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Clinical Perspectives of Domesticated Koji Mold - Probiotic Properties of Aspergillus oryzae: A Review Article

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ABSTRACT

Aspergillus oryzae, known as "Koji-kin" or "Koji mold," has been an indispensable organic part of traditional Japanese cuisine for over a thousand years. Due to its diverse metabolic activity, this filament fungus has distinctive characteristics uncovered via studies conducted over the past two decades. Cumulated shreds of evidence indicate that the unique substances produced by Koji mold have a curative effect on chronic inflammation, metabolic disorders (dyslipidemia, diabetes), cardiovascular and dermatologic diseases, cancer, COVID-19, etc. This article considered the prospects for using Aspergillus oryzae and its derivatives/metabolites in clinical practice as multipurpose preventive and therapeutic agents.

Keywords: Aspergillus orizae; Koji-kin; Koji mold; metabolites.

INTRODUCTION

The history of using the "domesticated" Koji-kin or Koji mold (Aspergillus oryzae) in the Japanese enzymatic industry and traditional cuisine Washoku dates back more than 1000 years.[1] This filamentous fungus and its products of activity (amino acids, fatty acids, simple sugars) are the key players in the production of famous Japanese traditional foods, such as saké (rice wine), amazake (sweet, low-alcohol or non-alcoholic beverage), mirin (type of rice wine), komezu (rice vinegar), miso (thick paste seasoning), takuan-zuke (pickled preparation of daikon radish), fukujinzuke (pickled vegetables), beni shōga (pickled ginger), umeboshi (pickled Japanese plums), etc.[1,2]

The unique properties of Aspergillus oryzae give products specific taste qualities and healing potential. For example, thanks to Koji-kin, miso contains elevated levels of isoflavones, mainly Genistein (soy isoflavone), with an anticancer effect due to Inhibition of NF-κB activation, and DNA methylation, enhancement of histone acetylation, inhibition of cell growth and metastasis, as well as anti-angiogenic, anti-inflammatory and antioxidant effects.[1]

Aspergillus oryzae has recognized GRAS (Generally Recognized as Safe) status since 1979[3] because during “domestication,” it lost functional genes encoding aflatoxin, a toxic substance that causes acute liver necrosis.[4]

It has also lost functional genes to produce less toxic cyclopiazonic acid, which has low-dose immunosuppressive activity and causes necrosis of various tissues.[5]

The safety secret of Aspergillus orizae can also be explained by the peculiarities of the distribution of genes encoding aflatoxin. The genome of A. oryzae consists of eight chromosomes with a genome size of 37.6 Mb, 25-30% larger than the genome of Aspergillus nidulans or Aspergillus fumigatus.[2] The genes responsible for producing aflatoxin are enriched with non-syntenic blocks (NSBs) distributed throughout the genome in a mosaic way.[2] Transcriptional expression of NSB genes is significantly weaker than syntenic block (SB) genes; this may explain the striking contrast in the expression of aflatoxin biosynthesis gene homologs.[2]

Recent research has revealed that Aspergillus oryzae produces unique substances with a curative effect on chronic inflammation, metabolic disorders (dyslipidemia, diabetes), cardiovascular and dermatologic diseases, cancer, COVID-19, etc.[6]

In the present review, we aimed to elucidate the potential medical applications of Aspergillus oryzae and its metabolites.

REVIEW

Metabolites of Aspergillus oryzae

Clinical perspectives of Aspergillus oryzae are based on the wide specter of its metabolic activity. This section will discuss the basic properties of certain substances with potential medical properties.

The complex effect of following active metabolites mediates the prebiotic properties of Koji-kin:

- Acid proteases stimulate the growth of the essential representatives of healthy gut microbiota - bifidobacteria and lactobacilli;[7,8]
- Bifidobacterium-stimulating peptides, such as glutamate, serine, and alanine, increase the

amount of commensal Bifidobacterium longum, Bifidobacterium adolescentis, and Bifidobacterium breve.[9]

- Oligosaccharides such as glucose, xylose, and arabinose also promote the growth of commensal microbes of the intestinal microflora and have beneficial effects in various diseases.[10] For example, oral administration of oligosaccharides stimulates the growth of Bifidobacterium infantis and Bifidobacterium adolescentis, which reduce intestinal mucositis in rats and exert antiproliferative effects on human colon cancer cell lines, respectively.[11,12] In addition to prebiotic activity, oligosaccharides positively affect the clinical course of ulcerative colitis and hepatic encephalopathy at the initial stage.[13-16]
- The uniqueness of this metabolite is not limited to the prebiotic effect alone; Koji glycosylceramide increases bile acid concentration and lowers liver cholesterol in obese mice.[17] The ability of Koji glycosylceramide to enhance the expression of genes involved in tight junctions and delivery of ceramide in normal human epidermal keratocytes and the ability to reduce transepidermal water loss (TEWL) in hairless mice determines the prospect of using this metabolite in dermatology and cosmetics.[18,19] The main components of Koji glycosylceramide - sphingolipids have a variety of biological functions, such as signal transduction and strengthening the immune system.[20,21]

Specific metabolites of Aspergillus oryzae have enzymatic activity and pronounced clinical effectiveness in various diseases. For example, isolated in 1911 by Professor Yokichi Takamine and named after him, taka-diastase (amylase, ribonuclease, phosphatase, proteases) is an enzyme complex that breaks down starch and has a favorable effect in functional gastrointestinal disorders, abdominal pain, heartburn, and overeating.[6,22]

Pre- and probiotic formulations containing enzymes derived from Koji mold are now successfully used in clinical practice to treat functional disorders of the gastrointestinal tract. For example, one such formulation, PROBACTO Enzyme (produced by LAMYRA), is a synergistic combination of probiotic bacteria (Lactobacillus rhamnosus [NCIMB 30373], L. casei [NCIMB 30371], and Lactobacillus acidophilus [NCIMB 30376]), four enzymes derived from Aspergillus oryzae (lactase, alpha-galactosidase, amylase, lipase), and two plantar enzymes - papain (a plant enzyme from the juice of unripe papaya fruits) and bromelain (a plant enzyme from fresh pineapple fruit and stem).[23]

Another metabolite of Aspergillus oryzae - Koji acid, is a competitive and reversible inhibitor of polyphenol oxidases, xanthine oxidase, and some amino acid oxidases. It is used as a food supplement to prevent enzymatic darkening or as a skin-lightening agent in cosmetic formulations.[24]

Another metabolite of Koji mold, ethyl-α-D-glucoside, improves skin impermeability in hairless mice under UVB irradiation,[25] and activates collagen I and fibroblast growth factor I and VII in cultured human skin fibroblasts.[26] Topical use of ethyl-α-D-glucoside increases intercellular lipid concentration, accelerates corneocyte differentiation, and reduces epidermal thickness, thus improving epidermal stratum corneum barrier functions.[27]

The skin's protective function has another metabolite of A. oryzae, Deferriferrichrizine, a small molecule iron chelating peptide, a natural antioxidant that prevents and inhibits skin inflammation.[28]

A wide range of therapeutic effects has Ferulic acid, which reduces lipid levels in hyperlipidemic and diabetic rats.[29] It prevents retinal degeneration[30] and protects against the toxic effects of amyloid peptides that cause Alzheimer's disease.[31]

Ergothioneine has antioxidative solid potential.[32,33] In addition to the reconstituted oxidative balance, it can prevent cisplatin-induced neuropathy and improve cognitive ability.[34]

Some of the remaining metabolites of Aspergillus oryzae attenuate the intensity of hepatitis and colitis in animal models (pyroglutamyl leucine),[35] or inhibit SARS-CoV-2 proteases and can be used to prevent COVID-19 (pyranonigrin A).[36]

Resistant proteins of Koji-kin, protected from exposure to pancreatic proteases, inhibit lipid absorption in the gut and improve the quantitative and qualitative performance of the gut microbiota.[37]

Beta-glucan, a potent hypotriglyceridemic metabolite, also has an antiallergic effect due to its immunomodulatory property and activation of macrophages through dectin 1 and CR3 (CD11b/CD18).[38] Due to its immunomodulatory effects, beta-glucan is also recommended as adjuvant therapy in cancer patients.[39]

Polyamines, especially agmatine, have revealed anti-cancer (reduction in the incidence of colorectal tumors) and anti-inflammatory properties, which are carried out through the regulation of aberrant DNA methylation.[40]

Another active metabolite of Aspergillus oryzae - angiotensin I converting enzyme inhibitor peptide, proved to be a powerful hypotensive agent in animal experiments.[41,42]

Aspergillus oryzae and experimental colitis

Ryo Nomura et al. studied the prebiotic properties of Aspergillus oryzae in mice with dextran sodium sulfate (DSS)-induced damage of colon tissues.[43]

16S rRNA gene sequencing of the gut microbiota revealed that the relative abundance of an anti-inflammatory Bifidobacterium pseudolongum strain

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increased 2.0-fold over the control when heat-killed A. oryzae spores were administered during the first stage of the study. In addition, a decrease in the number of Erysipelotrichaceae was revealed, including the Allobaculum genus.[43]

During the second stage of the investigation, it was revealed that besides the heat-killed Aspergillus oryzae spores, cell wall polysaccharides extracted from them also effectively mitigate colon tissue damage and superiorly enhance the growth of anti-inflammatory Bifidobacterium pseudolongum.[43]

Aspergillus oryzae and human antibodies

A study by Hung Hiep Huynh et al. demonstrated that Aspergillus oryzae might be a low-cost substrate for industrial-producing human antibodies.[44]

Recombinant monoclonal antibodies are typically produced using mammalian cell lines, such as Chinese hamster ovaries (CHO) cells. However, it should be noted that in addition to the high cost of this method, there is a risk of pathogenic contamination when using mammalian cells.[45]

The monoclonal anti-TNF-α antibody, adalimumab, widely used for treating immune-mediated inflammatory diseases, was expressed in Aspergillus oryzae by the fusion protein system with α-amylase AmyB with maximal productivity (39.7fmg/L) in the obtained from the ten-protease deletion strain Aspergillus oryzae.[44]

According to the study results, adalimumab generated by Aspergillus oryzae exhibited TNF-neutralizing and antigen binding capabilities comparable to those of the commercial product Humira®.[44]

Anti-tumor effects of Aspergillus oryzae in pancreatic cancer

Hiroaki Konishi et al. revealed a pancreatic tumor suppression effect of Aspergillus oryzae in vitro and in vivo in a xenograft model of pancreatic cancer cells.[46]

Heptelidic acid was identified as a potent anti-tumor compound produced from A. oryzae using low-resolution liquid chromatography with tandem mass spectrometry (LC–MS) and nuclear magnetic resonance (NMR) analysis.[46]

This study demonstrated that heptelidic acid could pass through the intestinal tract and induce an anti-tumor effect on the extra-intestinal tumors by the p38 MAPK signaling pathway.[46]

Aspergillus oryzae and Mycoplasma pneumoniae Pneumonia

Hui-Yu Lee et al. investigated the effect of A. oryzae fermentation extract (AOFE) on Mycoplasma pneumoniae pneumonia. According to the results, AOFE, mainly represented by Kojic acid, could cause in vitro growth and invasion of Mycoplasma pneumoniae into A549 lung epithelial cells. It is important that in mice preliminary treated with the extract of Aspergillus oryzae observed a significant decrease in neutrophil infiltration of the lungs after Mycoplasma pneumoniae infection.[47]

AOFE inhibited Mycoplasma pneumoniae-stimulated inflammatory response in murine MH-S alveolar macrophages by suppressing tumor necrosis factor (TNF-α) and interleukin (IL)-6 production.[47]

Besides suppressing the above-mentioned proinflammatory cytokines, AOFE inhibited the production of chemokines for MCP-1 monocytes and neutrophils in the bronchoalveolar lavage fluid (BALF).[47]

CONCLUSIONS

Given the unique metabolic profile of the filament fungus Aspergillus oryzae and reliable data on its beneficial effects, it and its metabolites/derivates have excellent potential to be developed into multipurpose preventive and therapeutic agents.

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