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Extract natural pigments from Trichoderma

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Abstract: Due to the progress of society, people pay more and more attention to health problems, so more and more people like natural pigments. Therefore, the efficient and safe extraction of natural pigments from microorganisms has become a problem we need to solve. This paper discusses how to extract natural pigments from microorganisms from the perspectives of optimizing media and extraction methods.

Keywords: Natural pigment; Safety; Extract.

1. Why extract natural pigments

Why extract natural pigments from microorganisms

The pigments available to us are mainly natural pigments and artificial pigments. Synthetic pigments have played a role in changing the color of food since 1856, when the first artificial pigment, aniline purple, was synthesized by the Englishman Parkin. In the 1950s and 1960s, most of the synthetic pigments used were derived from coal tar; now they are more commonly derived from petroleum products. Synthetic pigments are used most in the world, with more than 100 varieties. With the continuous development and progress of medical science and human society, the harm of artificial pigment to human body began to be exposed to people's eyeballs, the former Soviet Union in 1968-1970 on amaranth red (also known as edible red 2 (Japan), food pigment 9) this pigment for long-term animal experiments, found that the cancer rate is as high as 22%.[1] Researchers in the United States, Britain and other countries have also found that not only amaranth red, many other synthetic pigments may also cause diarrhea, fertility decline, teratogenesis and so on, some pigments in the human body may be converted into carcinogens. Therefore, artificial pigment is gradually eliminated by people, and people gradually pursue natural pigment.

Natural pigment, as its name suggests, is a kind of coloring material in nature, which is mostly obtained from plants, animals and microorganisms. Compared with artificial pigment, its biggest advantage is that it is safe and non-toxic, and some are even beneficial to human health. Therefore, it is a general trend to obtain natural pigments from microorganisms.

2. The three main sources of natural pigment

2.1. Plant natural pigments

Plant natural pigment is mainly extracted from the roots and leaves of some plants. It has the advantages of simple extraction and many raw materials, and most plant natural pigment contains a lot of vitamins, chlorophyll and so on, which is beneficial to health, so it is loved by everyone [2].

2.2. Animal natural pigments

The accumulation of naturally colored particles in the body and tissues of animals. Insects, fish, birds and animals, for example, come in a variety of colors, including protective and warning colors. The advantages are bright color, good quality, but the raw material acquisition cost is high.

2.3. Microbial natural pigments

Microbial natural pigment is mainly extracted from microorganisms. With short cycle and large yield, microbial natural pigment can be fermented into a large amount of fermentation liquid in a short time, so as to easily obtain a large amount of pigment, which is favored by people. Therefore, extracting pigment from Trichoderma is the general trend [3].

3. How to select the optimal medium

At present, PDA is the mainstream medium for Trichoderma culture. We can optimize by controlling carbon source, nitrogen source and other conditions, and finally select the best culture conditions. The methods are as follows:

3.1. Select the best medium by carbon source

Peel and shred the potatoes, boil for 20min, strain and add 20g/L of different carbon sources such as glucose, fructose, fibredisose, trehalose, lactose, maltose, galactose, sorbose and starch. Then AGAR was added, sterilized, and inoculated with Trichoderma inoculants on shift. The inoculants were static for 8 days. Observe the growth condition of the period, so as to select the optimal medium.

3.2. Select medium by controlling AGAR concentration

According to the references, some Trichoderma growth conditions and pigment production are related to the AGAR concentration of the medium. Therefore, we can also choose to control the growth and pigment production of Trichoderma by controlling the AGAR concentration of the medium.

For example, in PDA medium, with other components unchanged and AGAR concentration as the only variable, 0.5, 1, 2g AGAR was added to control AGAR concentration in a 200ml bottle, respectively. Trichoderma inoculant blocks were inoculated and stood still for 8 days. The growth condition was observed and the optimal AGAR concentration was selected.

3.3. Select the best medium by nitrogen source

The potatoes were peeled and shredded, boiled for 20min, filtered, and added 20g/L of different nitrogen sources such as ammonia, ammonium sulfate, ammonium chloride, nitrate, soybean cake powder, peanut cake powder, cotton seed cake powder, corn pulp, peptone, yeast powder, fish meal, mycelium and distiller's lees. Then AGAR was added, sterilized, and inoculated with Trichoderma inoculants on shift [4]. The inoculants were static for 8 days. Observe the growth condition of the period, so as to select the optimal medium.

3.4. Select the best medium by PH of medium

Different Trichoderma has different preferences and growth environment, so it is particularly critical to select the appropriate PH. Acid and base such as HCL and NaOH can be used to adjust the PH of the medium so as to select the optimal medium.

3.5. Select the appropriate culture temperature

The most suitable culture temperature for Trichoderma is generally 20℃-45℃, but different Trichoderma has different performance, so it is particularly important to choose the most suitable culture temperature. The main method was to place the culture medium inoculated with Trichoderma in a constant temperature incubator at different temperatures. After 8d of culture, the optimum culture temperature was determined by observing the growth of Trichoderma

4. Extraction of natural pigment

Now the mainstream pigment extraction is generally divided into water extraction, acid extraction, alkali extraction, ultrasonic extraction, microwave assisted extraction and organic solvent extraction, the three extraction methods can be matched with each other.

(1) Water extraction method: water as the solvent, can be used in hot water extraction or cold-water extraction (plant polysaccharide is mostly used in hot water extraction, can directly or centrifugal removal of impurities), because the polysaccharide is insoluble in ethanol, can be purified by precipitation. Water extraction does have disadvantages such as high temperature, long time and low extraction rate.

(2) acid extraction: some polysaccharides containing acid groups are not easy to dissolve under acidic conditions, can be treated with hydrochloric acid or acetic acid, and then ethanol or insoluble complex will precipitate polysaccharide out. Acid extraction is easy to destroy the spatial structure of polysaccharide, so it is rarely used.

(3) base extraction: some polysaccharides containing uronic acid and acid polysaccharides are relatively stable under alkaline conditions, can improve the extraction rate of polysaccharide, generally with sodium borohydride or potassium borohydride as solvent. The deficiency of alkali formulation lies in that some polysaccharides degrade when alkaline is strong, and the color and flavor of finished products are easily affected.

(4) Ultrasonic extraction method is mainly the use of ultrasonic mechanical wave, to accelerate its material propagation, ultrasonic extraction and other ultrasonic caused by cavitation effect, mechanical effect and thermal effect, according to the expansion of the movement rate of material molecules [5], expand the material penetration to extract the components of the sample. Compared with the basic extraction technology, ultrasonic extraction technology is rapid, high quality, low cost and high efficiency.

(5) Microwave extraction, also known as microwave-assisted extraction, is a new extraction technology with great potential for development. That is, the solvent in contact with the sample is heated by microwave energy, and the required compounds are separated from the sample matrix and into the solvent, which is a process to strengthen heat and mass transfer on the basis of the traditional extraction process. The extraction speed, extraction efficiency and extraction quality of the microwave-enhanced method are much better than the conventional process, so it has been developed rapidly in the extraction and separation of natural products [6].

(6) Organic solvent extraction method is the use of components in the system have different solubility in the solvent to separate the mixture unit operation. That is, it is a method to transfer a solute from one solvent to another by taking advantage of the difference in solubility or partition coefficient of a substance in two insoluble (or slightly soluble) solvents. Widely used in chemical, metallurgical, food and other industries, general used in petroleum refining industry. In addition, the operation of separating the two insoluble liquids after extraction is called liquid separation [7].

Solid-liquid extraction, also called leach, uses a solvent to separate components from a solid mixture, such as sugar in beets, by leach with water; Extracting soybean oil from soybean with alcohol to increase oil yield; The extraction of active ingredients from traditional Chinese medicine with water to produce fluid extract is called "leaching" or "leaching". Although extraction is often used in chemical experiments, its operation process does not cause the chemical composition of the extracted substance to change (or chemical reaction), so extraction operation is a physical process.

Extraction is one of the methods used to purify and purify compounds in organic chemistry laboratory. By extraction, the desired substance can be extracted from a mixture of solids or liquids.

Because of the different stability and properties of each pigment, we should learn to use a variety of methods to extract pigment, so as to achieve efficient and rapid extraction of the pigment we need.

5. Determine the stability of pigment extract

Natural pigments are usually unstable, so it is necessary to measure the pigment stability rate to obtain the preservation methods of pigments, which mainly include the following methods

(1) Explore the influence of light on the stability of pigment, design different lighting conditions: direct sunlight, indoor scattered light, ultraviolet light, fluorescent lamp and light avoidance treatment, observe the influence of light on the retention rate of pigment extract.

(2) Explore the influence of different temperatures on the stability of pigments, and design different temperatures (4, 25, 37, 55, 70℃) to observe the influence of temperature on the retention rate of pigments.

(3) Explore the influence of different pH levels on the stability of pigments, design hydrochloric acid or sodium hydroxide with different pH levels (0.125, 0.25, 0.5mol/L), and observe the influence of pH levels on the retention rate of pigments.

(4) Explore the influence of different oxidizing reducing agents on the stability of pigments, design different oxidizing reducing agents (H2O2, NaHSO3, EDTA-Na2), and observe the influence of different oxidizing reducing agents on the retention rate of pigments.

(5) Explore the influence of different organic acids on the stability of pigments, design different organic acids (citric acid, malic acid, succinic acid), and observe the influence of different organic acids on the retention rate of pigments.

6. Separation of secondary metabolites

Using the crude extracts obtained from secondary metabolites, thin layer chromatography was used to select the best solvent for the separation of crude metabolites, and the proportion of initial solvent for separation was determined. Then, the secondary metabolites of different components were isolated by silica gel column chromatography, and the monomer compounds were purified by semi-preparative liquid phase. The activity of different components of secondary metabolites was detected.

The chemical structure, molecular formula, molecular weight and absorption spectrum of the isolated high-purity monomer compounds were obtained by nuclear magnetic resonance, high resolution mass spectrometry, infrared spectroscopy, specific rotation and other detection methods. The natural product compound database and SciFinder database were searched for rearrangement of the compounds, and the analysis of each spectral data and corresponding literature information were integrated. The structure of the compound was identified. At the same time, the bacteriostatic and life-promoting activity of the identified monomer compounds was tested.The chemical structure, molecular formula, molecular weight and absorption spectrum of the isolated high-purity monomer compounds were obtained by nuclear magnetic resonance, high resolution mass spectrometry, infrared spectroscopy, specific rotation and other detection methods. The natural product compound database and SciFinder database were searched for rearrangement of the compounds, and the analysis of each spectral data and corresponding literature information were integrated. The structure of the compound was identified. At the same time, the bacteriostatic and life-promoting activity of the identified monomer compounds was tested.

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