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Review Article

Mold detection and environmentally friendly prevention technology for animal specimens

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Abstract

Animal specimens are easily invaded and corroded by molds, which seriously affects the beautful shape and integrity of biological specimens, It's led to a huge economic loss. And the traditional methods & agentia of molds controlled are always spoisonous and polluted agentia. In this paper, review the detecting methods of animal specimens infected molds, exploring methods and reagents of prevention of molds,which can make the animal specimens be preserved for a long time without mold damage. This way would be green, environmental-friendly, and protect the human health and reduce economic losses.

Introduction

For a long time, the preservation of biological specimens has been the primary and important task of the natural history museum and biology educator because the well-preserved biological specimens are conducive the development of teaching activities, science popularization and scientific research. At the same time, with the environmental polluted, many species are endangered, So there is great significance of preparation for the rare and endangered species.

Remodeling the shape and color of living creatures, not only make them lifelike forever, but it also can be permanently preserved. Perennially preserved the the dying or has died of endangered animals in another way in the natural world, It can be provided the raw materials and basis for the future scientific research, and for people to enjoy the sight of the extinct animals, In the mean time, it will be beneficial to alert people to protect the environment and biodiversity.It has important scientific and educational significance.

However, Because of biological specimens are contain mostly high protein and fat, it easy to be invaded and corroded by molds, bacteria and pests, which seriously affects the beautful shape and integrity of biological specimens, especially mold infection is more seriously. The traditional mouldproof methods and reagents have been generally toxic or harmful to the human body, and polluted the environmental, threatened the specimen preservation work, So, it has been not only a difficult problem for biological specimen production staff and scientific research workers, but also seriouslly polluted the environment of specimens exhibition, greatly endangered the health of visitors and researchers, as a result, biological specimen mold measurement technique and environmental control technology is of great significance.

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Therefore, this paper elaborated the detection and identification technology of mold and the existing prevention and control technology, so as to find out the prevention and control measures and biological specimens can be preserved well for a long time.

Mold and its hazards

Mold is a kind of eukaryotic microorganism, without the differentiation of root, stem and leaf, camp parasitic and saprophytic life. Its basic structure is a reproductive spore and a growth function of mycelia. Under the suitable environment, the spore grows out of the spore tube, which gradually extends into filamentary shape, and then spore grows out from the end of the hyphae. In this way, the circulation is continuous, and the offspring can be reproduced continuously. In addition, the growth rate of mold is very fast, and the survival rate is also very high [1].Thus, under the right conditions, mold can wantonly eroded many objects, such as food, furniture, specimens, etc. Dagnas [2], found that mycelia grew on the surface of mildew food, which was harmful to human body. Therefore, they wanted to ensure food safety by strengthening the strict inhibition of mold growth in food production, processing and packaging. Recently, Robert K Bush, et al. proposed that property losses caused by fungal infection in Korean apartment buildings are particularly obvious, which are all related to furniture mildew caused by thermal conditions around apartments. In the case of specimens, mold erosion can be seen everywhere. This not only poses a threat to the long-term preservation of specimens, but also endangers the health of herbarium staff. Julia Hullab studied dozens of diseases such as allergic rhinitis, allergic asthma and urticaria caused by mold on human body [3-5]. It can be seen that mold hazards human from several aspects. As for the prevention and control of mold in biological specimens, we must take measures to maintain the normal shape of the specimens by detecting and preventing mold, which is of great significance to the future scientific research activities and the health of workers.

Mold detection method of animal specimens

Plate method: Medium plate method is the standard method of mold detection. The commonly used medium for mold is PDA medium or potato medium. Multiple single colonies were obtained by cultivating molds scraped from mouldy specimens, and inoculated into PDA medium by plate marking method, and then cultured in a 37℃ incubator for 2 days. According to national standard GB4789.16-2016, the normal temperature of a constant temperature incubator was 25℃±1℃ for 5-14 days [6]. After mould to grow, can undertake the classification of morphology with the naked eye, you can also use 40 times observed with optical microscope, connecting the computer, use the software to get all sorts of images of the mold, then describes the cultivation of the characters, colony characteristics, spores, spore production structure form and characteristics, this preliminary identification has a certain understanding of the mold.

Rapid detection paper disk method

It was different from the medium plate method, the mold rapid detection paper method developed by dai changfang, et al. [7] It could detect mold quickly, accurately and safely. This technique was used to count the number of mold colonies (simultaneously growing yeast colonies) on the paper after inoculation samples were cultured at (36±1)℃ for 40~48h and then the total number of mold colonies per gram (ml) of the sample was converted by the formula. The number of bacterial colonies detected by the paper method is obviously more than that by the plate method, and it is clear and typical.

rDNA-ITS sequence analysis

To detect mold accurately, DNA amplification by PCR is also required, followed by rDNA-ITS sequence analysis to detect mold. In this process, the mold is screened and the primers are designed. Then PCR reaction was performed, pre-denaturation at 94℃ for 5 minutes, followed by denaturation at 98℃ for 30 seconds, annealing for 30 seconds at 58℃, followed by amplification at 68℃ for 2 to 3 minutes.After 25~30 cycles, agarose gel electrophoresis was used to detect and obtain DNA bands, which were finally sent to Shanghai Shenggong bioengineering company for sequencing. The obtained DNA sequences were input into GenBank, and the rDNA-ITS sequences were compared and analyzed by Blast program and DNAMAN tool, and the N-J phylogenetic tree was constructed to determine the mold species. Zhang Rui and Yang Yong [8,9] also conducted similar experiments, and the results showed that the fungi in biological specimens were mainly aspergillus and Streptomyces. Despite the application of rDNA-ITS sequence analysis has certain limitations, but the traditional fungal morphological identification method because of the influence of subjective experience and experiment condition made the appraisal work more difficult, unless research institutions and professionals, in the basic unit few comprehensive ability in fungal morphological identification, and rDNA - ITS sequence analysis for fungal identification is relatively more objective, simple, rapid. Therefore, it is widely used in fungal classification and appraisal research.

Computer vision detection

Computer vision is a kind of detection technology which simulates human vision system. It has the advantages of high detection speed, low cost, convenient maintenance and high visibility.At present, computer vision technology is mainly used for rapid detection of agricultural products, such as quality detection of grains, vegetables and fruits [10-12]. Computer vision technology is based on the computer machine learning model, in recent years, along with the rapid development of computer technology and the development of computer deep learning, Convolution Neural Network (CNN) and believe in the network (DBN) in the application of computer image analysis and classification of frequency is more and more high due to the deep learning technologyallows the original data input, so as to achieve higher classification accuracy [13]. Sun ke, et al. [10] explored and studied the application of computer vision technology based on traditional machine learning and deep learning technology in mold detection.Them use Support Vector Machine (SVM) and Back Propagation Neural Network (BPNN), Convolution Neural Network (CNN) and deep belief

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network model (DBM) mould to establish mode identification method by inoculating these five kinds of mold, aspergillus, degrees, aspergillus Niger, penicillium, aspergillus oryzae and aspergillus versicolor, and to develop and collect the sample images, which results show that the accuracy of computer vision detection technology is about 90%.

qPCR

PCR was often used to detect mold of samples and analyze their species [14] and qPCR is a new detection motheds of fungal abundance and diversity.

Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixedtemplate samples by qPCR and length heterogeneity analysis [15].

Identification of Aspergillus and Mucorales in formalin-fixed, paraffin-embedded tissue samples, Comparison of specific and broad-range fungal qPCR assays [16].

Mold control technology

Control of environmental conditions: The environmental conditions for mold growth and reproduction mainly include high temperature and high humidity. We control the environmental conditions to achieve the purpose of inhibiting mold growth and reproduction.In the herbarium, the temperature of staff flow is generally between 18℃ and 20℃. In the herbarium without staff working inside, the temperature should be maintained between 13℃ and 15℃, and the relative humidity should be controlled between 40% and 50% [17]. Therefore, in general, the specimen house is equipped with air conditioning, or exhaust fan and other ventilation equipment to ensure the air circulation. Some will be placed desiccant such as anhydrous calcium chloride, silica gel or dehumidifier, ensure lower humidity, prevent mold rapid growth and reproduction. In addition to the effects of humidity and temperature, the pH value and the proportion of components in the medium also have certain effects on the growth of mold. However, there are also some differences among different aspergillus. For example, after 7d culture, the optimal pH range for growth of prostrate aspergillus was 4.5~9.0, aspergillus aspergillus was 5.0~9.5 and sarva aspergillus was 5.5~9.0 (Huang Fuxin, et al. 2013).

Application of anti-mildew agent

Traditional fungicide

Formalin and potassium permanganate solution: The traditional fungicides are formalin and potassium permanganate solution. Shailavo, et al. [18] have studied it. They isolated the infected specimens and cleaned them with a clean wet rag or soft brush. Next, applying 10% formalin solution to the mold with a cotton ball, or injecting the mildew with a syringe.In this process, pay attention to the spread of mold to prevent secondary pollution. Furthermore, the amount of formalin solution should be controlled, and the residual liquid should be cleaned in time to prevent corrosion of specimens. This is because 10% formalin (4% formaldehyde) solution can quickly react with the protein to denaturate to achieve the purpose of sterilization, at the same time, converting the protein into insoluble resin that can prevent the decay of the specimen, thus preserving the specimen Zhang Dan, et al.[19,20] also proposed fumigating or applying formalin and potassium permanganate to moldy biological specimens, which can kill spores and prevent the generation of moldy. The application method of this kind of mildew proofing agent is simple and reliable, and it is widely used in the process of specimen mildew proofing, and it is one of the important daily management methods in the process of specimen preservation.However, the limitation of formalin is that it cannot control mold well. What'more, because formalin as the preservation solution will slowly volatilize, the concentration will be reduced, and formalin solution often forms paraformaldehyde in the preservation, so that the immersion solution becomes cloudy, which affects the observation and requires regular treatment.In addition, the strong pungent smell and toxicity of formalin immersion specimens will inevitably cause harm to the health of teachers and students (Xu yongxian, et al. 2011, Tang anke, et al. 2006). At present, more than 80% of the domestic and foreign scholars have conducted biomechanical tests with preserved specimens soaked in formalin, but most scholars believe that there are differences in biomechanical properties between fresh and preserved specimens. The test results of bone samples fixed by formalin cannot reflect their real mechanical properties. Although bones can be kept in ethanol/saline to minimize changes in mechanical properties, the method increases the compressive strength and young's modulus of cancerous bone, which has a certain impact on the mechanical properties of cancerous bone (Xun qinghe, 2019).

Mixture 84 disinfectant

To search a sort of disinfectors which can kill the mildew of specimens rapidly and powerfully Methods. Sunqinghe, et al. (Sun qinghe, 2019) developed 84 disinfectant mixture, which consists of 20% white vinegar, 0.3% benzoic acid, 5% glycerin, 5% 84 disinfectant, 1% natural flower dew and 68.7% water. By comparing with the antifungal effect of formalin, glycerol, alcohol, carbonic acid and mildew enemy, 84 disinfectant mixture was found to be superior.Because 84 disinfectant mixture is developed, acetic acid, which can reduce the PH of the solution and inhibit the growth rate of the mold through improvement, benzoic acid and dew can improve the anti-mildew performance and improve the air. Li Guofeng, et al. (Li Guofeng, et al. 2007) also did similar experiments, and also added the comparison with saturated salt water in his study. But both the results show that 84 disinfectant has good mildew resistance, and it can be widely used in the anti mildew and antisepsis of specimens.

Ethanol

An infection of Aspergillus fungus was discovered in the ichthyology and herpetology skeletal collections at the Natural History Museum of Los Angeles County (LACM) in October of 2003. Within our collections, 12% of fish and 4% of herpetological skeletons were visibly infected. We elected to use 70% ethanol as a fungicide because it is non-toxic,

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effective, inexpensive, and produces minimal damage. Atotal of 688 infected specimens were cleaned, and all 7,987 specimens were rehoused between June 2005 and May 2007. Treatments were carried out by a commercial fungus remediation firm, and the process was monitored by an environmental consultant. They recommend treatment of fungusinfested natural history collections with 70% ethanol, and storage in polyethylene boxes and polystyrene or polypropylene bags, to prevent infection and to contain the spread of infection if it does occur (Christine ET, Richard FF, Neftali AC, et al. 2008).

It conclude that ethanol is an effective fungicide, appropriate for treating museum fish, amphibian, and reptile osteological specimens. We also report that it is possible to perform large-scale fungus mitigation in a natural history collection by contracting with professional fungus remediation firms and consultants. In the case of toxic fungal species, this method may be the only choice for fungus removal. We recommend storage in polyethylene bags and polystyrene or polypropylene boxes, with both interior and exterior labeling. This combination makes the specimens easy to use and examine, safeguards the specimens against breakage, is inert to fungus infestation, and will contain fungal growth that initiates on the skeletal specimen.

Acetic acid solution

When studying the DNA in museum specimens(Sandra M, et al. 2019), the fresh samples were stored in 96% ethanol, while the specimens were stored in 70% ethanol. Due to the dehydrating effect of alcohol, some residual water will be lost when bone samples are stored in ethanol solution. For example, when bone samples are stored in 40% ethanol for 5-10 days, Young's modulus will decrease by 2.5-4%. So, when the samples are stored in ethanol, in order to restore the original humidity, the samples should be taken out and soaked in the same pressure saline several hours before the test, and then refrigerated (Zhang wen, et al. 2017) [1].

Preservatives for mammalian specimens

Small mammals can be treated with arsenic paste or other preservatives. The proportion of arsenic paste should be, soap 50g, arsenic 50g, water 150g, heated to paste shape and cooled before use. Large mammals can be treated with alum solution. The proportion of the mixture is 50kg water, 15kg salt and 2-5kg alum. In the container, add in turn water heat alum and salt to dissolve, and use after cooling.

And the fur is usually soaked for 5-30 days. In addition, the boric acid antiseptic powder which compounded by boric acid, alum and camphor can also be selected, which is safer to use.

New anti-mildew agent

In view of the advantages and disadvantages of the traditional anti-mildew agent, more and more researchers have invested in the research of biological specimen mould control technology, and developed a variety of new anti- agent. No matter in the effect of mould control or in the actual application of environmental protection, the new anti-mildew agent have shown great advantages. This is of great practical significance for the preservation of biological specimens, the development of teaching activities and the exploration of scientific research. According to the investigation, the new anti-mildew agent are mainly as follows.

Chitosan

Chitosan is a kind of broad-spectrum antibacterial agent, which has a good inhibitory effect on the growth of fungi and molds [21-24], so it has a broad application prospect in biological specimen control of molds. Xu qiyu, et al. [25] studied a new kind of specimen preservation solution with chitosan as the main component and the other components include diacetate, Na2EDTA, sodium chloride and glycerin. By inoculating three kinds of main fungi on fresh specimens, and placing them in the new specimen preservation solution and formalin preservation solution respectively, it is found that the two have different inhibition effects on different fungi, but on the whole, the new specimen preservation solution has a good antibacterial effect, and the corrosion stability and biological safety are better than the traditional formalin preservation solution.

Poly-hexamethylene guanidine

Polyhexamethylene guanidine, as a kind of mildew inhibitor for biological specimens, was invented by Zhang Rui (Zhang, Rui, et al. 2013) The preservative is mainly composed of polyhexamethylene with a total molarity of 0.010-100.0 molarity per liter. It can be applied, sprayed and soaked on biological samples, which has obvious effect on inhibition of mould growth, and the effect is stable and soluble in water. Compared with the traditional preservative, it is safer and more environmentally friendly.

Ionic liquid

Ionic liquid is a new type of mould inhibitor for tissue antisepsis and immobilization [26]. It mainly plays a role in the process of forming ion bond of DNA and RNA in tissue, preventing water from entering into tissue cells, and has bactericidal effect. In recent years, ionic liquids have become the focus and hot spot of green chemical industry that many experts and scholars at home and abroad pay close attention to [27]. In foreign countries, ionic liquids was found that they can be used as a fungicide instead of formaldehyde for tissue anticorrosion and fixation [28]. In China, ionic liquid was used as formaldehyde free corpse preservation solution and 5% formalin to immerse knee joint specimens [29]. The results showed that the anti-mildew effect of ionic liquid was significantly better than that of corpse preservation solution and formalin, which could be used for long-term preservation and anti mildew of specimens. And the volatility is very small, which means that it doesn't pollute the environment and damage the health of researchers. This new anti - mildew agent can be widely used in the anti mildew and anti-corrosion of cadaver specimens, including human body and animals, which plays an irreplaceable role in medical research and biological science research.

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Compound anti-mildew agent

In the research field of biological specimen anti-mildew technology, there is a kind of environmental protection compound anti-mildew agent with ingredients of 1-1500ppm chitosan, 1500ppm nano-silver, 1-100ppm glacial acetic acid and water. The compound anti-mildew agent was invented by Zhang Rui, et al. [30] and is suitable for mildew prevention and antisepsis of vertebrates, molluscs and mammals. Nano-silver is sprayed on the inner surface of sheepskin directly. After half a year to a year's observation, there was no obvious discoloration and mildew of sheepskin. The anti-mildew agent has more stable performance, broad-spectrum bactericidal effect, non-toxic and pollution-free. It is a new anti-mildew agent with irreplaceable effect, which is of great significance for the long-term preservation of specimens. Another new preservation solution developed with 50% glutaraldehyde 120g, polyoxyethylene fatty alcohol ether 1g, industrial ethanol 184g, benzalkonium bromide 11g, X reagent 10g, glycerin 3g, disodium EDTA 1g and water 670g. The preservation solution has small volatilization, no pungent smell, liquid element precipitation, low cost, wet specimen without mildew (Sha Z, et al. 2002), and better protection The physical and mental health of teachers and students is more suitable for experiment and teaching in Colleges and universities.

New specimen preservation form

Traditional biological specimens can be divided into peeled specimens, soaked specimens, bone specimens and embedded specimens according to their preservation forms, but they are still susceptible to fungal infection for a long time. For example, immerse the specimen in pure glycerin preservation solution and add a small amount of thymol (or 0.1% carbolic acid) to prevent corrosion, bacteria and mildew [31]. At present, the new forms of specimen preservation are resin embedding and plasticizing, which are suitable for small animals. Tang anke, et al. [32-65] studied a three in one transparent water-soluble resin embedding method for embedding biological specimens, mainly composed of urea, polyethylene glycol and formaldehyde. Compared with the traditional urea formaldehyde resin, it has the characteristics of large embedding capacity and high production success rate; compared with the traditional specimen preservation solution, it has the functions of long-term preservation, long-term mildew prevention and easy to carry or transport. The plastination technology of biology is the most advanced biological specimen preservation technology in the world. Through a vacuum process, it penetrates biological samples with active polymer polymers such as silicone rubber and epoxy resin. The types of polymers used determine the optical properties (transparent or opaque) and mechanical properties (soft and tough) of the samples. The plasticizing technology can keep the surface of the specimen in its original state. The plasticized specimen is dry, tasteless, durable and can be preserved for a long time [31]. Tang anke also developed a non-toxic method of specimen plasticization, which is mainly polyethylene glycol. First, the animals to be made into specimens were killed, and viscera, oil, eyeball and brain were taken out, then fixed and reshaped with iron wire, alcohol was injected into the muscle part, and then the fixed specimens were put into the dehydrator for dehydration. After dehydration, put it into a container filled with polyethylene glycol for plasticization. Turn it every other day. After 7-15 days, dehydrate and dry it. Then wash the polyethylene glycol on the surface with clear water, dehydrate and dry it again, and the specimen can be made. What's more the method has no peculiar smell, can prevent mildew, and can keep the skin smooth and color and is not easy to deform [65-82].

In addition, developed a gel composed of 1%~10% acrylamide compound, 15%~30% tris-hcl, 0.1%~0.5% N-N-methylene diacrylamide, 5%~20% sodium dodecyl sulfate, 0.5%~1.0% ammonium persulfate and 38.5%~78.5% water. Its pH value is 3.5~5.0, which is beneficial to inhibit the growth of mold, and has good effect of mildew prevention and sterilization (Ma hongfeng, et al. 2014).

Conclusion and prospect

Animal specimens are very important, It"s significant to controll and pretect the corrosion by molds. The traditional methods & agentia of molds controlled are always poisonous and polluted,such as Formalin and potassium permanganate solution, But new anti-mildew agent such as Polyhexamethylene guanidine, Ionic liquid, Chitosan and Compound anti-mildew agent are friendly to environmental and suitable protection for mildew prevention and antisepsis . The anti-mildew agent has more stable performance, broad-spectrum bactericidal effect, non-toxic and pollution-free. It is a new anti-mildew agent with irreplaceable effect, which is of great significance for the long-term preservation of specimens. New specimen preservation form, which can make the specimens be preserved for a long time without mold damage, and keep the shape and colour. With the development of mildew prevention technology, the environment-friendly anti-mildew agents for specimens has broad prospects for development.

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