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Microorganisms in food production: Fermentation, food spoilage and microorganism design

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Abstract: Food is one of the basic needs of human survival. In order to improve the safety and stability of food, human beings have mastered many food processing methods very early, some of which are to make the original perishable food to keep fresh longer, some are to give food a unique flavor. At the beginning, people did not understand the principles of these processing methods, but with the rapid development of biology, people realized that a large part of food processing methods cannot do without the action of microorganisms, so people began to try to understand and use these microorganisms and their use in the food industry. This article will mainly discuss the current application of microorganisms in the food industry, the screening of microorganisms and the design of a special yeast for coffee fermentation.

Keywords: microorganisms; fermentation; food spoilage; food industry introduction

1. Microorganisms in food production

1.1. Microorganisms in food fermentation

Among the microbial processing methods of food, fermentation is one of the main methods, The scientific rationale behind fermentation started with the identification of microorganisms in 1665 by Van Leeuwenhoek and Hooke [1]. As a food processing technology has been going for a long time in history [2], People use fermentation technology to make cheese, sausage processing and vegetables, fruit preservation [3]. Fermentation is a metabolic process that converts organic substrates (mainly carbohydrates) into useful products (organic acids, gases, or alcohol) using diverse groups of microbes [bacteria, fungi (yeasts and mold)]. Most microorganisms used in commercial fermentation need disaccharides or six-carbon sugars as substrates, even though the microbial world contains organism that can break down essentially any organic compound [4]. Several factors that mainly affect the fermentation process are pH value, temperature, the culture composition of medium, oxygen percentage, etc [4]. During the fermentation of food, lactic acid bacteria (LAB) are one of the most important microbial groups for food fermentation. Lactic acid bacteria include: a Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella [3]. In addition, microorganisms commonly used in fermentation include acetic acid bacteria and gluconic acid bacteria for vinegar and coffee fermentation, *Saccharomyces cerevisiae* for wine fermentation, *Aspergillus* for tea and soy sauce fermentation, *Micrococcus* used in cheese and meat fermentation [1]. *Propionibacterium* for Swiss Cheese and Olive Fermentation [3], In addition, two kinds of yeastlike molds have been found to be useful for the synthesis of vitamin B₂,

and some bacteria can be used for the synthesis of vitamin B12 [4], and *Streptococcus thermophilus* can be used to ferment meat and dairy products [5].

1.2. Microorganisms in food spoilage

In addition to participating in the fermentation process of food production, the spoilage process of food is mainly caused by microorganisms. In the food industry, it is necessary to especially guard against the microbial flora that can cause food spoilage. There exists a lot of complicated mechanisms in the processing of the activities of microorganisms cause the food spoilage, for instance, the protein of lipids' degradation by microorganisms will cause Presence of slime and this visible growing will decrease the food's Acceptance of sensory attributes. In theory, any microorganism (including microorganisms and pathogens used in food fermentation) that can multiply to a high level (spoilage detection level) in food has the ability to spoil food, in practice, however, only a few genera of bacterial species are associated with most food spoilage, this is determined by bacterial characteristics, food characteristics and storage conditions [6]. About this limited number of microorganisms presence in food spoilage, the predominant bacteria associated with spoilage are *Brochothrix thermosphacta*, *Carnobacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Streptococcus* spp., *Kurthia zopfii*, and *Weissella* spp. The main defects that these bacteria cause in foodstuffs are off-odors and off-flavors, discolorations, gas production, slime production, and decreases in pH.

1.3. Other things that microorganisms working in food

Meanwhile the acting in a direct way in food processing and food spoilage, microorganisms also can give some functional modification for food industry. For instance it can actor as a non-thermal approach for decontamination in food industry [7], Produce some medicinal or edible emulsifiers [8], and acting as probiotics protect intestine from the pathogen, improving digestibility by produce enzymes, and help digest with those hard-digesting food's nutrients(For example, lactose-intolerant consumers use lactase to hydrolyze lactose), Stimulate intestinal immune system, and improve intestinal peristalsis [9].

2. Screening of microorganisms used in the food industry and its main basis

2.1. Screening microorganisms for the food industry

In order to screening microorganisms used in food industry, first it needs the ability of identify different microorganisms, in general, in bacteriology a polyphasic approach is recommended. In practice, this means that a bacterial species is represented by a type strain, and strains with a high degree of phenotype and/or genotype similarity to the type strain are considered to belong to the same species [1]. In fungal taxonomy, different concepts are used to define microbial species, e.g. yeasts used to be usually identified by phenotype, but now they are identified by diagnostic sequence. Techniques using molecular biology are considered an alternative to

traditional methods because they analyze genomes independently of physiological traits, which can vary within species. Compared with traditional methods based on physiological and morphological traits, molecular techniques are more repeatable and faster. Additionally, these techniques prevent misclassification of species based on their traits [1].

With the ability of identification taken, now turns to how to screen microorganisms used in the food industry. Traditional screening relies heavily on manual separation/purification and flask culture, which is laborious, tedious and inefficient. While microbial selection is relatively straightforward when selecting for overt phenotypes (e.g., antibiotic/nutrient/tolerance selection pressure), selection for non-obvious phenotypes becomes more difficult, thus requiring new efficient screening techniques [10]. To date, microtiter plate (MTP)-based screening technology is favored because of its moderate to high throughput and automation abilities and cost efficiency, making it a suitable technology platform for preliminary screening [10]. Flow cytometry (FC) enables counting, monitoring, enriching, and sorting of microbial cells suspended in fluid streams. FC technology uses fluorescence and microfluidic techniques for high-throughput characterization, identification, and screening of food microorganisms. Among them, fluorescence coupled with FC allows fast and real-time screening of the desired microbial strains. Fluorescent signals that correlate with cellular chemical or physical characteristics of the target strain enable microbial selection through fluorescence-activated cell sorting (FACS) [10].

2.2. Main factors for screening microorganisms used in the food industry

2.2.1. Degree of fermentation

The most important thing for an ideal starter culture strain is to use a broad spectrum of substrates in the metabolic process, to release inhibitory metabolites faster and to resist the stressful conditions of the fermentation process. Having these qualities ensures well-characterized and defined processing and a certain product certainty.

2.2.2. Nutritional

Nutritional should be one important factor because when fermenting microbial species are carefully applied to enrich the nutritional quality of fermented food substrates, it is cost-effective and efficient with regard to consumer acceptance.

2.2.3. Bacterial resistance to miscellaneous bacteria

Fungal contamination of fermented beverages and food products is a common occurrence, leading to dramatic declines in revenue and healthcare challenges. Recently, fermenting microbial strains with antifungal activity were isolated and identified, which are of great benefit to food preservation.

2.2.4. Biological activity

High biological activity under a wide range of conditions is crucial for the economics of food processing and can also contribute positively to the overall microbial product yield.

3. A possible idea of special bacteria

3.1. Significance of application of special bacteria in food industry

When processing food, traditional food-use microorganisms have the potential to spread antibiotic resistance or produce unwanted metabolites such as D-lactic acid, ammonia, biogenic amines, etc. In addition, traditional food-used microorganisms may not be specific to different pathogens because the antimicrobial substances they release are limited to specific microorganisms. Also, when ingesting food-use microorganisms such as probiotics, some people may experience side effects associated with ingesting probiotics, the effects tend to be mild and digestive problems (such as gas or bloating); however, more serious consequences have been reported in certain high-risk groups, especially if they are among the disadvantaged of receiving antibiotics or have severely compromised immunity [11]. There are several ways we can improve food microorganisms and give them the functions we want.

3.2. Major strategies for making specialty bacteria

Generation of specialty bacteria can be achieved by mutagenesis screening method [10], Protein Engineering Methods [12], and genome editing methods, mutagenesis screening methods mainly include atmospheric and room temperature plasma (ARTP)-mediated mutagenesis, UV/chemical mutagenesis and combined mutagenesis [10], protein engineering methods are often used to optimize the function of enzymes (or other proteins), this may be to increase catalytic activity, but more to make the enzyme work more efficiently under applied conditions which may involve temperatures, pH or salt concentrations far outside the optimal range of the enzyme. For instance, amylases for baking can be designed to withstand blast furnace temperatures for longer periods of time, so that the same number of catalyzed reactions can be achieved with a lower initial enzyme concentration before the enzyme becomes ineffective during baking [12]. The genome editing methods are mainly divided into two strategies i) top-down method (genome reduction to delete unnecessary genes) ii) bottom-up method (genome synthesis to add necessary gene parts) Using these strategy, based on efficient and precise genome editing tools, the biochemical pathways of traditional microorganisms can be altered to promote, enhance or terminate specific biological activities [11]. In this article, I intend to use a mutagenesis screening approach and a genomic approach to engineer a specific lactic acid bacterium for coffee bean fermentation.

3.3. Idea about special yeasts used in coffee bean fermentation

At present, the main treatment methods for coffee beans in the world are drying or frying, which may reduce the possibility of the flavor of coffee beans. Author thinks using special yeasts could improve presents coffee's flavor and quality.

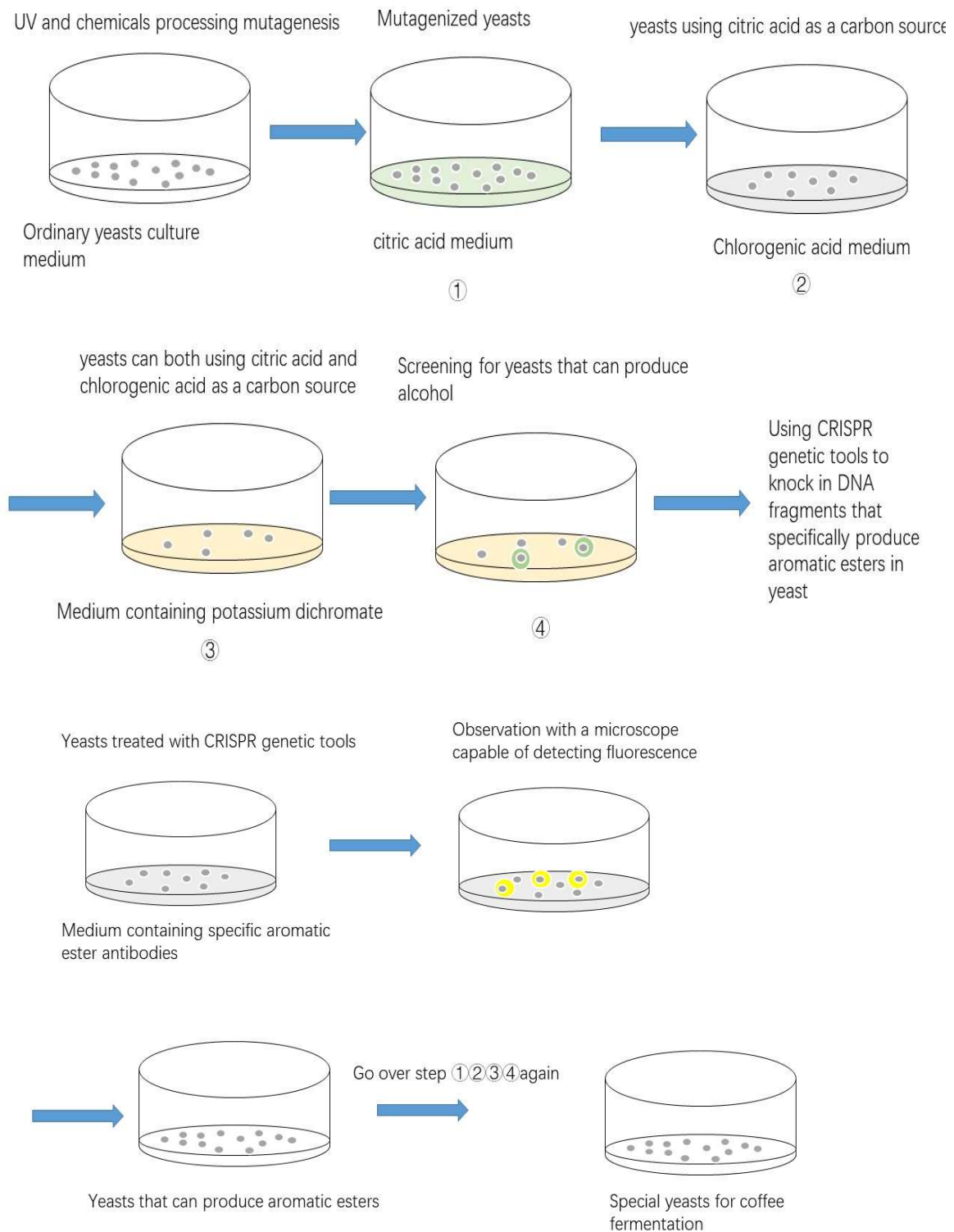


Figure 1. A simplified flow chart describing the special lactic acid bacteria designed for the fermentation of coffee beans.

First induce ordinary yeast cells with UV light and chemicals. After obtaining a large number of induced microorganisms, using using microtiter plate (MTP) screening technology and the medium that uses citric acid as a carbon source it is capable to screen out those special yeast cells that use citric acid as a carbon source.

Then put chlorogenic acid as the carbon source medium for directional screening, and screen to obtain the special yeast that can use both citric acid as carbon source and chlorogenic acid as carbon source. With the step of putting it into the medium mixed with potassium dichromate solution to screen out the special yeast capable of producing alcohol. Then adopt a bottom-up strategy to add necessary gene parts to the yeast, and use the CRISPR gene tool to add a dna fragment that can produce aromatic ester products to the special yeast, Put the DNA-added yeast into the fluorescent antibody medium containing the aromatic ester, and use fluorescence detection technology to detect the special yeast that successfully expresses the gene. Finally, repeat the first 3 steps of screening to obtain the special yeast that meets the requirements [12].

After the above design, such strains should be able to effectively reduce the sourness and astringency of the coffee itself through anaerobic fermentation, and at the same time make the coffee have a certain mellow aroma of alcohol. Aromatic esters can provide such coffee with a unique flavor.

4. Conclusion and future outlook

4.1. Future outlook

The rapid development of biotechnology in recent decades has made many expensive biological methods become more economical, at the same time, with the development of science and technology, policies have gradually shown an acceptable attitude towards biotechnology, author believes that in the near future, as people's understanding of microorganisms increases, microorganisms applied to the food industry will be more widely. This will lead to more non-thermal processing methods in the food industry, making microbial treatment of food (mainly fermentation products) more widespread. This will also benefit people's dining habits, because fermented products are usually ready-to-eat foods that do not require consumers to reprocess, which will improve consumers' sense of convenience and reduce the cleaning cost of completing a meal.

4.2. Conclusion

Based on the above, researchers have gained a certain understanding of microorganisms in the food industry and have begun to apply advanced biotechnology to them. Although there are still doubts about genetically processed foods in the market today, it can be seen that more and more stable and safe genetic methods have been used in laboratories. In this article author also adopts a relatively stable and mature method to design this new special yeast.

Conflict of interest: The authors declare no conflict of interest.

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