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Identification of *Aspergillus nidulans* from cooked eggs produced by permitted factory

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Abstract

Fungus generally doesn't produce toxic or harmful substances so it has low chances to cause food poisoning. However it leads to change appearance, odor and characteristics of the contaminated foods and result in sanitary risk problems. Therefore the contamination of fungi should be prevented since they are not proper for human consumption. Green fungi with white outline raised from the air cell of cooked eggs which were collected by Gyeongi Livestock Veterinary Service in August, 2006. The results came out after the cultivation using Sabouraud's Dextrose Agar(SDA). The conidium appeared white and monospore, the shape of colony was round and oval. Conidiophore was brown and granulated and wrinkles and formed. It was confirmed as *Aspergillus nidulans* based on the dying using Lactophenol cotton blue, the observation of septum and vesicle from the grown spores, and rDNA sequencing

Key words : Fungi, spore, Lactophenol cotton blue, rDNA sequencing, *Aspergillus nidulans*

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Introduction

Generally the mold very well grow in everywhere with proper moisture, temperature and nutrition. When condition is not good for themselves, they overcome it by forming spore. Mold spores are scattered in everywhere such as air, soil, utensils, clothes, food, and they germinate in moist and warm circumstance. According to the report[1] we live with the mold every day even though there were slight seasonal differences in the density of the mold spore. In April, for instance, the density of indoor and outdoor were 17.1sp/m^3 and 46.0sp/m^3,

respectively. In July, that of indoor was 287.4sp/m^3 and outdoor 475.8 sp/m^3. In October, the former was 474.7 sp/$m^3$and the latter 1105.9sp/m^3

The unsatisfied cases of mold contamination in eggs were not known in Korea because the individual standard of it is not set up in Food Hygiene Act or Livestock Processing Act. In addition because mold contamination case is classified as foreign material case, there is no case which reported just mold pollution in eggs.

This study would like to report the results of identification *Aspergillus nidulans* from cooked eggs produced by legal factory.

Methods and Results

Cultivation and macroscopic finding

The eggs collected from Gyonggi province in August, 2006, had thick green brimmed whitish fluffy colony mold in airsack (Fig 1). The mold was examined by common lab protocol[2], then cultivation and microscopic observation was performed as usual. The mold was inoculated in Sabouraud Dextrose (SD) agar and observed. It formed fluffy colony of 2 cm diameter (Fig 2) 2 days after inoculation, and bright green center (Fig 3) was shown 3 days after culture. Following a week, it formed granular wrinkle, and the center of that became thick green with white brim (Fig 4).

Microscopic examination

For microscopic examination the slide cultivation samples (Fig 5) were prepared. The mold was inoculated in the end of SD agar and it was covered with cover glass and cultivated with it. The inoculated glass was stained with a drop of lactophenol cotton blue (BD, USA) and it was investigated with microscope (× 40). The mold was suspected of genus Aspergillus because ascus was formed in conidiospore and septum (Fig 6).

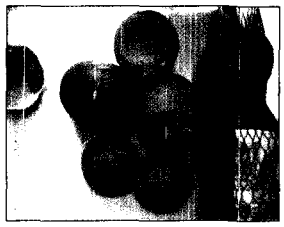

Fig 1. *Aspergillus nidulans* on cooked egg

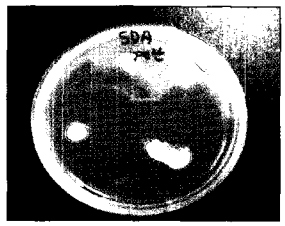

Fig 2. *Aspergillus nidulans* on SDA 2 days after culture

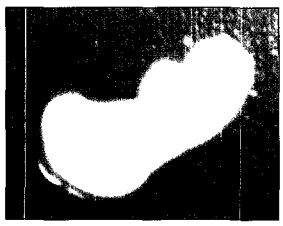

Fig 3. *Aspergillus nidulans* on SDA 3 days after culture

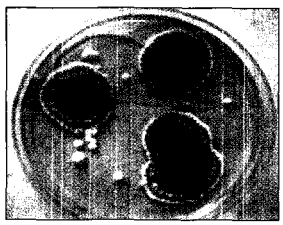

Fig 4. *Aspergillus nidulans* on SDA 7 days after culture

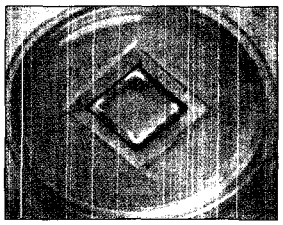

Fig. 5. Slide culture

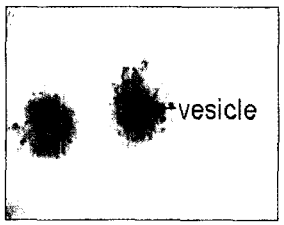

Fig 6. Slide culture of *Aspergillus nidulans* mounted in lactophenol cotton blue

Analysis of rDNA sequencing

The additive analysis for identification

was conducted by the Korean Microbe Preservation Center. The mold was identified as *A nidulans* because of very high homology (99%) after ITS-5.8S rDNA sequencing (Fig 7, 8, 9).

>06-105
GGCTGCCTCCGGGGCGCCCAACCTCCCACCCGTGAATACCTAACACTGTTGCTTCGGCGGGGAACCCCCTCGGGGGCGAGCCGCCG
GGGACTACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGTCTGAATATAAAATCAGTCAAAACTTTCAACAATGGATCTCTTG
GTTCCGGCATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCA
CATTGCGCCCCCTGGCATTCCGGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCCCGGCTTGTGTGTTGGGTCGTCG
TCCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCGACTA
GGGCCGGCCGGGCGCCAGCCGACGTCTCCAACCATTTTTCTTCAGGT

Fig 7. ITS-5.8S rDNA sequencing result (sample: 06-105)

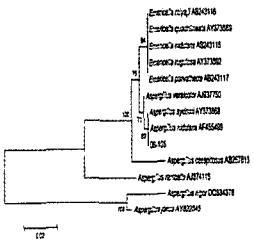

Fig. 8. Dandrogram 1 (sample: 06-105)

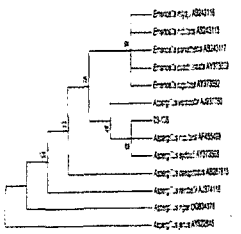

Fig. 9. Dandrogram 2 (sample: 06-105)

Discussion

Aspergillus belongs to the Ascomycota and has the common feature of holding diploid ascospore in the ascus and has the characteristics of having ascus in reproducing process. Today there are about 1,000 species in the world. Because of having diploid ascospore, some can get syngenesis process and generally others are forming 8 spores. Also, hypha is independent in each cell and feature is determined by the characteristics of septum.

Aspergillus is frequently found from nature like indoor air and easily discovered even from the food by-product or soil. This mold can corrupt the foods and others. It doesn't cause disease, but sometimes the disease could be developed if the immunity is weakened[3,4]. For instance *Aspergillus nidulans* is the etiologic agent of cutaneous aspergillosis[5], maxillary sinus disease[6,7], osteomyelitis[8], pulmonary disease[9,10], guttural pouch mycosis in a horse[11,12], and a cerebral abscess[13].

As the resistance mechanism of eggs against contamination of the mold, the surface could prevent the infiltration of microbes by the cuticula layer which is originated from the protein mucus secreted from the oviduct. Air sac is located in the blunt end of egg which has a lot of pores, thus through these microbes can penetrate into the egg. The shell can protect the egg from microbes because it is composed of keratin and reticular system[14]. So, in order to protect this system in the course of egg heating procedure, we have to treat the egg with special care.

In conclusion, the mold of the cooked egg collected from 2006 August Gyoenggi province represented the similarity with *A nidulans* by the rDNA sequencing.

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