SPECTRAL CHARACTERIZATION OF AN EXTRACELLULAR PIGMENT SYNTHESIZED BY THE HALOTOLERANT ISOLATE T3SII 7.2

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Abstract. In this study, a microorganism was isolated that has the ability to grow on lytic support as shown by the results obtained from SEM and AFM microscopy analyses and to synthesize bioactive compounds such as melanin pigments. The results obtained showed that the isolate is a Gram-negative bacillus that exhibits catalase but not oxidase activity. Optimal growth was obtained on a culture medium supplemented with NaCl in concentrations of 0.5M and 1M, as well as at concentrations of 0.1M and 0.5M $MgCl_2$. Following the testing of four experimental variants of the Na^+/Mg^{2+} ionic ratio, the optimal pH value range was established between 5 and 9 units. Considering the fluctuations in temperature values in the ecosystem from which the investigated isolate comes, its development and the synthesis of melanin pigments were evaluated at temperatures of 25, 30, 37, 40 and 45 degrees Celsius. It was observed that the optimal growth temperature is 25^0C, and an increase in it negatively affected the development of the microorganism and pigment synthesis. Spectrophotometric analyses revealed absorption spectra specific to melanin, similar to those in other specialized studies.

Keywords: melanin pigments, halotolerant bacteria, spectral studies.

Rezumat. Caracterizarea spectrală a unui pigment extracelular sintetizat de izolatul halotolerant T3SII 7.2. În acest studiu a fost izolat un microorganism care are capacitatea de a se dezvolta pe suport litic după cum arată rezultatele obținute în urma analizelor de microscopie SEM și AFM și de a sintetiza compuși bioactivi precum pigmenții melanici. Rezultatele obținute au arătat că izolatul este un bacil Gram-negativ care prezintă activitate catalazică dar nu și oxidazică. Creșterea optimă a fost obținută pe un mediu de cultură suplimentat cu NaCl în concentrații de 0,5M și 1M, precum și la concentrații de 0,1M și 0,5M $MgCl_2$. În urma testării a patru variante experimentale ale raportului ionic Na^+/Mg^{2+} a fost stabilit intervalul optim al valorii pH cuprins între 5 și 9 unități. Având în vedere fluctuațiile valorilor de tempratură în ecosistemul din care provine izolatul investigat, dezvoltarea acestuia și sinteza de pigmenți melanici a fost evaluată la temperaturi de 25, 30, 37, 40 si 45 grade Celsius. S-a observat că temperatura optimă de creștere este de 25^0C, iar o creștere a acesteia a afectat negativ dezvoltarea microorganismului și sinteza de pigment. Analizele spectrofotometrice au pus în evidență spectre de absorbție specifice melaninei, similare cu cele din alte studii de specialitate.

Cuvinte cheie: pigmenți melanici, bacterii halotolerante, studii spectrale.

INTRODUCTION

The importance and role of microorganisms living in environments with polyextremophilic conditions have been ignored for a long time by the scientific community. In recent years, studies on their importance and significance for applied sciences have developed greatly, highlighting the involvement of extremophilic microorganisms belonging to the domains Archaea and Bacteria in various biochemical processes in nature. Polyextremophily can be correlated with the evolution of life on Earth by relating to the main physicochemical parameters that define them (OMELON et al., 2006; OMELON, 2016; MERINO et al., 2019).

Pigments are molecules that are also defined by the characteristic absorption at a certain wavelength of the visible spectrum, usually in the 380-750 nm range. Their synthesis is one of the characteristic features of certain microorganisms. Microbial pigments are also defined as a mixture of diverse chemical components with multiple potential biological activities (SOLIEV & ENOMOTO, 2013). For example, the importance of microbial pigments is noted in various applications, such as the cosmetic, food, pharmaceutical and textile industries, but their potential cytotoxic, antioxidant, antimicrobial, antitumor activity and even as antifouling agents is equally well known (VENIL et al., 2013; NUMAN et al., 2018).

Lactoflavin is considered the first yellow pigment obtained from milk in 1879 and in 1932 studies led to the isolation of a pigment with a similar color from aqueous extracts of yeasts which after fractionation was identified as riboflavin (REVUELTA et al., 2016). During the 1970s, bacteriorhodopsin was identified, a pigment considered specific to the genus *Halobacterium*. A series of studies have so far reported over 65 pigmented compounds produced by *Monascus* sp. some with antimicrobial, anticancer and obesity-related activities (JONGRUNGRUANGCHOK et al., 2004; AGBOYIBIOR et al., 2018).

Microbial pigments can be secreted into the extracellular environment or are retained in cells due to biological properties important for their metabolic activity. From the point of view of chemical structure, they can be mainly grouped into four categories, namely carotenoid pigments which are also the most abundant (RAMESH et al., 2019), flavin pigments, melanic pigments and those with heterocyclic structure.

Bacterial melanic pigments act as cell protectors by neutralizing toxic chemical compounds such as xenobiotics but also contribute to adaptation to modified physiological conditions under the influence of certain ecological factors perceived as sources of stress (PLONKA & GRABACKA, 2006). A series of studies have shown that

melanic pigments produced by *Rhizobium* species are involved in the detoxification of polyphenolic compounds accumulated in senescent nodules. Fungal melanins are also known to have a protective role against the action of UV and solar radiation but also to inhibit enzymes that degrade cell walls.

The role of microbial pigments in the form of antioxidant activities, in the process of photosynthesis, cell signaling, radiation protection, UV capture, antibiotic activities, antiviral factors and membrane stabilization is known. Also, microbial pigments are used as biological markers for taxonomic identification and classification of different categories of microorganisms (OREN, 2011).

Bacterial pigments have extensive applications in textile dyeing, cosmetics, food coloring, painting, pharmaceuticals, plastics, etc. and are considered to be predominant in industrial applications in the near future. Considerably, consumer demand for important natural microbial pigments with food quality, such as β-carotene, riboflavin and phycocyanin is increasing in specific markets (VENIL et al., 2013; 2014). Food pigments act as preservatives and have antioxidant roles (NIGAM & LUKE, 2016).

The main purpose of this work was represented by studies on the spectral characterization of an extracellular pigment synthesized by the halotolerant isolate T3SII 7.2. In this regard, a study ecosystem represented by the zeolitic volcanic tuff from the Malul Alb area (Buzău county) located in the vicinity of the Meledic salt plateau was chosen, characterized by extremophilic conditions, respectively with low water activity and reduced nutrient availability, from which the isolation, purification and identification of microorganisms was carried out, by applying investigation methods such as electron microscopy (SEM, AFM), optical microscopy, microbiology and biochemistry techniques. The production of pigments and their spectral characterization were highlighted.

MATERIAL AND METHODS

The investigated samples were represented by fragments of volcanic tuff rock from the Malul Alb area, Buzau County. These were transported to the laboratory in sterile containers where they were ground using a hammer in a sterilized cotton material. Fragments with dimensions of approximately 0.5 mm were obtained, suitable for subsequent inoculations on culture media for the isolation of microorganisms present in the lithic substrate under investigation. Nutrient broth culture medium containing beef extract, yeast extract, peptone and sodium chloride was used. Approximately 2 grams of ground material were inoculated in 25 ml of medium and incubated at 28^0C until the appearance changed as a result of the disturbance, which denotes the active development of microorganisms. Subsequently, inoculations were performed on solidified medium in order to isolate microorganisms producing active principles.

The chemical composition of the investigated lithic substrate was carried out by XRF analysis according to previous descriptions (BĂTRÎNESCU-MOTEAU et al., 2022). The surface of the studied lithic substrate was imaged by AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy). In principle, AFM can achieve a higher resolution in x/y (plane) than SEM, without special sample preparation, but is limited to a few micrometers in vertical z direction, while SEM can achieve a field depth of the order of millimeters.

In the case of the purified isolate selected for this study, a series of microbiological and physicochemical analyses were performed, including Gram staining, catalase and oxidase tests, optical microscopy, SEM, AFM. The evaluation of the degree of pigment production in different cultivation conditions was performed by UV-VIS spectral measurements at 660 nm and 740 nm in different cultivation conditions.

To highlight the presence of catalase, the microbial culture was homogenized with 2% hydrogen peroxide. The appearance of gas bubbles is considered a positive reaction. The catalase test is a frequently used method to distinguish different families or orders of microorganisms (REINER, 2010).

For the oxidase test, the "oxidase reagent" - N,N-Dimethyl-p-phenylenediamine hydrochloride - was used, which acts as an artificial electron donor for cytochrome c. Once the reagent is oxidized by cytochrome c, the color of the reagent, initially colorless, evolves to shades of dark blue or purple.

The Gram character was demonstrated using the potassium hydroxide test that distinguishes between Gram-negative and Gram-positive bacteria (BUCK, 1982). The KOH solution has the ability to break down the cell wall of gram-negative bacteria due to its structure. Change in the texture of the solution to a viscous, mucilaginous consistency indicates a positive test result. If no change in the texture of the solution is observed, the test is considered negative, signifying the presence of gram-positive bacteria.

RESULTS AND DISCUSSIONS

Volcanic tuff is a natural resource with multiple applications. It is a type of rock that is formed by volcanic eruptions, and the pyroclastic materials resulting from these events are carried by the wind and sedimented in different environments. It is a relatively soft rock type and can be classified as igneous or sedimentary rock (MOCANU et al., 2008). The chemical composition of the investigated lithic substrate was determined by X-ray fluorescence, using the Rigaku ZSX100e Supermini XRF system (Rigaku, Japan), following the manufacturer's protocol (LEWIS et al., 2012).

The results obtained revealed the presence in the form of oxides of several elements, namely, SiO_2 – 72%, Al_2O_3 – 10%, CaO -6.8%, K_2O – 4.7%, Fe_2O_3 – 3.8%, TiO_2 – 1.2% as well as traces of rare elements from the lanthanide and actinide

groups in a total percentage of 1.5%. It is observed that the major elements of the analyzed lithic substrate are silicon, aluminum and calcium. Similarly, in a study conducted by Pabiś-Mazgaj et al., 2021 comparable data to those obtained in this study were recorded, SiO_2 and Al_2O_3 being the major components, specific to zeolites. In the study conducted, Pabiś-Mazgaj and collaborators compared the composition of rocks from several regions, their composition being similar.

Electron microscopy techniques (SEM and AFM) demonstrated the development of microorganisms on the surface of the lytic support in the form of biofilms (Fig. 1). The experiments for scanning electron microscopy involved high vacuum conditions. In order to be investigated, milled rock samples have been pre-treated by dispersion in 30% ethanol solution, and 10 µl of suspension were deposited on the microscope slide and sputtered with a gold layer. After the evaporation of ethanol, the prepared slides were dried in a vacuum followed by metal coating sputter. The used instruments are Jeol JSM-6610LV and coater JFC-1300 (BĂTRÎNESCU-MOTEAU et al., 2022).

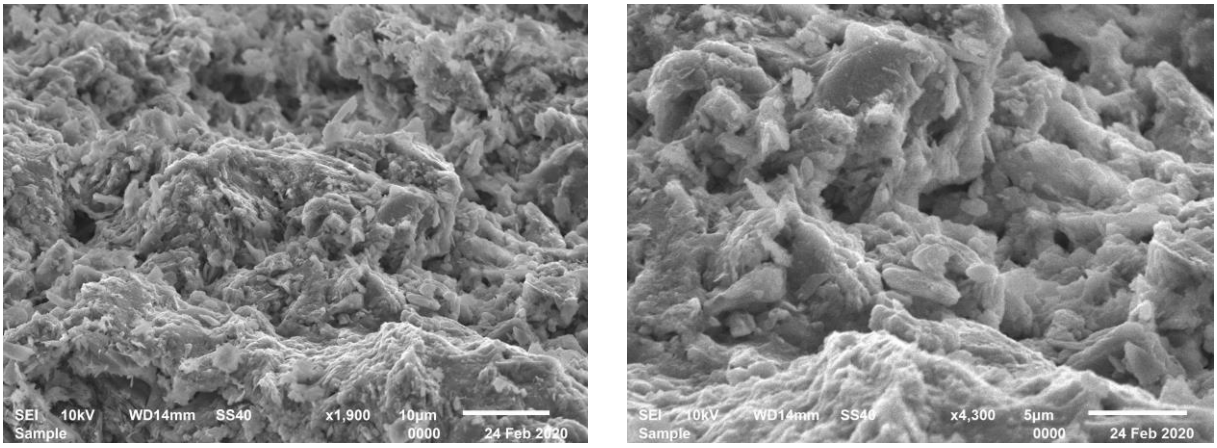

Figure 1. Biofilms revealed on the surface of the investigated litic sample by SEM (original).

Atomic force microscopy (AFM) measurements were performed in non-contact mode with a Park Systems XE-100 microscope, using sharp tips (NCLR type from Nanosensors™). The XE100 microscope (Park Systems, Suwon, Republic of Korea) used arch-guided scanners with eliminated crosstalk, thus minimizing tip-sample interaction. AFM images were processed with the XEI program (v 1.8.0 - Park Systems). Fig. 2 presents bi-dimensional (2D) and tri-dimensional (3D) AFM images, at the scale of 8µm x 8µm, presented in clasic mode (one color gradient image) – first two images, as well as enhanced contrast mode (two colors gradient image) – last two AFM images. Fig. 2 show the microbial growth on the rock surface under analysis, consisting in well-dispersed small particles-like protruding features (few tens of nm in diameter), medium sized (1-200 nm) as well as agglomerated ares (1-2 microns in diameter). As can be observed from Fig. 2, the "enhanced contrast" AFM images better enhances the topographic details of the microbial population.

A number of 1.5 X 10^5 CFU/g were quantified from the investigated rock sample. A microorganism with brown pigmentation was randomly selected for further studies. The morphology of the isolated and purified microorganism was highlighted by microscopic visualization with a 40x magnification objective. The data presented in Fig. 3 highlight a microorganism in the form of slightly curved, ungrouped bacilli. This is Gram-negative, with positive catalase activity and negative oxidase activity.

The tolerance of the investigated isolate to the ionic strength generated by the presence of NaCl in the growth medium as well as the production of melanic pigments under these conditions was evaluated in the range of 0 – 4M NaCl. The results obtained in table no. 1 demonstrate a development of the isolate in the range of 0 – 1M NaCl in the other experimental variants, no growth being observed. These data show that the isolate cannot tolerate high concentrations of NaCl in the growth medium. The synthesis of melanic pigments is observed in the range in which the microorganism develops with significant values of absorbance at 740 nm at 96 hours of cultivation (Fig. 4).

Taking into account the environmental conditions offered by the investigated lytic support and previous results regarding tolerance to low salt stress, the culture medium of the isolate was supplemented with $MgCl_2$ concentrations ranging from 0 to 1M. Some previous studies have demonstrated the influence of the ratio between Na and Mg on the biological activity of some bacterial metabolites under conditions of low salt stress (ENACHE et al., 2009; ZHOU et al., 2020). The results obtained recorded up to 48 hours of growth and 9 days respectively (Tables 2 and 3) demonstrate the development of the investigated isolate on the culture medium supplemented with up to 0.3M $MgCl_2$ accompanied equally by the synthesis of melanic pigments.

The estimation of the pH value range on the growth and synthesis of melanic pigments was carried out for the range of 5 – 9 pH units for four variants of ionic ratio, namely A (0.5 M NaCl and 0.1M $MgCl_2$), B (0.5 M NaCl and 0.5 M $MgCl_2$), C (1 M NaCl and 0.1 M $MgCl_2$) and D (1 M NaCl and 0.5 M $MgCl_2$). The data presented in Figures 5 - 12 show that at pH value 5 significant development of the isolate and pigment production was recorded for experimental variants D and C and at pH value 7 for study variants A, C and D.

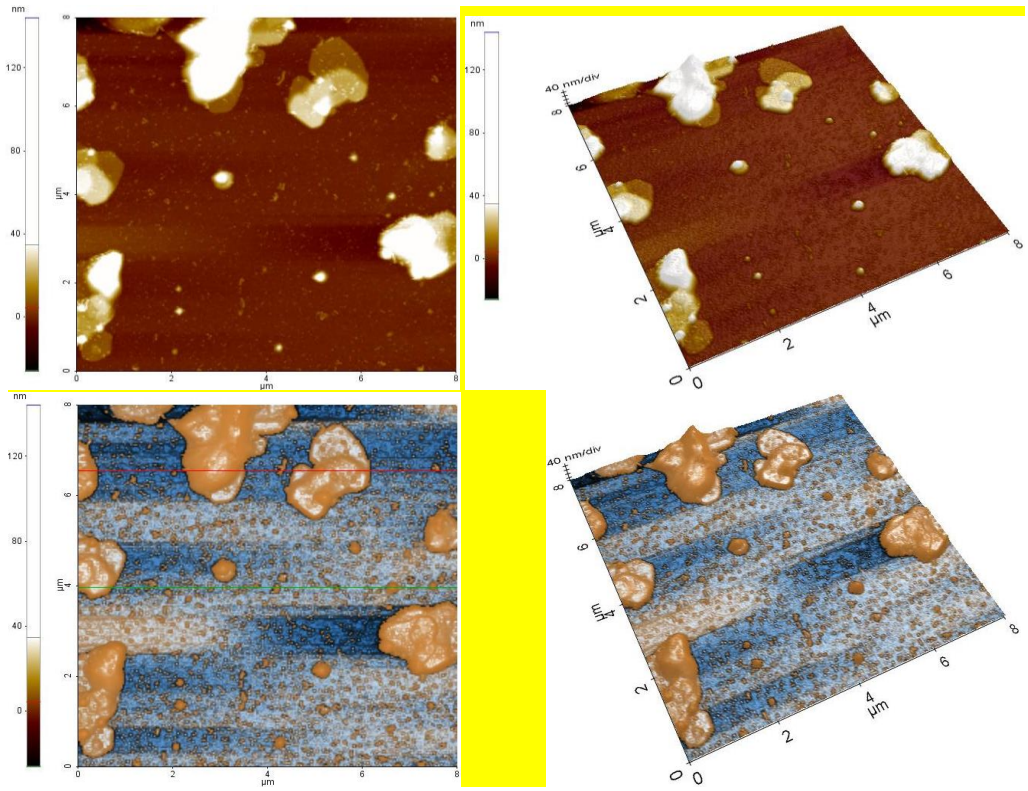

Figure 2. 2D (left) and 3D (right) AFM images, scanned over an area of 8µm x 8µm, in classic view mode (first row) as well as enhanced contrast (second row) showing the development of microorganisms on the surface of the lytic support (original).

Table 1. Dynamics of bacterial culture development (660 nm) and pigment synthesis (740 nm) as a function of NaCl concentration (NaCl – molar concentrations); N = data not recorded.

	0 h		24 h		48 h		72 h		96 h	
NaCl	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm
0	0,019	0,015	0,272	0,220	0,168	0,131	1,260	1,190	1,156	1,452
0,5	0,018	0,015	0,210	0,173	0,508	0,427	0,738	0,651	0,667	0,585
1	0,023	0,019	0,246	0,203	0,059	0,048	0,0675	0,057	1,195	1,132
1,5	0,019	0,014	0,0072	0,0049	0,0064	0,0038	0,0031	0,0021	0,0145	0,0123
2	0,024	0,019	0,0078	0,0053	0,0097	0,0077	0,0019	0,007	N	N
2,5	0,022	0,017	0,028	0,022	0,010	0,0083	0,0033	0,0023	N	N
3	0,015	0,011	0,018	0,015	0,011	0,0088	0,0027	0,0015	N	N
3,5	0,018	0,012	0,016	0,013	0,020	0,019	0,0022	0,0004	N	N
4	0,018	0,013	0,013	0,010	0,0045	0,0021	0,0282	0,0218	N	N

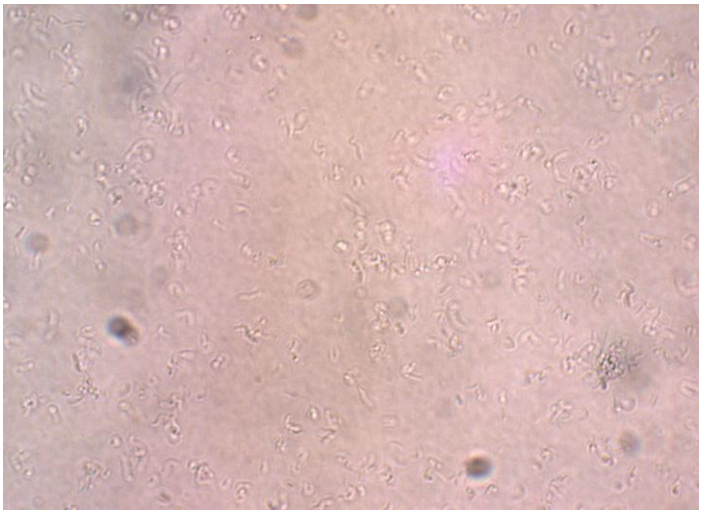

Figure 3. Image taken with an optical microscope (40x). Isolated, slightly curved bacilli can be observed (original).

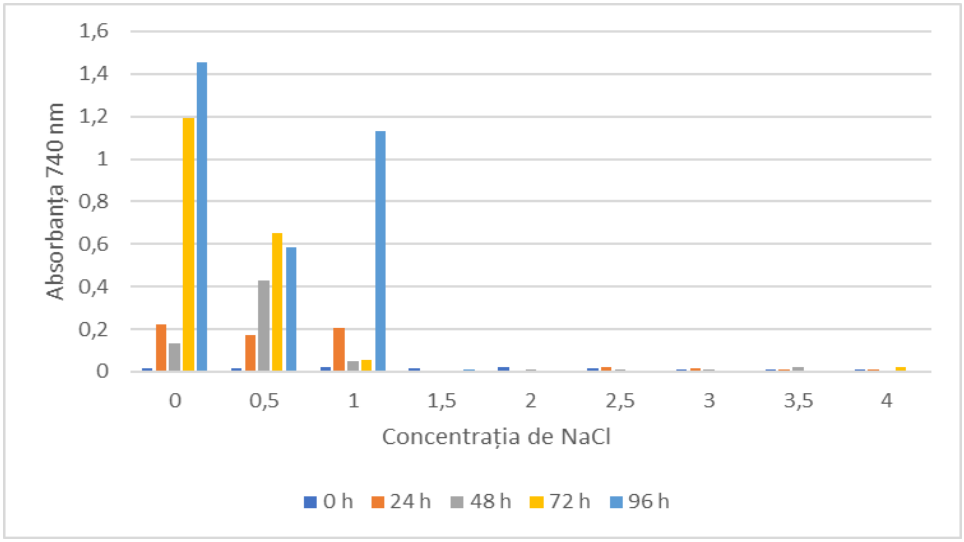

Figure 4. Pigment synthesis as a function of NaCl concentration.

Table 2. Dynamics of bacterial culture development (660 nm) and pigment production (740 nm) depending on $MgCl_2$ concentration in the interval 0 – 24 h; - = data not recorded.

	0 h		**0 h**		**24 h**		**24 h**	
	660 nm	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**
$MgCl_2$	**0,5M NaCl**	**0,5M NaCl**	**1M NaCl**	**1M NaCl**	**0,5M NaCl**	**0,5M NaCl**	**1 M NaCl**	**1 M NaCl**
0	0,219	0,186	0,216	0,186	0,257	0,239	0,116	0,103
0,1	0,217	0,175	0,202	0,170	0,232	0,208	0,252	0,226
0,3	0,190	0,161	0,239	0,202	0,365	0,335	-	-
0,5	0,192	0,161	0,160	0,133	0,137	0,117	0,097	0,081
0,8	0,211	0,178	0,102	0,085	-	-	0,135	0,116
1	0,196	0,164	0,134	0,111	0,093	0,077	0,091	0,074

Table 3. Dynamics of bacterial culture development (660 nm) and pigment production (740 nm) depending on $MgCl_2$ concentration at 48 h and 9 days; - = data not recorded.

	48 h		**48 h**		**9 days**		**9 days**	
	660 nm	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**
$MgCl_2$	**0,5M NaCl**	**0,5M NaCl**	**1M NaCl**	**1M NaCl**	**0,5M NaCl**	**0,5M NaCl**	**1 M NaCl**	**1 M NaCl**
0	1,359	1,295	0,697	0,654	1,655	1,587	1,562	1,502
0,1	0,886	0,840	1,314	1.252	1,455	1,391	1,321	1,255
0,3	1,200	1,138	-	-	1,512	1,450	-	-
0,5	1,015	0,958	0,108	0,090	1,348	1,289	0,150	0,130
0,8	-	-	0,827	0,766	-		1,192	1,126
1	0,105	0,087	0,104	0,086	0,200	0,175	0,159	0,138

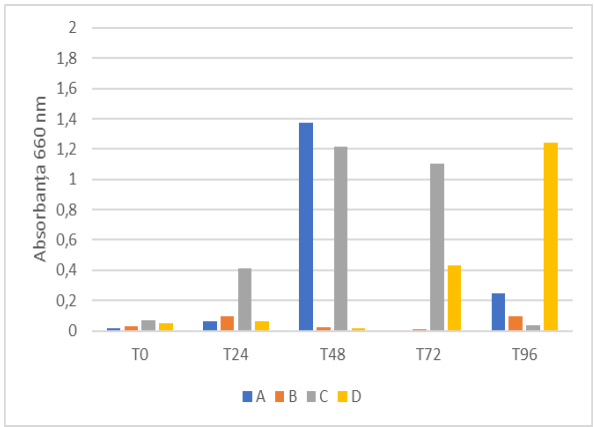

Figure 5. Development of the isolate according to experimental conditions at pH 5.

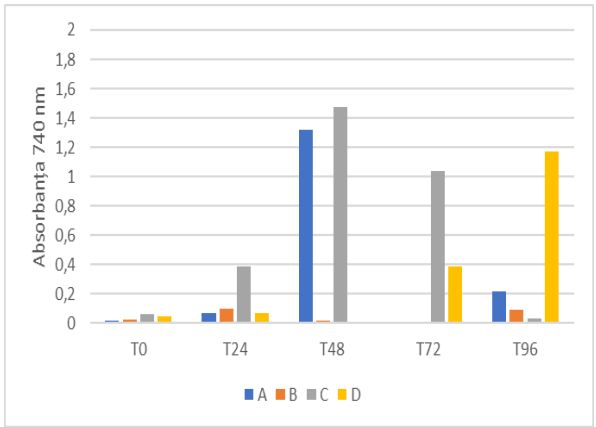

Figure 6. Pigment synthesis depending on experimental conditions at pH value 5.

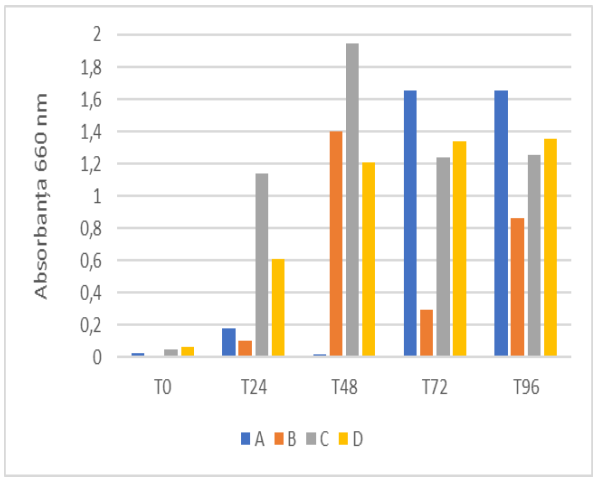

Figure 7. Development of the isolate according to experimental conditions at pH 7.

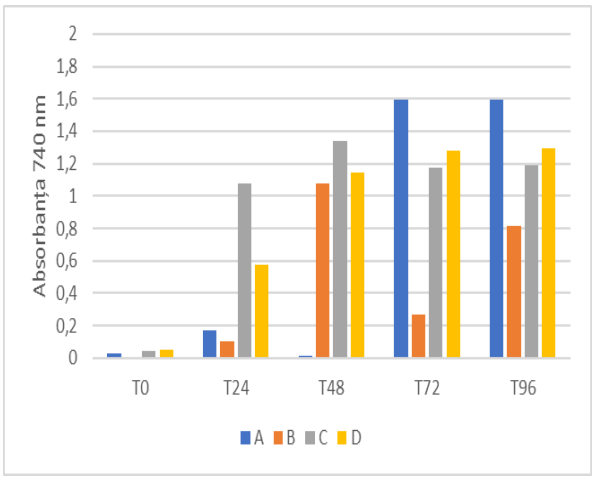

Figure 8. Pigment synthesis depending on experimental conditions at pH value 7.

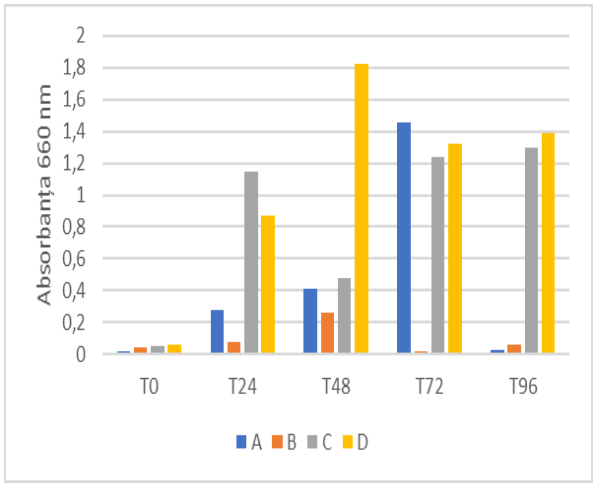

Figure 9. Development of the isolate according to experimental conditions at pH 8.

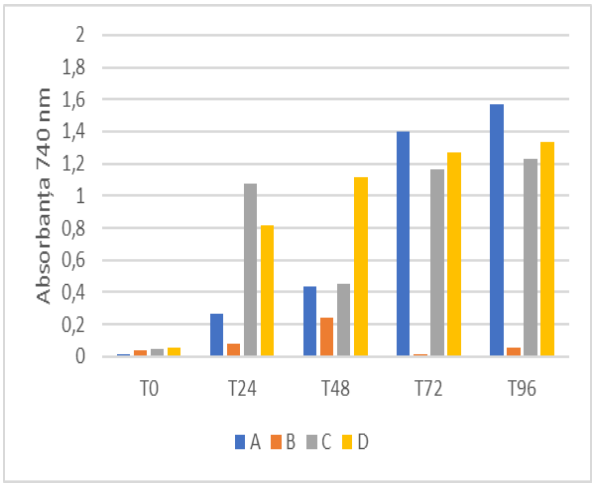

Figure 10. Pigment synthesis depending on experimental conditions at pH value 8.

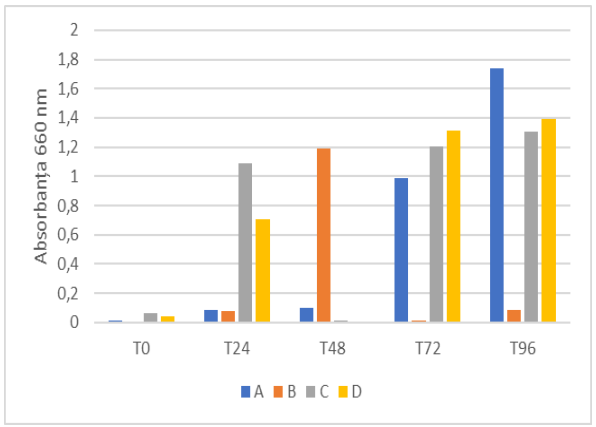

Figure 11. Development of the isolate according to experimental conditions at pH 9.

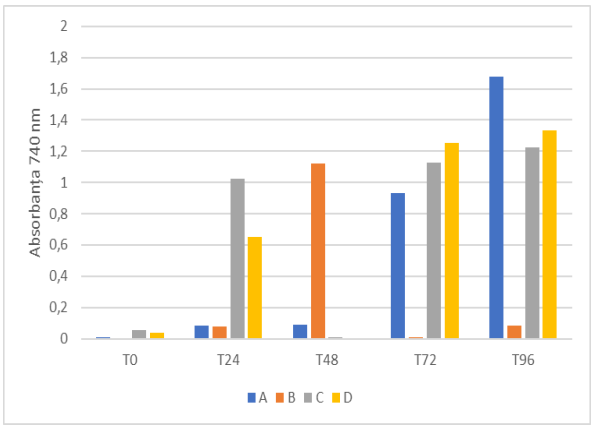

Figure 12. Pigment synthesis depending on experimental conditions at pH value 9.

Table 4. Dynamics of bacterial culture development at 24 hours at different incubation temperatures.

Temperature (^{0}C)	**25**		**30**		**37**		**40**		**45**	
	660 nm	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**
A 7	0,447	0,403	1,028	0,983	0,655	0,606	0,722	0,657	0,126	0,106
B7	1,118	1,050	1,373	1,310	1,122	1,057	0,867	0,800	0,120	0,104
B8	1,099	1,032	1,235	1,173	1,343	1,068	0,979	0,909	0,228	0,200
B9	0,971	0,899	1,263	1,194	1,050	0,972	0,749	0,685	0,092	0,081
C5	0,214	0,192	0,914	0,845	0,933	0,858	0,192	0,171	0,083	0,069
C9	0,470	0,428	1,044	0,973	1,002	0,932	0,576	0,529	0,048	0,042

Another parameter that can influence both growth and synthesis of metabolites with biological activity is temperature. In the case of the studied isolate, six variants of culture medium were tested based on previous experimental data (influence of NaCl, $MgCl_2$ concentrations and pH value) at temperatures of 25^0C, 30^0C, 37^0C, 40^0C and 45^0C. Thus, experimental variants A (0.5 M NaCl and 0.5 M $MgCl_2$ with pH 7 - experimental variant B from establishing the pH value range), B (1M NaCl and 0.1M $MgCl_2$ with pH 7, 8 and 9 - variant C from establishing the pH value range) and C (1M NaCl and 0.5M $MgCl_2$ with pH 5 and 9 - variant D from establishing the pH value range) were tested (SHIELDS & CATHCAR, 2010).

At a temperature of 25^0C, the best results were recorded at both 660 nm absorbance (isolate development) and 740 nm (melanin pigment synthesis), as shown in the data presented in Tables 4 – 7. As the temperature increases, a reduction in bacterial culture development is observed, which implicitly leads to a decrease in pigment synthesis.

Table 5. Dynamics of bacterial culture development at 48 hours at different incubation temperatures.

Temperature (^{0}C)	**25**		**30**		**37**		**40**		**45**	
	660 nm	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**
A 7	1,113	1,063	1,353	1,300	1,031	0,956	1,506	1,441	0,382	0,338
B7	1,533	1,468	1,619	1,549	1,120	1,051	0,658	0,594	0,687	0,627
B8	1,239	1,174	1,227	1,162	1,073	0,996	0,869	0,790	0,817	0,743
B9	1,199	1,132	1,184	1,114	0,862	0,780	0,779	0,703	0,106	0,093
C5	0,782	0,722	1,326	1,262	1,205	1,135	0,711	0,645	0,059	0,049
C9	1,186	1,122	1,391	1,329	1,133	1,069	0,562	0,513	0,049	0,044

Table 6. Dynamics of bacterial culture development at 72 hours at different incubation temperatures.

Temperature (^{0}C)	**25**		**30**		**37**		**40**		**45**	
	660 nm	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**	**660 nm**	**740 nm**
A 7	1,397	1,339	1,516	1,454	1,052	0,973	0,638	0,584	N	N
B7	1,713	1,636	1,655	1,584	1,152	1,073	1,024	0,951	N	N
B8	1,264	1,199	1,212	1,145	1,064	0,986	0,634	0,573	N	N
B9	1,216	1,146	1,153	1,080	0,890	0,803	0,820	0,740	N	N
C5	1,238	1,173	1,577	1,517	1,238	1,170	0,440	0,390	N	N
C9	1,746	1,702	1,478	1,413	1,117	1,057	0,055	0,045	N	N

Table 7. Dynamics of bacterial culture development at 96 hours at different incubation temperatures.

Temperature (^{0}C)	**25**		**30**		**37**		**40**		**45**	
	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm	660 nm	740 nm
A 7	1,619	1,565	1,542	1,479	1,250	1,174	1,215	1,139	0,989	0,914
B7	1,738	1,661	1,643	1,576	1,057	0,979	0,608	0,543	0,725	0,660
B8	1,283	1,215	1,195	1,127	1,098	1,019	0,902	0,818	0,351	0,331
B9	1,212	1,144	1,128	1,054	0,876	0,788	0,726	0,651	0,188	0,167
C5	1,410	1,347	1,538	1,475	1,260	1,191	1,019	0,951	0,100	0,093
C9	1,457	1,395	1,516	1,454	1,083	1,015	0,752	0,692	0,056	0,052

The influence of additional carbon sources on the development and production of pigments by the investigated isolate was tested on nutrient broth culture medium in the presence of 1M NaCl and 0.1M $MgCl_2$, at pH values of 7 and 8, a temperature of 25^0C and stirring conditions of 150 rpm. The carbon sources tested were represented by glucose, sucrose and ammonium nitrate, added in an amount of 5 grams per liter of cultivation medium. The growth and intensity of the culture pigmentation were estimated visually, after 96 hours of cultivation very good development was noted in all experimental variants, but without a significant change in color in the sense of the appearance of a slightly brown coloration.

The bacterial cultures obtained after 96 hours of cultivation under optimal conditions of NaCl, $MgCl_2$, temperature and pH value were centrifuged at 10000 rpm, 40C and the supernatant was subjected to spectral and fluorescence analyses. The absorption spectra were recorded in the spectral range 400 – 800 nm with the Jasco V-630 Spectrophotometer, using quartz cuvettes with an optical path of 1 cm. The samples were read against the reference - ethanol 80%. The samples were numbered as follows: Sample 1 – broth reference, sample as such, Sample 2 – broth reference, sample diluted with broth 10x, Sample 3 – broth reference, sample diluted with broth 5x, Sample 4 – MH reference, sample as such, Sample 5 – MH reference, sample diluted with MH 10x, Sample 6 – MH reference, sample diluted with MH 5x. The recorded spectra (Fig. 13) show the characteristics of the absorption spectrum of melanin, namely absorption in the UV – VIS range, a strong absorption in the UV range that decreases exponentially as one reaches the visible range (300 – 600 nm) and a linearity in the range 600 – 700 nm. This type of spectrum without an absorption maximum is not characteristic of organic molecules and rather semiconductors and can be explained based on the complex structure of melanin and its aggregation.

In other studies in the specialized literature (CAPOZII et al., 2006; TARAGANI & MISHRA, 2014), absorption spectra characterized by the presence of peaks in the UV-VIS region (200-300 nm) were presented. This absorption is consistent with the photoprotective role of melanin.

The fluorescence spectra were recorded in the spectral range 200 – 800 nm with the Jasco V-630 Spectrofluorimeter, using a quartz cuvette with an optical path of 1 cm. The excitation wavelength λex = 250 nm. The fluorescence spectra of samples 1 – 4 show two maxima: one in the UV range at 250 nm and another in the visible range at 468 nm. Also, several shoulders are observed in the range 300 – 450 nm. The analyzed samples were numbered as follows: Sample 1 – sample diluted with 100x broth; Sample 2 – sample diluted with 420x broth; Sample 3 – sample diluted with 20x broth and Sample 4 – sample diluted with 15x broth (Fig. 4) (STAFSNES & BRUHEIM, 2013).

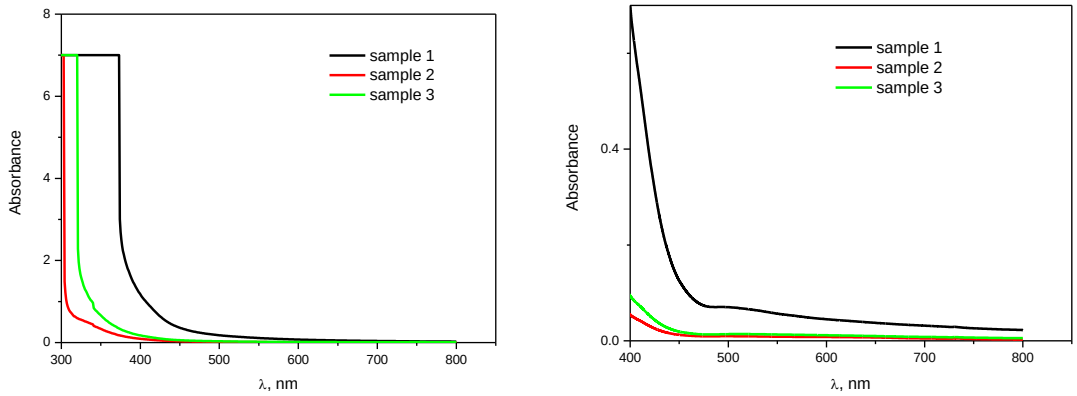

Figure 13. Absorption spectra of the analyzed samples.

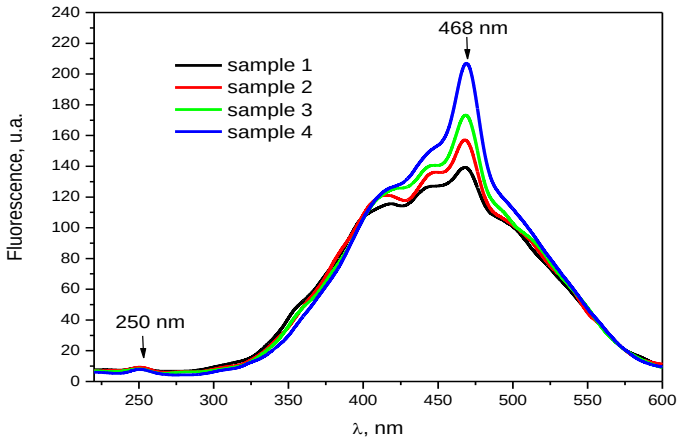

Figure 14. Fluorescence spectra (200-600 nm) of samples 1-4.

CONCLUSIONS

The synthesis of bioactive compounds by various microorganisms has been the subject of numerous researches in recent years, due to their potential in various fields, such as industry and medicine. Microbial pigments, in particular, have a wide range of applications. Therefore, in this study, a microorganism was isolated that has the ability to grow on lytic support as shown by the results obtained from SEM and AFM microscopy analyses and to synthesize bioactive compounds such as melanic pigments.

The results obtained showed that the isolate is a Gram-negative bacillus that exhibits catalase but not oxidase activity. Optimal growth was obtained on a culture medium supplemented with NaCl in concentrations of 0.5M and 1M, as well as at concentrations of 0.1M and 0.5M $MgCl_2$. After testing four experimental variants of the Na^{+}/Mg^{2+} ionic ratio, the optimal pH range was established between 5 and 9 units.

Considering the fluctuations in temperature values in the ecosystem from which the investigated isolate comes, its development and the synthesis of melanic pigments were evaluated at temperatures of 25, 30, 37, 40 and 45 degrees Celsius. It was observed that the optimal growth temperature is 25 degrees Celsius, and an increase in temperature negatively affected the development of the microorganism and pigment production.

Spectrophotometric analyses highlighted absorption spectra specific to melanin, similar to those in other specialized studies.

In general, a direct link between the development rate of the microorganism and pigment synthesis could not be established. It can be assumed that this phenomenon is more likely influenced by other factors, such as day/night alternation, lighting, agitation.

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