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BIOSYNTHESIS OF PIGMENT BY STRAIN *ARTHROBACTER AGILIS* WB28 DURING FERMENTATION OF SECONDARY RAW MATERIALS

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KEY WORDS:
Arthrobacter agilis, bacterioruberin, pigments, carotenoids, secondary agricultural raw materials, hydrolases

ABSTRACT

The article presents the results of studies aimed at developing a cost-effective technology for producing microbial carotenoids using secondary raw materials from the agro-industrial sector. The article provides data on the fermentation of media made from wheat bran, soybean meal, rapeseed cake, and the biosynthesis of bacterioruberin, a rare microbial carotenoid. The article uses the *Arthrobacter agilis* wb28 strain, isolated from wheat bran, as the producer. Cultivation was carried out on solid and liquid media; in rocking flasks and a bioreactor. The pigment was isolated from the biomass using a well-known extraction method, modified to suit the specific characteristics of the producer. When cultured on medium with agar, visible growth of colonies was observed after 48 hours. In liquid media, the stationary growth phase was reached within 16–24 hours. Active aeration during fermentation in a bioreactor increased the pigment yield compared to the process in rocking flasks. Active aeration during fermentation in a bioreactor increased the pigment yield compared to the process in rocking flasks. The dependence of pigment synthesis on the composition of the nutrient medium and the aeration regime was revealed. It was shown that *A. agilis* wb28 selectively consumes substrates. It has the ability to hydrolyze poly- and oligosaccharides, lipids, and fatty acid esters, as well as complex proteins. It can use citrate as the only source of carbon and energy. Glucose is poorly assimilated by the strain's enzyme system. The main stress factor in the composition of nutrient media from secondary raw materials for pigment formation was the concentration of carbohydrate. Protein components were mainly used for biomass production. The highest pigment yield was observed during fermentation of media from wheat bran (4.28 mg/g), while the lowest yield was observed from rapeseed cake (1.16 mg/l). The resulting bacterioruberin yield was comparable to the yield of carotenoids for known *Arthrobacter* strains.

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БИОСИНТЕЗ ПИГМЕНТА ШТАММОМ *ARTHROBACTER AGILIS* WB28 ПРИ ФЕРМЕНТАЦИИ ВТОРИЧНОГО СЫРЬЯ

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КЛЮЧЕВЫЕ СЛОВА:
Arthrobacter agilis, бактериоруберин, пигменты, каротиноиды, вторичное сельскохозяйственное сырье, гидролазы

АННОТАЦИЯ

В статье представлены результаты исследований, направленных на разработку экономически выгодной технологии получения каротиноидов микробного происхождения с использованием вторичного сырья агропромышленного производства. Приведены данные по ферментации сред из пшеничных отрубей, соевого шрота, рапсового жмыха и биосинтезу бактеориоруберина — редкого каротиноида микробного происхождения. В качестве продуцента исследовали штамм *Arthrobacter agilis* wb28, выделенный из пшеничных отрубей. Культивирование проводили на плотных и в жидких средах; в качалочных колбах и биореакторе. Пигмент выделяли из биомассы известным методом экстракции в модификации с учетом особенностей продуцента. При культивировании на агаризованных средах видимый рост колоний отмечен через 48 ч. В жидких средах наступление стационарной фазы роста выявлено уже через 16–24 ч. Активная аэрация при ферментации в биореакторе способствовала увеличению выхода пигмента по сравнению с процессом в качалочных колбах. Выявлена зависимость синтеза пигмента от состава питательной среды в совокупности с режимом аэрации. Показано, что *A. agilis* wb28 избирательно потребляет субстраты. Он обладает способностью гидролизовать поли- и олигосахариды, липиды и сложные эфиры жирных кислот, а также сложные белки. Может использовать цитрат как единственный источник углерода и энергии. Глюкоза слабо ассимилируются ферментной системой штамма. Основным стрессовым фактором в составе питательных сред из вторичного сырья для образования пигмента являлась концентрация углевода. Белковые компоненты расходовались в основном на биомассу. Наибольший количественный выход пигмента наблюдался при ферментации сред из пшеничных отрубей (4,28 мг/г), а наименьший — из рапсового жмыха (1,16 мг/л). Полученный выход бактериоруберина находился на уровне, сопоставимом с уровнем выхода каротиноидов для известных штаммов *Arthrobacter*.

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1. Introduction

The agro-industrial sector generates significant amounts of waste, which are products of plant raw material processing [1]. If not properly prepared for disposal, these materials can have a detrimental impact on the environment. Quite often, they are either incinerated or disposed of in landfills [2]. Their use as secondary raw materials in livestock feed production is of interest; however, there is a risk of reduced nutritional value in such products [3]. Nevertheless, the overall content of proteins, fats, carbohydrates, and minerals in secondary raw materials presents a prospect for their application as a substrate for microbial synthesis [4,5].

Pigments are among the valuable products of microbiological synthesis. They can be produced through the bioconversion of secondary raw materials [6]. These substances possess a range of beneficial properties that give them advantages over dyes produced by chemical synthesis. They exhibit biological activity, such as anticancer, antioxidant, and antibacterial effects [7,8].

A common group of natural pigments is carotenoids. Chemically, they belong to isoprenoids, which are classified according to the number of isoprene units. Currently, chemical synthesis is the predominant method for producing carotenoids, which involves the use of petroleum products as starting materials. Methods for microbial synthesis using renewable natural resources are also known [9,10].

One of the rare carotenoids that can be obtained through biosynthesis is bacterioruberin (BR). This compound imparts a color ranging from pale pink to bright red to microbial cells. BR is an important component of haloarchaeal membranes, enabling them to adapt to high salinity and oxidative stress [11,12]. The pigment is a C50-carotenoid, and its molecule contains 13 conjugated double bonds. This structure grants BR high antioxidant activity.

Red extracts derived from haloarchaeal biomass exhibit a range of beneficial properties, including antimicrobial activity, potential for tumor treatment, support for sperm viability, and antiproliferative activity against human hepatocellular carcinoma HepG2 cell lines [13,14].

The influence of various factors on biomass growth and bacterioruberin concentration in dry cell weight has already been the subject of extensive research. Most studies in this field focus on the effects of thermal stress and osmotic shock induced by high NaCl concentrations in the nutrient medium [15–17].

The carotenoid bacterioruberin (BR) has also been identified in prokaryotes belonging to the genus *Arthrobacter* [18–20]. These microorganisms are capable of surviving in extreme conditions and can tolerate low temperatures; their representatives are numerous and often occupy a dominant position in the microbiota of terrestrial and aquatic ecosystems on our planet [18]. *Arthrobacter* bacteria are Gram-positive, exhibit exponential growth as both rods and cocci during the stationary phase, can grow under aerobic and anaerobic conditions, and belong to the *Actinobacteria* phylum [19]. Various *Arthrobacter* species are involved in plant growth promotion, synthesize industrially significant enzymes, and are a source of xeroprotectants. These data indicate that *Arthrobacter* species possess genes encoding enzymes that could be useful in fields such as industry, agriculture, and biotechnology [20,21].

This bacterial carotenoid is now well-studied, and its application in the food and feed industries is highly probable [15]. The synthesis of the bacterioruberin carotenoid is characteristic of certain members of the *Arthrobacter* genus, one of which is *A. agilis*.

Furthermore, BR synthesis is essential for the survival and reproduction of *A. agilis* at low temperatures, as evidenced by the suppression of one of its biosynthesis genes [21].

However, a significant knowledge gap currently exists regarding the influence of nutrient medium composition on the pigmentation of *A. agilis* and the role played by the enzymes synthesized by the bacterium in the biosynthesis process. Although information is available on the use of *A. agilis* for BR biosynthesis using secondary raw materials from cheese production, specifically whey, there is still a need to optimize the nutrient medium composition to achieve a higher yield of the target product [17].

This research investigated the application of common plant raw material processing wastes. Wheat bran, whose production is increasing annually, was used for this purpose. Consequently, they have become a traditional substrate for microbial synthesis [22,23]. Rapeseed meal, a byproduct of rapeseed oil production (the third most common oil worldwide), was studied as a raw material. Thus, the disposal of this meal is a major concern [24]. Soybean meal, whose composition includes substrates for the biosynthesis of various metabolites, was also investigated as a nutrient medium [25].

The aim of this study was to investigate pigment biosynthesis by the strain *A. agilis* wb28, isolated from wheat bran, during the fermentation of secondary raw materials.

2. Objects and methods

2.1. Research objects

The cold-resistant strain *Arthrobacter agilis* wb28 (RCAM 05966), isolated by researchers from the All-Russian Research Institute for Food Additives (VNIPI) from wheat bran intended for food and feed, was investigated as a producer of bacterioruberin (BR). The strain was identified using 16S rRNA gene sequencing. It exhibits active growth at +4 °C (making it a psychrotroph) and was isolated from a source uncharacteristic for cold-tolerant microorganisms. Furthermore, it is a halophile and an organotroph.

The following secondary raw materials were studied as substrate sources for pigment biosynthesis: wheat bran (Petersburg Mill Combine, Russia), soybean meal (Zolotaya Niva, Russia), and rapeseed meal (Grandpa Nikita's Recipes, Russia).

2.2. Preparation and composition of nutrient media

The nutrient medium was prepared from the raw material, which was preliminarily ground using an A11 basic laboratory mill (IKA, Germany), and distilled water. Wheat bran was placed in a beaker, mixed with 600 ml of water, and incubated for two hours at a temperature of 90 ± 3 °C. After the hot water extraction, the volume was adjusted to 1 L (resulting in a hydro-module of 1:20).

For submerged cultivation in a bioreactor, the media were filtered to avoid inaccuracies in determining the solution's turbidity. Coarse filtration was performed using cotton cloth, and the residue was washed with 100 ml of distilled water. The medium was then filtered through a "blue ribbon" filter (Reacon Plus, Russia) with a pore size of 2–3 μm. The volume of the solution was adjusted to 1 L with distilled water using a measuring cylinder.

For solid nutrient media, agar-agar (AGAT-MED, Germany) was added at a concentration of 15 g/L after bringing the volume to 1 L. Filtration was not performed in this case. The media were sterilized at 115 °C for 30 minutes.

2.3. Cultivation

For surface cultivation, the bacteria were streaked onto the surface of the nutrient medium and incubated for 48 hours in a thermostat at a temperature of 28 ± 1 °C.

For submerged inoculation, the strain *A. agilis* wb28 was first cultivated on slanted LB agar for 48 hours at 28 ± 1 °C in a forced convection thermostat (Daihan Labtech LIB-080M, South Korea). Submerged cell cultivation was carried out in 750 ml baffled flasks containing 60 ml of the nutrient medium. For inoculation, a suspension was prepared by washing cells from the slanted agar tubes with the nutrient medium using a microbiological loop. The culture was grown at 28 ± 1 °C in a Multitron Standard shaker-incubator (INFORS, Switzerland) at 180 rpm for 16 hours.

For the 1 L bioreactor (BIOSTAT® A, Germany), a working volume equal to 0.6 of the total volume was used. For inoculation, 60 ml of the overnight culture, prepared using the submerged cultivation method described above, was added to 540 ml of the sterile medium. During cultivation, the dissolved oxygen content was maintained at 30 ± 5 % by adjusting the supply of sterile air and varying the agitation speed.

During the stationary growth phase of *A. agilis* wb28, a sample of the culture broth was taken to determine the dry biomass weight and bacterioruberin (BR) content.

2.4. Growth curve description

The standard logarithmic model was applied to describe the growth curve of *A. agilis* wb28. The specific growth rate, μ (abs.units/h), was calculated using the following equation:

$$\mu = \frac{\ln OD - \ln OD_0}{t - t_0}, \quad (1)$$

where: OD_0 — is the optical density (abs. units) characterizing the biomass quantity at the initial time of a specific growth period, t_0; OD — is the optical density (abs. units) characterizing the biomass quantity at the final time of a specific growth period, t; t_0 — is the initial time of the growth period, h; t — is the final time of the growth period, h.

The doubling time (generation time) was determined using the following equation:

$$\tau_{1/2} = \frac{\ln 2}{\mu} \quad (2)$$

where: $\tau_{1/2}$ — is the doubling time, h; μ — is the specific growth rate, abs. units/h; ln 2 — is the natural logarithm.

2.5. Determination of dry cell weight

To determine the biomass quantity after the onset of the stationary growth phase, a 50 mL aliquot of the culture broth was centrifuged for 20 minutes using a Centrifuge 5804 (Eppendorf, Germany). The supernatant was decanted, and the pellet was resuspended and washed with 30 mL of physiological saline. The resulting suspension was centrifuged again. The pellet was then dried to constant weight for 24 hours at 105 °C in a UF260 forced-air drying oven (Memmert, Germany). The biomass was weighed on a GR-200 analytical balance (AND, Japan).

2.6. Determination of starch in the nutrient medium

The starch content in the nutrient medium was determined using an iodometric method [26]. An iodine reagent was used, prepared by dissolving 3 g of potassium iodide (KI) and 2.54 g of crystalline iodine (I_2) in 100 mL of water. To 1 mL of the sample, 30 μL of the iodine reagent was added, and the optical density was measured at a wavelength of 600 nm.

A standard 0.1% solution, prepared by heating native starch for 3 hours at 90±3 °C, was used to construct the calibration curve. The curve was generated using six data points, and the starch measurement in the samples was performed in triplicate.

2.7. Determination of simple sugars in the nutrient medium

For sample preparation, the samples were centrifuged at a relative centrifugal force (RCF) of 15,000 *g* for 2 minutes. Analysis was performed using a Kapel'-205 capillary electrophoresis system (Lumex, Russia). The analysis conditions were as follows: capillary internal diameter — 50 μm; total capillary length — 75 cm; temperature — 20 °C; sample injection — 30 mbar for 5 s; detection wavelength — 230 nm; voltage — negative 25 kV; electrophoregram registration time — 15 minutes.

2.8. Determination of protein in the nutrient medium

The protein content in the nutrient medium was quantified using the standard Lowry method [27]. For this purpose, two solutions (A and B) were prepared. Solution A consisted of 2% sodium carbonate (Na_2CO_3) dissolved in a 0.1 M sodium hydroxide (NaOH) solution. Solution B contained 0.5% copper sulfate ($CuSO_4$) and 1% potassium sodium tartrate tetrahydrate ($C_4H_4O_6KNa \cdot 4H_2O$).

Immediately prior to analysis, reagent C was prepared by mixing 50 mL of reagent A with 1 mL of reagent B. Subsequently, 5 mL of reagent C was added to 1 mL of the nutrient medium sample, and the mixture was incubated in a dark place for 30 minutes. Then, 0.5 mL of Folin's reagent, previously diluted twofold, was added. The sample was mixed thoroughly and incubated for an additional 30 minutes in the dark.

The optical density was measured at a wavelength of 750 nm using a BioSpectrometer basic spectrophotometer (Eppendorf, Germany).

2.9. Evaluation of enzymatic activity of A. agilis wb28

Nutrient differential-diagnostic and selective media were used; the pH of the media was adjusted using 0.1 N NaOH or 0.1 N HCl solutions. The sterilization regime was 15 minutes at 121 °C. Bacterial cells of *A. agilis* wb28 were streaked onto the surface of agarized media in Petri dishes or stabbed into the depth of agarized media in microbiological tubes (stab inoculation) and incubated at 28±1 °C for 24–48 hours.

Activities were assessed based on changes in coloration, the formation of precipitates and lysis zones around colonies, liquefaction of the medium during strain cultivation on solid media, and by changes in color, gas production, and turbidity in liquid and semi-liquid media.

The saccharolytic activity of *A. agilis* wb28 was tested by inoculation onto semi-solid Hiss medium with the following composition (g/L): peptone — 5, agar — 3, NaCl — 3.5, carbohydrates — 3.5, Na_2HPO_4 — 0.2, bromothymol blue — 0.03 (using 3 mL of a 1% solution). The composition of the Hiss medium's "color series" included: sucrose, glucose, maltose, fructose, raffinose, and soluble starch [28]. The studied culture was inoculated into Hiss media and cultivated for 24 hours. The presence of saccharolytic activity was detected by changes in the pH of the medium and gas production, which could result from the action of enzymes on carbohydrates. Changes in pH were recorded using the property of bromothymol blue to change color from yellow to blue through shades of green as the pH value varied from 5.8 to 7.6; the green color corresponded to neutral values, from 6.8 to 7.2 pH units.

Amylase activity was determined using a modified starch agar medium containing (g/L): agar — 12, soluble starch (amylodextrin) — 10, meat extract — 3, distilled water — 1 L; pH = 6.8±0.2. After incubation of the bacterial cells, 10 mL of a 0.1 N iodine solution was poured onto the agar surface to cover it completely, left for 20 minutes at room temperature, and then decanted. A clear zone of hydrolysis around the microbial culture indicated the presence of amylase activity [29]. Lipolytic activity was assessed on agarized media containing olive oil and on agarized media with Tween 80 [30]:

- **Medium with olive oil and rhodamine solution** contained (g/L): agar — 15, peptone — 5, NaCl — 4, yeast extract — 3. After autoclaving, the medium was cooled to 60 °C, and then 31.25 mL/L of autoclaved olive oil and 10 mL/L of a sterile rhodamine solution (1 mg/mL) were added; pH = 7.0±0.2. After incubation, the synthesis of lipase was inferred from the formation of orange fluorescent halos around bacterial colonies, visible under UV irradiation at a wavelength of 365 nm (using a TCP-20.LC transilluminator, Vilber, France).
- **Agar with olive oil and phenol red** was prepared from (g/L): agar — 15, peptone — 5, yeast extract — 3, $CaCl_2$ — 1, with the addition of 10 mL/L of phenol red (1 mg/mL) and 10 mL/L of olive oil; pH = 7.4±0.2. After incubation of the microbial cells, a color change from orange to pink indicated the release of fatty acids due to lipolysis.
- **Medium with Tween 80** consisted of (g/L): agar — 20, peptone — 10, NaCl — 5, $CaCl_2 \cdot 2H_2O$ — 0.1, with the addition of 10 mL/L of Tween 80 (5 g/L); pH = 7.0±0.2. After incubation, the formation of a white precipitate around the bacterial colony indicated the presence of lipase activity.

Proteolytic activity was determined on milk agar and gelatin medium:

- **Milk agar** contained (g/L): skim milk powder — 50, agar — 10, peptone — 5, yeast extract — 2.5, glucose — 1; pH = 6.8±0.2. Specifically, 25 g of skim milk powder was dissolved in 250 mL of distilled water. Separately, a 2.5% agar suspension was prepared in 500 mL of distilled water. The medium components were autoclaved separately for 15 minutes at 121 °C, cooled to 50 °C, mixed, and poured into Petri dishes. Bacterial cells were distributed in a circular pattern on the medium surface, incubated, and subsequently the plates were kept in a refrigerator at +4 °C for 36 hours. Proteolytic activity was determined by the appearance of clear zones of hydrolysis around the bacterial colonies [31].
- **The gelatin medium** consisted of (g/L): gelatin — 120, peptone — 5, meat extract — 3; pH = 6.8±0.2. Bacterial cells were inoculated via stab inoculation. After incubation, the tubes were placed in a refrigerator at +4 °C for 36 hours. Liquefaction of the gelatin indicated the presence of proteolytic activity [32].

Urease activity

- Urease activity was assessed on Christensen's medium, containing (g/L): urea — 20, agar — 10, glucose — 10, NaCl — 5, KH_2PO_4 — 2, peptone — 1, phenol red — 0.012; pH = 6.8±0.2. The initial medium is orange-colored; it turns yellow at lower pH and pink, then red, at alkaline pH due to the hydrolysis of urea, which releases ammonia and carbon dioxide [33].

Catalase activity

- Catalase activity was tested by adding 1.0 mL of 3% H_2O_2 directly to a suspension of bacterial cells grown on a solid Lysogeny Broth (LB) medium in Petri dishes. Positive reactions were indicated by the immediate formation of bubbles, demonstrating the presence of catalase enzyme, which hydrolyzes hydrogen peroxide [34].

Citrate permease activity

- Citrate permease activity was determined on solid Simmons' citrate agar, containing (g/L): agar — 20, NaCl — 5, $MgSO_4 \cdot 7H_2O$ — 0.2, K_2HPO_4 — 1, $NH_4H_2PO_4$ — 1, $Na_3C_6H_5O_7 \cdot 5H_2O$ (trisodium citrate pentahydrate) — 3, bromothymol blue — 0.08 (using 40 mL of a 0.2% solution), distilled water — 1 L; pH = 7.2±0.2. The medium was dispensed into tubes (5–7 mL per tube), sterilized, and slanted after autoclaving. The composition of the medium supports abundant growth of bacteria utilizing sodium citrate as the sole carbon source; this growth is accompanied by a color change of the medium from green to blue [35].

2.10. Pigment extraction and determination of bacterioruberin (BR) content

For pigment extraction, a known method [36] was used as the basis with modifications. First, the collected sample was lysed using sonication for 30 minutes while maintaining a temperature not exceeding 20 °C. For this purpose, a Sonorex Digital 10p ultrasonic bath (Bandelin Electronic, Germany) was employed. The sample was then centrifuged using a Centrifuge 5804 (Eppendorf, Germany), resulting in a dense, red-colored pellet of *A. agilis* wb28 cells. The supernatant was decanted; this step was repeated if necessary, and any residual liquid above the cell pellet was removed using an automatic pipette. The biomass was not dried to avoid temperature exposure and subsequent degradation of BR.

Ten milliliters of methanol were added to the pellet. The pellet was then intensively homogenized using a Lab Dancer vortex mixer (IKA, Japan) and an automatic pipette to prevent cell aggregation from interfering with uniform extraction. In the next stage, the pigment was extracted from the cells using a Multi Bio RS-24 multi-rotator (Biosan, Latvia) set to 60 revolutions per minute for 10 minutes, with periodic checks to prevent renewed adhesion. The resulting extract was centrifuged, yielding a fully formed, discolored pellet of cellular debris at the bottom.

Subsequently, 10 mL of acetone were added to the pellet, followed by dispersion and another centrifugation step. The pigment concentration in the final extract was then determined spectrophotometrically using an Eppendorf BioSpectrometer (Germany) at a wavelength of 495 nm.

The concentration of the pigment, calculated as bacterioruberin (BR), was determined using the Beer-Lambert law with an extinction coefficient of 183,000.

2.11. Statistical analysis

The standard deviation method, adjusted with Student's t-test, was used to evaluate the error in determining optical density, dry biomass weight concentration, pigment, protein, and starch content, as well as in calculating the constants describing growth curves and cell doubling time. For three measurements and a confidence interval of $\alpha = 0.05$, the t-value was 4.30. For simple sugars (mono- and disaccharides), whose content was determined by capillary electrophoresis, the expanded uncertainty is indicated. Student's t-test was applied for the statistical comparison of the target product yield when using different substrates.

3. Results and discussion

The degree of pigmentation was initially assessed visually following surface inoculation and incubation of the cell culture. On the medium with soybean meal, the colonies of the *A. agilis* wb28 strain exhibited a bright red color, indicating a high concentration of pigment in the biomass. In contrast, on the medium with wheat bran, the bacterial cells displayed a pink hue during growth. When cultivated on the medium with rapeseed meal, the cells showed less pronounced pigmentation and a pale pink color (Figure 1).

Apparently, the synthesis of the pigment is significantly influenced by the structure of the carbohydrate, which may potentially participate in building the pigment's carbon backbone.

In a comparative analysis, the studied wheat bran exhibits a greater qualitative diversity of sugars and is superior in total carbohydrate content to both rapeseed meal and soybean meal (Table 1).

When comparing the prevalence of sugars based on the complexity of their chemical structure, the highest total sugar content is also characteristic of wheat bran. Wheat bran contains the polysaccharide starch, the trisaccharide raffinose, and the disaccharides sucrose and maltose. Although rapeseed meal contains both complex and simple sugars, maltose was not detected, and sucrose dominates. Soybean meal, among the identified carbohydrates, contains only starch.

The aforementioned hypothesis regarding the influence of the carbon source's structure on pigment synthesis by the *A. agilis* wb28 strain is, to some extent, supported by the results of studying its consumption of individual carbohydrates. It was revealed that incubation of the strain on certain carbohydrate-containing media leads to changes in the color and pH of the medium, indicating the activity of the microorganism's enzyme system and carbohydrate consumption (Table 2).

According to the obtained data, the *A. agilis* wb28 strain actively utilizes oligo- and polysaccharides, suggesting the inducible synthesis of specific hydrolases. Based on literature sources, *A. agilis* bacteria can produce hydrolases such as lipase, amylase, protease, chitinase, and β-galactosidase [37].

The studies revealed that the *A. agilis* wb28 strain possesses saccharolytic, lipolytic, and proteolytic capabilities to hydrolyze complex substrates and transform simple molecules of various chemical natures (Table 3). It should be noted that *A. agilis* wb28 consumed substrates selectively. For instance, among the numerous components of the nutrient media, glucose, gelatin, and urea were either not assimilated or were poorly assimilated by the strain's enzyme system.

Glucose, at the concentration used in the nutrient medium, apparently inhibited the growth of the studied strain and was assimilated more slowly. It is possible that the bacterium cannot efficiently utilize glucose as an energy source due to a lack or absence of enzymes for its assimilation. Another possible reason is the presence of more preferable carbon sources. This assumption is based on the fact that on a medium containing only agar (composed of the polysaccharides agarose and agaropectin) as a carbon source, the strain exhibited active growth, unlike on a medium of the same composition but with additional glucose (Table 2).

Table 2. **Saccharolytic properties of the *Arthrobacter agilis* wb28 strain**
Таблица 2. **Сахаролитические свойства штамма *Arthrobacter agilis* wb28**

Carbohydrate	Sign			
	Change in Medium Color	Change in Medium pH		Growth (Pellicle Formation)
		Acidification	Alkalization	
Sucrose	++	++	–	+
Glucose	–	–	–	+/–
Maltose	++	–	+	+
Fructose	++	++	–	+
Raffinose	++	++	–	+
Mannitol	++	++	–	+/–
Sorbitol	+	–	++	+/–
Amyldextrin	+	–	+	+
Cellulose	++	–	+	+
Agarose+Agaropectin	++	–	++	++

Note: «+» Positive reaction, «++» Strong positive reaction, «–« Negative reaction, «+/» Weak or variable reaction.

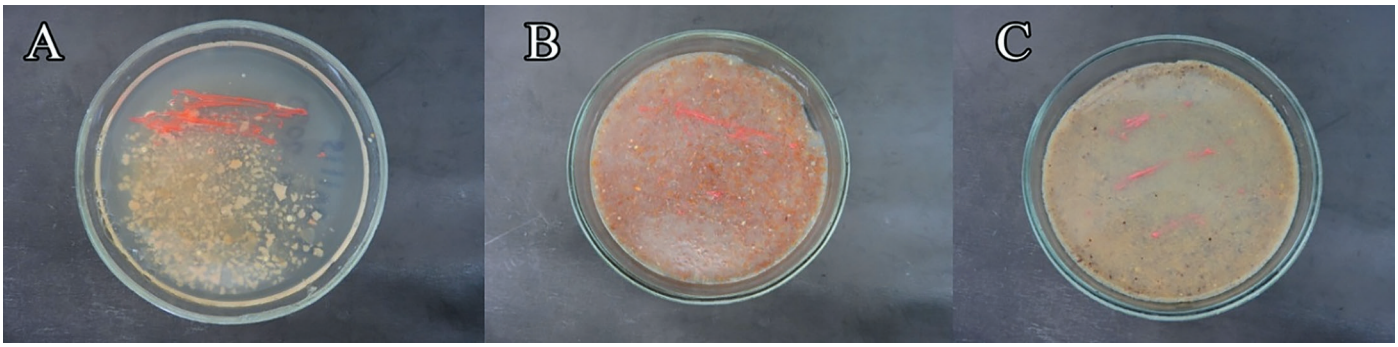

Figure 1. **Growth of *Arthrobacter agilis* wb28 cells on solid agarized medium: A — soybean meal, B — wheat bran, C — rapeseed meal**
Рисунок 1. **Рост клеток *Arthrobacter agilis* wb28 на плотной агаризованной среде: A — соевый шрот, B — пшеничные отруби, C — рапсовый жмых**

Table 1. **Carbohydrate and protein composition of the nutrient medium**
Таблица 1. **Углеводный и белковый состав питательной среды**

Raw Material	Mass Concentration of Component, g/L						
	Fructose	Glucose	Maltose	Sucrose	Raffinose	Starch	Protein
Soybean Meal	–	–	–	–	–	1,86±0,09	5,49±0,21
Wheat Bran	0,090±0,010	0,170±0,010	0,430±0,030	0,800±0,060	0,540±0,040	3,25±0,13	3,39±0,14
Rapeseed Meal	0,040±0,003	0,051±0,004	–	3,060±0,240	0,77±0,06	0,46±0,03	2,18±0,08

Note: «–» — not detected.

Table 3. **Enzymatic activity of the *Arthrobacter agilis* wb28 strain**
Таблица 3. **Ферментативная активность штамма *Arthrobacter agilis* wb28**

Enzymatic Activity	Specific Substrate (Source)	Chemical Nature of Substrate / Class of Compounds	Activity
Saccharolytic	Sucrose	Carbohydrate / Disaccharide	+
	Glucose	Carbohydrate / Monosaccharide	–
	Maltose	Carbohydrate / Disaccharide	+
	Fructose	Carbohydrate / Monosaccharide	+
	Raffinose	Carbohydrate / Trisaccharide	+
	Mannitol	Carbohydrate / Sugar alcohol	+
	Sorbitol	Carbohydrate / Sugar alcohol	+
	Agarose and Agaropectin (Agar)	Carbohydrate / Polysaccharide	+
Amylolytic	Amyldextrin (Soluble Starch)	Carbohydrate / Polysaccharide	+
Lipolytic	Esters of Fatty Acids and Glycerol (Olive Oil)	Lipids / Fats (Triglycerides)	+
	Polyoxyethylene (20) sorbitan monooleate — Ester of oleic acid, sorbitol, and its anhydrides (Tween 80)	Nonionic Surfactant	+
Proteolytic	Casein (Skim Milk Powder)	Protein	+
	Partially Hydrolyzed Collagen (Gelatin)	Polypeptides	–
Urease	Urea	Diamide of Carbonic Acid	–
Citrate Permease	Sodium Citrate	Salt of Citric Acid	+
Catalase	Hydrogen Peroxide	Peroxide	+

Many members of the *Arthrobacter* genus lack proteolytic activity. However, *A. agilis* wb28 peptonized milk. This indicates the synthesis of caseinase for hydrolyzing casein, the predominant complex protein in milk.

However, *A. agilis* wb28 does not liquefy gelatin, which consists of a mixture of low-molecular-weight peptide fractions or their aggregates, the most common of which is the tripeptide ProHypGly (proline — hydroxyproline — glycine). Most *Arthrobacter* strains do not synthesize gelatinase, which hydrolyzes gelatin.

Urea (diamide of carbonic acid) is not utilized by *A. agilis* wb28 as a nitrogen source; the strain does not synthesize urease, which catalyzes the hydrolysis of urea.

As the experimental results showed, the bacterium is capable of utilizing citrate as the sole source of carbon and energy for its growth. This indicates that the strain synthesizes an enzyme-like protein, citrate permease. In this case, this protein is involved in the transport of citrate into the bacterial cell. Inside the cell, citrate is incorporated into the reaction chain to produce energy.

The *A. agilis* wb28 strain exhibits a pronounced ability to hydrolyze lipids, triglycerides, and plant-derived fatty acid esters.

The aforementioned results on the hydrolytic capacity of *A. agilis* wb28 indicate the occurrence of biochemical processes primarily characteristic of the *Arthrobacter* genus. The biosynthesis of secondary metabolites by the bacterium, particularly the pigment, is a response to stressful conditions. For arthrobacters, known stress factors that significantly influence the intensity of pigment synthesis are low environmental temperature and osmotic pressure. The experiments maintained the same temperature regime for the fermentation of the nutrient media. Regarding the osmotic factor, the studied secondary raw materials differ in chemical composition, including salt content. The content of mineral components can vary depending on the characteristics of the agricultural crop from which it was derived. For example, the main mineral elements present in feed wheat bran are, g/kg: potassium — 10.6; phosphorus — 7.4; magnesium — 4.5; calcium — 0.9, while other elements constitute only 1% [38]. Soybean meal contains, g/kg: potassium — 25.2; phosphorus — 7.7; magnesium — 4.9; calcium — 4.7; sodium — 1.1, while other elements (manganese, zinc, iron, copper) constitute 3% [39]. Rapeseed meal contains, g/kg: potassium — 11.1; phosphorus — 7.9; calcium — 4.8; sulfur — 4.5; magnesium — 4.4; iron — 0.5; the rest are less than 1% (manganese, zinc, copper, cobalt, iodine) [40]. Accordingly, the trend of potassium and phosphorus predominance in the nutrient media prepared from the studied raw materials persists. However, while the phosphorus content in the raw materials is at a similar level, potassium is 2.5 times more abundant in soybean meal. In other words, the likelihood of ion pump operation involving potassium ions is higher for bacterial cells when cultivated in a liquid medium with soybean meal. In aerobic bacterial cells, nutrient transport into the cell occurs via symport with protons involving specific carrier proteins, permeases (paired transport of two different organic molecules or ions across the membrane) [41]. The A. agilis wb28 strain is capable of synthesizing permease for the assimilation of, in particular, sodium citrate (Table 3). Theoretically, the quantitative salt composition should not sharply provoke pigmentation of the culture fluid. Depending on the cationic and anionic composition of the salts, as shown by studies of other scientists, the pigment content in the biomass of *A. agilis* can fluctuate both towards increase and decrease [42].

As a result of fermenting nutrient media from the studied raw materials in shake flasks under shaker-incubator conditions and extracting the pigment from the bacterial biomass, the following quantitative data were obtained, mg/L: soybean meal fermentation — 24.46±0.62, wheat bran — 10.19±0.46, rapeseed meal — 2.39±0.03. Apparently, during the cultivation of the *A. agilis* wb28 strain in a liquid medium with soybean meal, the predominance of potassium contributed to active nutrient transport. Combined with a relatively higher protein content, metabolic processes proceeded more intensively, stimulating the accumulation of biomass and the biosynthesis of metabolites, including the pigment.

In a comparative aspect, the obtained results somewhat diverge from the visual assessment of the degree of pigmentation of *A. agilis* wb28 colonies when cultivated on solid agarized media from the studied secondary raw materials (Figure 1). Most likely, this is due to slowed and unevenly occurring diffusion processes in the agar medium. Considering that *A. agilis* wb28 is an aerobe, the uniformity of oxygen distribution throughout the volume of the culture medium significantly affects both biomass accumulation and metabolite biosynthesis. It is known from literature sources that the induction of carotenoid biosynthesis, particularly BR, occurs as a result of exposure to such physicochemical environmental factors as the intensity of light irradiation of microbial cells, changes in medium salinity, and the level of aeration also has a significant influence [9,43]. The biosynthesis of carotenoids and their derivatives by bacteria, including BR, is associated with redox reactions [44]. In shake flasks under shaker-incubator conditions, the rate of oxygen supply to bacterial cells is not always uniform, which can significantly affect the research results. When cultivating *A. agilis* wb28 in a liquid medium in a bioreactor, a stable saturation level necessary for bacterial viability was maintained. The study of growth kinetics, traditionally characterized by the level of optical density, showed that, regardless of the substrate, the stationary growth phase occurs after 16–24 hours of cultivation (Figure 2).

The obtained data indicate rapid culture growth under laboratory bioreactor conditions simulating an industrial biotechnological process. The lag phase for all studied raw materials lasted approximately 8 hours and was less pronounced during rapeseed meal fermentation. The highest biomass accumulation was observed during soybean meal fermentation, which is consistent with the shake flask cultivation results. However, pigment productivity was higher when *A. agilis* wb28 was cultivated on wheat bran-based medium (Table 4).

Aerobes utilize oxygen for respiration, which is linked to the activity of oxidoreductases that catalyze redox reactions. During oxygen consumption for respiration, hydrogen peroxide is also produced in the cells of aerobes as a byproduct of oxidative phosphorylation. Bacteria, including *Arthrobacter*, possess various defense mechanisms against hydrogen peroxide due to its potential toxicity. These mechanisms involve the action of oxidoreductases such as catalase and peroxidase, resulting in the decomposition of hydrogen peroxide into water and oxygen.

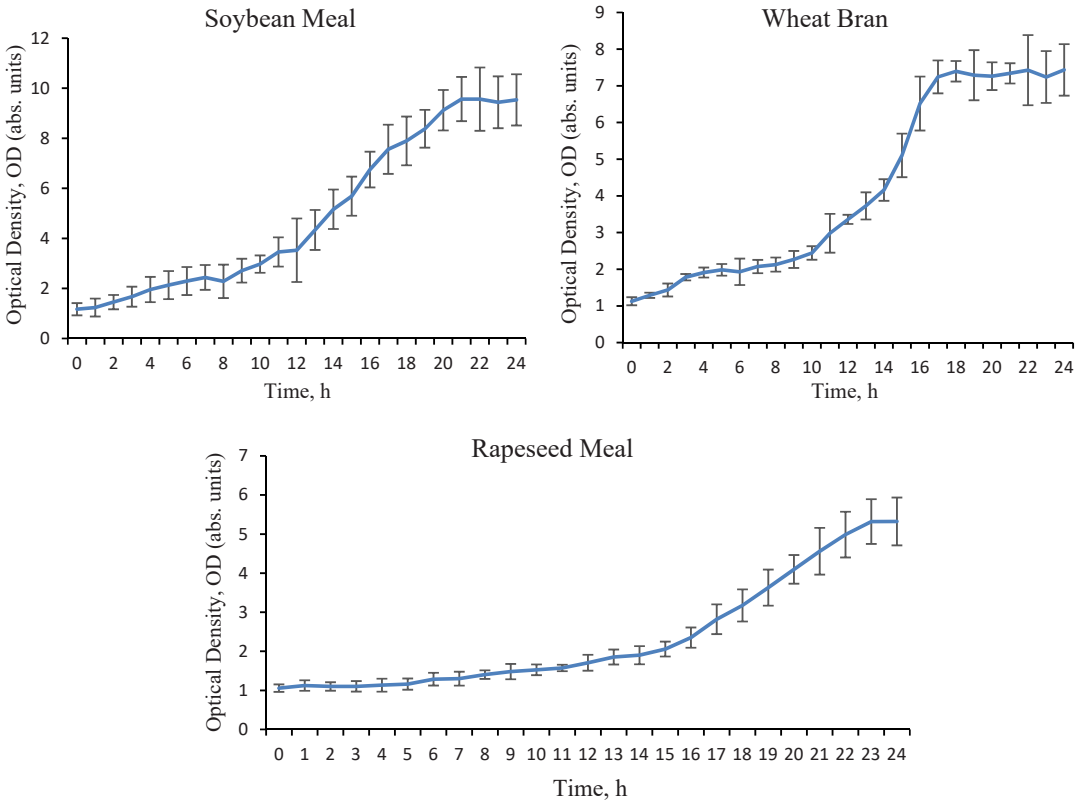

Figure 2. **Growth kinetics of *Arthrobacter agilis* wb28 cultured on media from secondary raw materials**
Рисунок 2. **Кинетика роста штамма *Arthrobacter agilis* wb28, культивированного на средах из вторичного сырья**

Table 4. **Fermentation performance indicators of secondary raw materials**
Таблица 4. **Показатели эффективности ферментации вторичного сырья**

Indicator	Raw Material		
	Soybean Meal	Wheat Bran	Rapeseed Meal
Dry cell weight concentration, g/L	1,37±0,14[a]	0,98±0,07[b]	0,78±0,04[c]
Cell doubling time, h	1,67	2,13	3,85
Pigment (BR) concentration in culture liquid, mg/L	2,59±0,21[a]	4,28±0,37[b]	1,16±0,08[c]

a, b, c — Mean values within a column sharing common superscript letters are not significantly different ($p < 0.05$).

As indicated above, besides hydrolytic activity, the studied strain *A. agilis* wb28 exhibited catalase activity (Table 3). The oxidoreductase activity of some *Arthrobacter* bacteria increases under the influence of extreme factors [45]. It is plausible that the oxidation of carbohydrates in the culture medium under intense aeration is accompanied by excessive formation of hydrogen peroxide, which can induce oxidative stress. Consequently, *A. agilis* wb28 initiates the biosynthesis of catalase to decompose hydrogen peroxide.

Oxidative stress can negatively impact carotenoid biosynthesis, as it may alter the properties of enzymes and other components essential for this process. On the other hand, carotenoids themselves act as antioxidants and play a protective role by neutralizing reactive oxygen species (ROS), including those formed during peroxide decomposition. It is possible that under oxidative stress conditions, the bacterium *A. agilis* wb28 synthesizes an increased amount of carotenoids.

It is known that in haloarchaea, during bacterioruberin (BR) synthesis, oxidoreductases catalyze the oxidation of carotenoid precursors — isoprene units. The precursor of BR is the isoprenoid compound lycopene, which belongs to the carotenes [46]. The conversion of lycopene into BR occurs through numerous chemical processes such as elongation, hydration, dehydrogenation, and hydroxylation. As a result of these transformations, carotenoid molecules can contain epoxy, hydroxyl, and carbonyl groups, as well as carry methoxy- and carboxy-radicals. BR is an oxygen-containing carotenoid and contains 13 conjugated double bonds, which contributes to its efficient elimination of hydroxyl radicals [46].

Collectively, the biosynthesis of both catalase and BR by the strain *A. agilis* wb28 represents a comprehensive response of the bacterial antioxidant system to stress associated with carbohydrate transformation under conditions of active aeration of the culture medium.

The relatively high content of carbohydrate components in the initial nutrient media compared to mineral substances indicates that the primary stress factor in the nutrient medium composition for pigment formation by the studied strain is the carbohydrate concentration. Protein components were primarily utilized for biomass production. The significant amount of protein in the nutrient medium based on soybean meal explains the more productive accumulation of biomass during the cultivation of *A. agilis* wb28 (Table 1 and Table 4). The initial nutrient medium based on rapeseed meal was characterized by a lower protein content, which accounts for the reduced “Mass concentration of dry biomass” indicator.

The results of assessing the feasibility of using the studied secondary raw materials for the biotechnological production of BR showed that the strain *A. agilis* wb28 is capable of synthesizing carotenoids when cultivated on nutrient media from wheat bran, soybean meal, and rapeseed meal. The quantitative indicator obtained from the extraction of BR is comparable to the yield levels of pigments synthesized by representatives of the genus *Arthrobacter* during the fermentation of various types of secondary raw materials (Table 5).

Known pigment-synthesizing bacteria from other genera (such as *Bradyrhizobium* sp. *Flavobacterium* sp., *Sphingobacterium multivorum, Sphingomonas paucimobilis, Paracoccus* sp., etc.), including halophilic species (e. g., *Bacillus clausii*), are characterized by their ability to grow on various substrates and synthesize a relatively wide spectrum of carotenoids [50]. However, no producers of bacterioruberin (BR) are found among them.

Compared to representatives of halophilic archaea, the BR yield obtained during the cultivation of *A. agilis* wb28 is relatively lower. However, it is important to note that in such studies [e. g., 49], individual carbohydrate sources were used as substrates. The productivity of halophilic bacteria in terms of carotenoids is generally on par with that of filamentous fungi and yeasts, potentially constituting less than 1 % and reaching up to 4.5 % of the dry biomass [50]. When calculated per dry biomass, the BR yield for *A. agilis* wb28 was 0.2 % when cultivated on media from soybean and rapeseed meal, and 0.5 % on wheat bran.

Compared to filamentous fungi and yeasts, the advantages of using bacterial producers of carotenoids include a shorter fermentation cycle, a less robust cell wall, and consequently, greater accessibility for diffusion processes during extraction. The aforementioned factors create prerequisites for establishing a faster, simpler, and less costly carotenoid production process.

Table 5. **Comparison of the yield of target carotenoids during the cultivation of bacteria of the genus *Arthrobacter* on media derived from secondary raw materials and their processing products**

Таблица 5. **Сравнение выхода целевых каротиноидов при культивировании бактерий рода *Arthrobacter* на средах из вторичного сырья и продуктов их переработки**

Substrate	Producer	Pigment	Yield	Source
Soybean meal	*A. agilis* wb28	Bacterioruberin	2.59 mg/L	Present study
Wheat bran	*A. agilis* wb28	Bacterioruberin	4.28 mg/L	Ibid.
Rapeseed meal	*A. agilis* wb28	Bacterioruberin	1.16 mg/L	—
Whey	*A. agilis* NP20	Bacterioruberin	5.13 mg/L	[42]
Molasses	*A. agilis* A17	β-Carotene	100 mg/g	[47]
Starch	*Arthrobacter* sp. QL17	Arthroxanthin	25 mg/g	[48]
Starch-containing fraction	*Haloferax mediterranei*	Bacterioruberin	97.39 ± 1.86 μg/mL	[49]

4. Conclusion

Thus, bacteria of the genus *Arthrobacter* are of interest as promising producers for the microbiological synthesis of carotenoids. The bacterial isolate *A. agilis* wb28 is capable of synthesizing the carotenoid bacterioruberin (BR) when cultivated on complex media derived from secondary raw materials. It should be noted that the studied strain synthesizes BR with a higher yield on a nutrient medium made from the raw material from which it was originally isolated, namely wheat bran.

The quantity and structure of carbohydrates in the nutrient medium significantly influence pigment synthesis by the strain *A. agilis* wb28. Wheat bran medium contains predominantly starch as well as simple sugars. These serve as a carbon source, which is necessary, in particular, for building the carbon skeleton of carotenoids. Under conditions of carbohydrate deficiency, as observed in the soybean meal medium, pigment formation in the bacterial cells is reduced. At the same time, the high protein content, compared to other media, positively affected biomass growth.

It is important to consider that pigment synthesis by the strain *A. agilis* wb28 is influenced not only by low temperature and osmotic pressure but also by the composition of the nutrient medium in combination with the aeration regime.

The presented experimental data indicate the prospective potential for using three types of secondary raw materials for the biosynthesis of BR. The obtained results contribute to expanding knowledge about the biosynthetic properties of bacterial isolates, particularly representatives of the genus *Arthrobacter*. The identified patterns will allow for the development of targeted fermentation technologies for secondary raw materials to achieve productive biosynthesis of carotenoids. These carotenoids could subsequently be recommended for study as potential feed and food micro-ingredients.

Since bacteria, besides carotenoids, also synthesize other biologically active substances (such as enzymes with various specificities) and are characterized by a short life cycle, their widespread use in industrial production is highly feasible.

БИБЛИОГРАФИЧЕСКИЙ СПИСОК / REFERENCES

1. Singh, R., Das, R., Sangwan, S., Rohatgi, B., Khanam, R., Peera, S. K. P. G. et al. (2021). Utilisation of agro-industrial waste for sustainable green production: A review. *Environmental Sustainability*, 4(4), 619–636. https://doi.org/10.1007/s42398-021-00200-x
2. Sadh, P. K., Duhan, S., Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: A review. *Bioresources and Bioprocessing*, 5(1), Article 1. https://doi.org/10.1186/s40643-017-0187-z
3. Malenica, D., Kass, M., Bhat, R. (2023). Sustainable management and valorization of agri-food industrial wastes and by-products as animal feed: For ruminants, non-ruminants and as poultry feed. *Sustainability*, 15(1), Article 117. https://doi.org/10.3390/su15010117
4. Astudillo, Á., Rubilar, O., Briceño, G., Diez, M. C., Schalchli, H. (2023). Advances in agroindustrial waste as a substrate for obtaining eco-friendly microbial products. *Sustainability*, 15(4), Article 3467. https://doi.org/10.3390/su15043467
5. Rodríguez-Espinosa, T., Voukkali, I., Pérez-Gimeno, A., Candel, M. B. A., Hernández-Martich, J. D., Zorpas, A. A. et al. J. (2024). Waste as a sustainable source of nutrients for plants and humans: A strategy to reduce hidden hunger. *Sustainability*, 16(16), Article 7185. https://doi.org/10.3390/su16167185
6. Lopes, F. C., Ligabue-Braun, R. (2021). Agro-industrial residues: Eco-friendly and inexpensive substrates for microbial pigments production. *Frontiers in Sustainable Food Systems*, 5, Article 589414. https://doi.org/10.3389/fsufs.2021.589414
7. Rao, M. P. N., Xiao, M., Li, W.-J. (2017). Fungal and bacterial pigments: Secondary metabolites with wide applications. *Frontiers in Microbiology*, 8, Article 1113. https://doi.org/10.3389/fmicb.2017.01113
8. Shakeri, A., Soheili, V., Karimi, M., Hosseininia, S. A., Bazzaz, B. S. F. (2018). Biological activities of three natural plant pigments and their health benefits. *Journal of Food Measurement and Characterization*, 12(1), 356–361. https://doi.org/10.1007/s11694-017-9647-6
9. Martínez-Cámara, S., Ibañez, A., Rubio, S., Barreiro, C., Barredo, J.-L. (2021). Main carotenoids produced by microorganisms. *Encyclopedia*, 1(4), 1223–1245. https://doi.org/10.3390/encyclopedia1040093
10. Yaqoob, S., Riaz, M., Shabbir, A., Zia-Ul-Haq, M., Alwakeel, S. S., Bin-Jumah, M. (2021). Commercialization and Marketing Potential of Carotenoids. Chapter in a book: Carotenoids: Structure and Function in the Human Body. Springer International Publishing, 2021. https://doi.org/10.1007/978-3-030-46459-2_27
11. Hwang, C. Y., Cho, E.-S., Kim, S., Kim, K., Seo, M.-J. (2024). Optimization of bacterioruberin production from *Halorubrumruber* and assessment of its antioxidant potential. *Microbial Cell Factories*, 23, Article 2. https://doi.org/10.1186/s12934-023-02274-0
12. Salvador-Castell, M., Tourte, M., Oger, P. M. (2019). In search for the membrane regulators of archaea. *International Journal of Molecular Sciences*, 20(18), Article 4434. https://doi.org/10.3390/ijms20184434
13. Gianni, M., Monteiro-Lobato, Z., Garbayo, I., Vilchez, C., Vega, J.M., Martinez-Espinosa, R.M. (2021). Haloferax mediterranei cells as C50 carotenoid factories. *Marine Drugs*, 19(2), Article 100. https://doi.org/10.3390/md19020100
14. Giani, M., Garbayo, I., Vílchez, C., Martínez-Espinosa, R. M. (2019). Haloarchaeal carotenoids: Healthy novel compounds from extreme environments. *Marine Drugs,* 17(9), Article 524. https://doi.org/10.3390/md17090524
15. Merlino, G., Barozzi, A., Michoud, G., Ngugi, D. K., Daffonchio, D. (2018). Microbial ecology of deep-sea hypersaline anoxic basins. *FEMS Microbiology Ecology*, 94(7), Article 85. https://doi.org/10.1093/femsec/fiy085
16. Flores, N., Hoyos, S., Venegas, M., Galetović, A., Zúñiga, L.M., Fábrega, F. et al. (2020). *Haloterrigena* sp. strain SGH1, a bacterioruberin-rich, perchlorate-tolerant halophilic archaeon isolated from halite microbial communities, Atacama Desert, Chile. *Frontiers in Microbiology,* 11, Article 324. https://doi.org/10.3389/fmicb.2020.00324
17. Guo, S., Song, Q., Song, X., Zhang, C., Fei, Q. (2024). Sustainable production of C50 carotenoid bacterioruberin from methane using soil-enriched microbial consortia. *Bioresource Technology*, 412, Article 131415. https://doi.org/10.1016/j.biortech.2024.131415
18. Agarwal, H, Bajpai, S, Mishra, A, Kohli, I, Varma, A, Fouillaud, M. et al. (2023). Bacterial pigments and their multifaceted roles in contemporary biotechnology and pharmacological applications. *Microorganisms*, 11(3), Article 614. https://doi.org/10.3390/microorganisms11030614
19. Silva, T. R., Tavares, R. S. N., Canela-Garayoa, R., Eras, J., Rodrigues, M. V. N., Neri-Numa, I. A. et al. (2019). Chemical characterization and biotechnological applicability of pigments isolated from Antarctic bacteria. *Marine Biotechnology*, 21(3), 416–429. https://doi.org/10.1007/s10126-019-09892-z
20. Mussagy, C. U., Caicedo-Paz, A. V., Farias, F. O., de Souza Mesquita, L. M., Giuffrida, D., Dufossé, L. (2024). Microbial bacterioruberin: The new C50 carotenoid player in food industries. *Food Microbiology*, 124, Article 104623. https://doi.org/10.1016/j.fm.2024.104623
21. Flegler, A., Lipski, A. (2021). The C_{50} carotenoid bacterioruberin regulates membrane fluidity in pink-pigmented *Arthrobacter* species. *Archives of Microbiology*, 204(1), Article 70. https://doi.org/10.1007/s00203-021-02719-3
22. Di Fidio, N., Carmassi, L., Kasmiarti, G., Fulignati, S., Licursi, D., Galletti, A. M. R. et al. (2024). Chemical and enzymatic hydrolysis of waste wheat bran to sugars and their simultaneous biocatalytic conversion to valuable carotenoids and lipids. *Catalysis Today*, 442, Article 114941. https://doi.org/10.1016/j.cattod.2024.114941
23. Kataleviciute, A., Plakys, G., Budreviciute, A., Onder, K., Damiati, S., Kodzius, R. (2019). A sight to wheat bran: High value-added products. *Biomolecules*, 9(12), Article 887. https://doi.org/10.3390/biom9120887
24. Song, Y., Sun, L., Zhang, S., Fan, K., Wang, H., Shi, Y. et al. (2022). Enzymes and microorganisms jointly promote the fermentation of rapeseed cake. *Frontiers in Nutrition*, 9, Article 989410. https://doi.org/10.3389/fnut.2022.989410
25. Gohil, N., Bhattacharjee, G., Kalariya, R., Pandya, V., Khambhati, K., Gohil, J. et al. (2021). Biovalorization of agro-industrial waste soybean meal for the production of prodigiosin by Serratia marcescens. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-02102-8
26. Mari, A., Fafalis, C., Krokida, M. (2024). Evaluation of edible coatings from components from chlorella vulgaris and comparison with conventional coatings. *Coatings*, 14(5), Article 621. https://doi.org/10.3390/coatings14050621
27. Shen, C.-H. (2019). Quantification and Analysis of Proteins. Chapter in a book: Quantification and analysis of proteins in diagnostic molecular biology. Academic Press, 2019. https://doi.org/10.1016/B978-0-12-802823-0.00008-0

28. Lebedeva, E. G., Panichev, A. M., Kiselev, K. V., Ryseva, Yu. Yu, Zaitseva, E. A. (2024). Taxonomic composition and physiological and biochemical properties of cultivated microorganisms isolated from kudurite rocks of the Primorsky Krai and the Republic of Altai (Russia). *The Microbe*, 5, Article 100214. https://doi.org/10.1016/j.microb.2024.100214
29. Ilesanmi, O. I., Adekunle, A. E, Omolaiye, J. A., Olorode, E. M., Ogunkanmi, A. L. (2020). Isolation optimization and molecular characterization of lipase producing bacteria from contaminated soil. *Scientific African*, 8, Article e00279. https://doi.org/10.1016/j.sciaf.2020.e00279
30. Sarmah, N., Revathi, D., Sheelu, G., Rani, K. Y., Sridhar, S., Mehtab, V. (2018). Recent advances on sources and industrial applications of lipases. *Biotechnology Progress*, 34(1), 5–28. https://doi.org/10.1002/btpr.2581
31. Zhang, Y., Xia, Y., Lai, P. F.-H., Liu, X., Xiong. Z., Liu, J. et al. (2019). Fermentation conditions of serine/alkaline milk-clotting enzyme production by newly isolated *Bacillus licheniformis* BL312. *Annals of Microbiology*, 69, 1289–1300. https://doi.org/10.1007/s13213-019-01513-3
32. Aryal, S. (2022). Gelatin Hydrolysis Test — Principle, Procedure, Uses and Interpretation. Retrieved from https://microbiologyinfo.com/gelatin-hydrolysis-test. Accessed September 25, 2024
33. Dahlén, G., Hassan, H., Blomqvist, S., Carlén, H. (2018). Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. *BMC Oral Health*, 18, Article 89. https://doi.org/10.1186/s12903-018-0541-3
34. Rivera-Morales, L. (2022). Catalase test for bacterial identification V.2 Retrieved from https://www.protocols.io/view/catalase-test-for-bacterial-identification-n2bvj6ozxlk5/v2. Accessed February 12, 2022.
35. Aryal, S. (2022). Citrate Utilization Test- Principle, Media, Procedure and Result Retrieved from https://microbiologyinfo.com/citrate-utilization-test-principle-media-procedure-and-result/. Accessed August 10, 2022
36. Churio, M. S., Cerletti, M., De Castro, R. E. (2022). Carotenoids from Haloarchaea: Extraction, fractionation, and characterization. Chapter in a book: Archaea: Methods and Protocols. Springer US, 2022. https://doi.org/10.1007/978-1-0716-2445-6_21
37. Annapure, U.S., Pratisha, N. (2022). Psychrozymes: A novel and promising resource for industrial applications. Chapter in a book: Microbial extremozymes novel sources and industrial applications. Academic Press, 2022. https://doi.org/10.1016/B978-0-12-822945-3.00018-X
38. Krikunova, L. N., Dubinina, E. V., Zakharov, M. A. Lazareva, I. V. (2021). To the question of the grain bran mineral composition evaluation. *Polzunovskiy Vestnik*, 2, 27–35. https://doi.org/10.25712/ASTU.2072–8921.2021.02.004 (In Russian)
39. Ibáñez, M.A., de Blas, C., Cámara, L., Mateos, G.G. (2020). Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. *Animal Feed Science and Technology*, 267, Article114531.https://doi.org/10.1016/j.anifeedsci.2020.114531
40. Renzyaev, A.O., Kravchenko, S.N., Kryuk, R.V. (2022). Rapse cake quality increasing method in AIC. *Bulletin KSAU*, 99(186), 245–251. https://doi.org/10.36718/1819-4036-2022-9-245-251 (In Russian)
41. Raval, S. Y., Arya, P., Jain, M., Sosa, T., Trivedi, P., Dabhi, R. et al. (2024). Sustainable biosynthesis of β-carotene utilizing sugarcane bagasse: Depiction and biotechnological implications. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-05815-8
42. Noby, N., Khattab, S. N., Soliman, N. A. (2023). Sustainable production of bacterioruberin carotenoid and its derivatives from *Arthrobacter agilis* NP20 on whey-based medium: Optimization and product characterization. *Bioresources and Bioprocessing*, 10(1), Article 46. https://doi.org/10.1186/s40643-023-00662-3
43. White, M. F., Allers, T. (2018). DNA repair in the Archaea — an emerging picture. *FEMS Microbiology Reviews*, 42(4), 514–526. https://doi.org/10.1093/femsre/fuy020
44. Shariati, S., Zare, D., Mirdamadi, S. (2019). Screening of carbon and nitrogen sources using mixture analysis designs for carotenoid production by *Blakeslea trispora*. *Food Science and Biotechnology*, 28(2), 469–479. https://doi.org/10.1007/s10068-018-0484
45. Rodríguez, M.F., Gomez, A.P., Parra, C.M. (2022). Molecular and proteomic identification of *Arthrobacter gandavensis* isolated from cows with subclinical mastitis in a dairy farm. *Malaysian Journal of Microbiology*, 18, 309–314. https://doi/10.21161/mjm.221407
46. Jiang, Y., Song, Y., Jiang, C., Li, X., Liu, T., Wang, J. et al. (2022) Identification and characterization of *Arthrobacter nicotinovorans* JI39, a novel plant growth-promoting Rhizobacteria strain from Panax ginseng. *Frontiers in Plant Science*, 13, Article 873621. https://doi/10.3389/fpls.2022.873621
47. Özdal, M., Özdal, Ö.G., Gürkök, S. (April 18–21, 2017). *Statistical optimization of beta-carotene production by Arthrobacter agilis A17 using response surface methodology and Box-Behnken design*. AIP Conference Proceedings: II. International conference on advances in natural and applied sciences: ICANAS2017, 1833(1), Article 020101. https://doi.org/10.1063/1.4981749
48. Yu, X., Jiang, K., Zhang, W., Dong, S., Wu, Y., Zhang, G. et al. (2022). Purification, identification, and properties of a novel carotenoid produced by *Arthrobacter* sp. QL17 isolated from mount Qomolangma. *Antioxidants*, 11(8), Article1493. https://doi.org/10.3390/antiox11081493
49. Giani, M., Pire, C., Martínez-Espinosa, R. M. (2024). Carotenoid production by Haloferax mediterranei using starch residues from the candy industry as a carbon source. *Current Research in Biotechnology*, 8, Article 100265. https://doi.org/10.1016/j.crbiot.2024.100265
50. Yaderec, V.V., Karpova, N.V., Glagoleva, E.V., Petrova K. S., Shibaeva A. S., Javakhia V. V. (2024). Carotenoids: Review of major biotechnological methods and conditions for production. *Proceedings of Universities. Applied Chemistry and Biotechnology*, 14(1), 41–54. https://doi.org/10.21285/achb.905 (In Russian)

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