Protein calculation was performed by obtaining the necessary formulation. The standard graphic and the amount of protein were given in Figure 2 and Table 3, respectively.

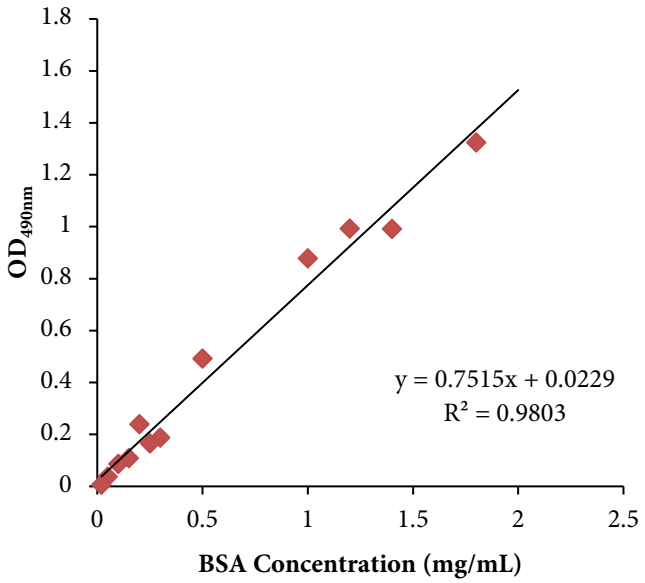

Figure 2. Bradford assay standard curve for the absorbance of BSA concentration

It has been determined that the amount of extracellular protein produced by *V. gigantis* strain FU9 (gill) obtained from the intestinal contents of *D. annularis* is higher than the amount of protein obtained from S2W42, the strain obtained from sea water (Table 3).

Detection of Reducing Sugar

The maltose standard graph obtained as a result of the measurements performed spectrophotometrically at 546 nm wavelength was shown in Figure 3. With the formulation obtained on the graphic, the amount of α-amylase and the values of specific activity were calculated and given in Table 3.

Extracellular enzyme amounts and activities were calculated using the graphic created based on the determination of reducing sugar. Although the amount of extracellular protein produced by S2W42 was less, it was found that the amount and activity of α-amylase produced as expected was higher. Although, as a result of the experiment performed on agar plates, a zone diameter of 5 mm was obtained for S2W42 and 7 mm for FU9 (gill); as a result of the activity determination performed by spectrophotometric method, it was determined that the opposite situation was in question. When we compared these two strains in terms of the amount of α-amylase produced, it was revealed that there was a 5.5% difference. The same ratio was observed in enzyme activity values. The difference of 35.6% in the amount of extracellular protein produced by bioluminescent *V. gigantis* strains showed its effect in specific activity. It was determined that the S2W42 strain had 39% higher specific activity (Table 3).

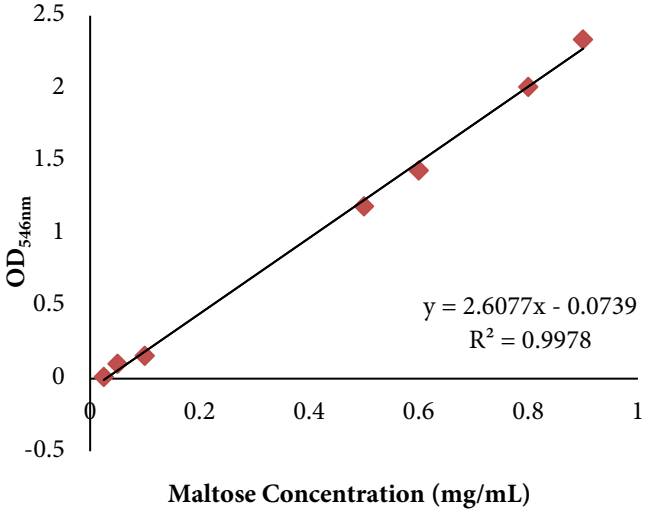

Figure 3. Standard curve for maltose determination assay

Table 3. Extracellular protein amount, α-amylase amount, α-amylase enzyme activity, specific activity and bioluminescence values of *V. gigantis* strains

Strain No	Strain	Extracellular Protein Amount (mg/mL)	α-amylase Amount (mg/mL)	α-amylase Enzyme Activity (IU/mL)	Specific Activity (IU/mg)	Bioluminescence (17th hour) (RLU)
6	**S2W42**	0.0551	0.0586	0.2344	4.254	11.5
20	**FU-9 (gill)**	0.0856	0.0554	0.2216	2.5887	27.3

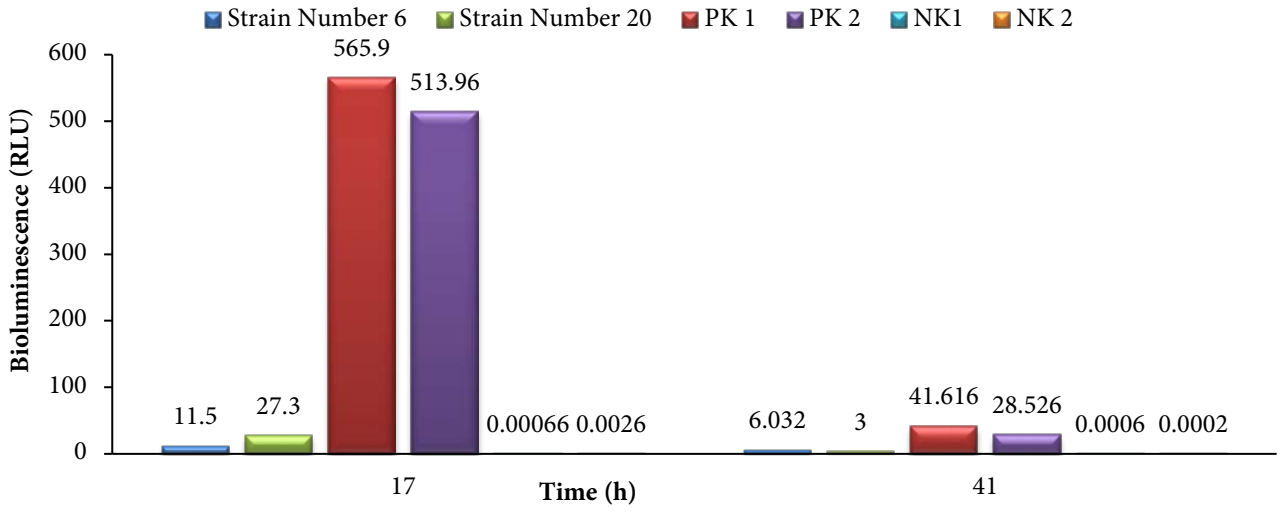

Figure 4. 17th and 41th hours measurement results performed with luminometer. PK1: Positive control 1 (NB+*V.gigantis* (S2W42) strain number 6), PK2: Positive control 2 (NB+*V.gigantis* (FU-9 gill) strain number 20), NK1: Negative control 1 (SB), NK2: Negative control 2 (NB)

Bioluminescence Measurement

The results of the measurements performed with the bioluminometer at 17 hours after inoculation and 24 hours after the first measurement are shown in Figure 4.

It was observed that there is an inverse relationship between α-amylase enzyme activity and bioluminescence in the bioluminescent strains selected within the scope of the study. As a common feature for both strains, it was determined that there was a significant decrease in the amount of bioluminescence at the 41st hour of incubation both in the experimental environment and in the positive controls. Especially, it was determined that the longer the incubation time had a more negative effect on FU9 (gill) (Figure 4).

Discussion

Amylases as extracellular enzymes that hydrolyze the starch constitute a significant 30% of the world enzyme market. Cold active amylases are suitable for application in detergents for cold washing purposes, as well as in various industrial areas such as food industry, textile, paper, molecular, pharmaceutical, bioremediation, alcohol production and biofuels. They come into prominence with their low energy consumption due to their active activity at low temperatures and economic benefits (Arabacı & Arıkan, 2018; Al-Maqtari et al., 2019).

Considering detergents that have an important share in the industrial field, it is thought that it is possible to achieve an environmentally friendly approach by using enzymes compatible with detergents instead of harmful chemicals as additives and The addition of α-amylase enzyme to detergents to effectively remove starch-based dirt and stains is an example of applications in this context. In this direction, the isolation and feasibility studies of cold active amylases to be used as detergent additives have gained continuity (Al-Ghanayem & Joseph, 2020) and therefore, microorganisms that can be a source of cold active enzymes are very valuable (Arabacı & Arıkan, 2018).

Studies on amylase obtained from bacteria, yeast and fungi continue in industrial fields and scientific research. Bacteria that are among the groups of organisms that show amylase production come to the fore because they give faster results and require less cost, and they have a wide application potential that includes the production of recombinant enzymes for genetic engineering studies. But, the amylase production level varies from microorganism to microorganism even between the same genus, species and strains (Gopinath et al., 2017). Therefore, organism selection constitutes an important step in enzyme production.

Based on this point, in order to be used bioluminescence in the selection of the advantageous strain in terms of enzyme production; within the scope of our study, 20 different bioluminescent strains of *V. gigantis* were examined in terms of biological luminescence and extracellular α-amylase production activities. As a result of the pre-screening, 2 bioluminescent strains that showed a high amount of biological radiation and obtained a high zone diameter due to extracellular enzyme activity, so that it was seen to be advantageous by comparing both criteria. Trials were continued to explain the relationship between bioluminescent and extracellular enzyme activity with the selected strain number 6 *V. gigantis* (S2W42) and strain number 20 *V. gigantis* (FU-9 gill). Such that; for this study, our initial research hypothesis was that as bioluminescence increases, extracellular α-amylase activity will increase, so the organism with high bioluminescence will show a high level of enzymatic activity. Thus; based on this hypothesis, and by identifying the organisms that can be used in the production of cold active α-amylase enzyme, the first step of producing α-amylase enzyme, the pre-screening step, was considered to be completed.

If the data obtained as a result of our study are evaluated, differences were observed in both organisms that did not comply with our hypothesis. First, when we look at the data of strain number 6 *V. gigantis* (S2W42), when the organism is inoculated into SB medium other than SWC agar, which is the closest nutrient medium to its own environment, there was a decrease in its bioluminescence, but an increase in enzyme production.

Secondly; when the data of *V. gigantis* (FU-9 gill) are examined, it was determined that its bioluminescence was 3 times higher than *V. gigantis* (S2W42) by showing the opposite characteristics when they were inoculated into SB medium instead of SWC agar, which is the medium closest to its own growth medium. Considering that bioluminescent microorganisms need to use 20% of their metabolic energy to perform bioluminescent activity; it is concluded that when strain number 6 *V. gigantis* (S2W42) is inoculated in a different environment, it spends most of its energy on enzyme production, but strain number 20 *V. gigantis* (FU-9 gill) consumes its energy in bioluminescence when under the same conditions.

It is thought that there are many reasons that will produce these results. The first reason is that the organisms are only 80% similar as stated by Ersoy Omeroglu & Karaboz (2012). This situation is in line with the knowledge in the literature that there is a difference in enzyme production even between species and strains among organisms (Gopinath et al., 2017). Another reason is that the isolation sources of organisms are different.

Strain number 6 *V. gigantis* (S2W42) was isolated from sea water. Therefore, it is thought that the bioluminescence is high in the SWC agar medium, which is the closest to its own habitat, but there is a decrease in its bioluminescence and an increase in enzyme production when taken to another environment. The strain number 20 *V. gigantis* (FU-9 gill) differs in that it is isolated from the gill of *D. annularis*.

Another possibility that may cause this difference is that reproductive temperatures differ between bioluminescent microorganisms. It was revealed by Ersoy Omeroglu & Karaboz (2012) that strain number 6 *V. gigantis* (S2W42) reproduced at 30°C and strain number 20 *V. gigantis* (FU-9 gill) at 27°C. At the same time, considering the temperature grouping of microorganisms from which cold-active enzymes are isolated, it is stated in the literature that most of the cold-active enzymes are isolated from psychrophil and psychlotolerant microorganisms, but also from microorganisms with mesophilic and even thermophilic properties (Santiago et al., 2016). According to an information based on the literature; the cold active α-amylase enzyme was isolated from *Bacillus subtilis* N8 at 15°C and pH 10, and the enzyme showed the highest activity at 25°C and remained stable at pH 8. It also showed a very high stability of 96% in a wide temperature range of 10-40°C (Al-Ghanayem & Joseph, 2020). With this information, the idea of using cold active enzymes for the production of bioluminescent bacteria used in our study is supported due to the similarity of the temperature values at which they reproduce. In terms of the convenience it will provide in the isolation and subsequent examination studies, it is thought that it would be advantageous to use bioluminescent bacteria used in this study as a model instead of extreme organisms that survive at low temperatures.

As a result of this study, it has been proven that both bioluminescent *V. gigantis* strains produce α-amylase and can be used as model organisms in cold active enzyme production. The data obtained and the approaches put forward will form the basis for future studies to be carried out in the relevant field.

Conclusion

Nowadays, concerns about harmful chemicals used in the industry are increasing and efforts are being made to replace these chemicals with enzymes that provide advantage with their high specificity and low by-product formation. The high temperatures used in the detergent industry, which has a very wide market, reduced detergent effectiveness and high temperatures negatively affected energy use, which required alternative solutions to be found. Cold active enzymes come to the fore with their high activity at low temperatures. Therefore, obtaining α-amylase, one of the cold active enzymes, has gained importance. Microorganisms, especially bacteria, constitute an important source for the α-amylase enzyme, with their advantages such as high-speed growth and low cost. At this point, it is thought that bioluminescence as a method to be used in the selection of organisms that differ in enzyme production will accelerate the related studies.

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Compliance With Ethical Standards

Authors' Contributions

EEO designed the study. EEO and AB wrote the manuscript. EEO, AB and BST performed laboratorial work. EEO critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

Ethical Approval

For this type of study, formal consent is not required.

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