YUN XIONG
HAO YANG
PENG ZHU
HAILONG HUANG
and
QIANQIAN HE

Evaluation the influence of Aspergillus penicillioides on storage jet fuel and develop a rapid and accurate method for its detection

Aspergillus penicillioides is one of the main contaminating fungi in jet fuel result in many damage. This work aims to study the influence of Aspergillus penicillioides on the thermal oxidation stability and corrosivity of jet fuel, meanwhile, developing a repaid and accurate method for detection of Aspergillus penicillioides in jet fuel. The experiment results showed that Aspergillus penicillioides could not only significant weaken the thermal oxidation stability of jet fuel, but also enhance the corrosion ofA3 steel. In this study, a loop-mediated isothermal amplification (LAMP) combined with a lateral flow dipstick (LFD) method has been developed to detect the 18S ribosomal DNA of Aspergillus penicillioides. The whole detection procedure of LAMP-LFD needed only 90 minutes and detection limit of LAMP-LFD for pure A. penicillioides DNA is 0.82pg/ ¼lwhich was 100 times more sensitive than PCR method. LAMP-LFD could specifically detect A. penicillioides isolates and did not occur non-specific amplification. The LAMP-LFD assay established in this study was proved to be a simpler, more sensitive and accurate method for A. penicillioides genomic DNA detection than PCR and LAMP method, therefore, it is expected to be a new method to detect A. penicillioides in jet fuel.

Keywords: *Aspergillus penicillioides; thermal oxidation stability; corrosivity; isothermal amplification (LAMP); a lateral flow dipstick (LFD).*

1. Introduction

Jet fuel as one of a strategic reserve has an important impact on national security. Therefore, the oxidation stability of storage jet fuel is very important. However, it has been proved that microbial contamination can accelerate the fuel deterioration (Rosenberg et al., 1979; Morton and Surman, 1994) .In addition, microbial contamination resulting in many damages, including microbiologically influenced corrosion (MIC) and fouling (Passman., 2013). The consequences of microbial growth in fuel systems were listed by Christine (1999), such as surfactant production, corrosion of storage tanks and lines, fouling of injectors et al. Meanwhile, Christine (1999) also emphasized that some microbial contamination may cause disease. Aspergillus penicillioides as one of the main contaminating fungi has been identified by the next-generation DNA sequencing (NGS) techniques which was used to study the microbial diversity in storage jet fuel of southwest China. And that has never been reported before. The absolute abundance of Aspergillus penicillioides is 9.8%, and it is one of the typical kinds of xenophilia species and widely distributed (Nazareth and Gonsalves, 2013). Meanwhile, Samson (1978) has proved that Aspergillus penicillioides is one of the main pathogens of sick building syndrome and will threat the health of people related to jet fuel.

Thermal oxidation stability of the jet fuel is not only as one of the dominate parameter in modern aircraft, but also very important for fuel storage. The standard test method used to estimate the thermal oxidation stability of jet fuel is Rapid Small scale Oxidation Test (RSSOT) (ASTM D7545). Sicard (2013) has employed Rapid Small Scale Oxidation Test (RSSOT) to investigat the sensibility to oxidation of the n-dodecane. Tao (2006) has indicated that jet fuel itself did not result in the corrosion of A3 steel, but contaminating microorganism did. The method that Tao (2006) used for investigating the influence of Cladosporium on the corrosion of A3 steel will be a reference for this study.

The main methods for detection of contaminating microorganism were traditional methods (Ferrari et al 1998) and methods based on PCR (Raikos et al 2011), and the same applies to the A. penicillioides (Nazareth et al, 2013; Asad et al, 2007). But the two methods have their limitations: 1) traditional methods were based on cultivation, however, most of the microbial in environment could not be separated and identified, so the traditional identification methods can only distinguish between 0.1% to 10% of the total number of

Messes. Yun Xiong, Hao Yang, Peng Zhu, Hailong Huang and Qianqian He, Dept. of Oil Application & Management Engineering, Logistical Engineering University, Chongqing 401311, China, Key Laboratory of Applied Marine Biotechnology, Ningbo University, Ningbo 315211, China, Academic section, Logistics Academy, Beijing, 100000, China

environmental micro-organisms (Sharkey et al, 2004); 2) methods that based PCR need expensive equipment, highly trained operators and strict experimental environment (Xu et al, 2015). All the backwards have limited the above methods field applications.

In order to make up the defects of the above two methods, Notomi (2000) has firstly development the new method Loop-mediated isothermal amplification (LAMP). It is a new method for the amplification of DNA and performed in isothermal condition, therefore, it does not need special equipment comparing to PCR methods. In addition, the LAMP method also has the advantages of easy operation and time-saving, so it has already begun to replace the PCR as a new method for detecting microorganisms. Lateral flow dipstick (LFD) as a substitution of tradition methods (usually gel electrophoresis) for detection of LAMP production has been widely used (Deng et al, 2015; Thongkao et al, 2015). The biotin labelled LAMP amplification could specially hybridize with the probe in LFD which labelled fluorescein isothiocyanate (FITC). LFD combined with LAMP do not need the expensive equipment and easy to operation comparing to gel electrophoresis method. But above all, LFD could accomplish the detection within 10min for visualization. Therefore, LFD maximum limit reduces the influence of artificial factors and makes it possible to apply the LAMP-LFD method to the field quickly detection.

In this paper we evaluated the thermal oxidation ability and corrosivity change of jet fuel which has been contaminated with Aspergillus penicillioides. Meanwhile, we aimed to develop a rapid and special method for Aspergillus penicillioides detection in jet fuel using the loop-mediated isothermal amplification (LAMP) combined with lateral flow dipstick (LFD)".

Actually, as mentioned in the abstract section, it will be rather easy to follow these rules as long as you just replace the "content" here without modifying the "form".

2. Experimental section

2.1 Materials

Fungi sample and DNA extraction

A total of 6 fungal strains including Cladosporium resinae isolated were used in this study (Table 1). These fungi were cultured using Sabouraud liquid medium at 25□ for 4d. The DNA of fungi were abstract by the General Genomic DNA extraction kit (Takara Biotechnology Co., Ltd, Dalian, China) according to the manufacturer's instructions. The DNAs were stored at -20□ until be used.

Table 1 Fungi strains used in this study

Fungi	Source
Aspergillus penicillioides	CICC2153a
Penicllium restrictum	CICC40688
Trichoderma viride	CGMCC3.3875b
Aureobasidium pullulans	CGMCC3.2942
Khuskia oryzae	CGMCC3.8851
Cladosporium resinae	Laboratory isolated

[a] China Center of Industrial Culture Collection, CICC
[b] China General Microbiological Culture Collection Center, CGMCC

2.2 Method

2.2.1 Thermal oxidation ability and corrosion test of jet fuel

2.2.1.1 A. penicillioides Cultivation

Jet fuel was filtrated by 0.22 μm filter membrane before adding to the BH agar being as the sole carbon source for the growth of A. penicillioides .The proportion of BH medium and jet fuel for the last culture medium is 1:3 and the final volume is 200ml. Then, A. penicillioides was inoculated in Fuel-BH medium and cultured at 25°C for different days range from 30 to 90 d, numbering as A, B and C. Meanwhile, the one without A. Penicillioides being ascontrol. The composition of BH agar was shown as Table 2.

Table 2: The composition of BH agar

Ingredients	Gms/Litre
Magnesium sulphate ($MgSO_4$)	0.2g
Calcium chloride ($CaCl_2$)	0.02g
Monopotassium phosphate (KH_2PO_4)	1.0g
Dipotassium phosphate ($(NH_4)_2$ HPO_4)	1.0g
Ammonium nitrate (KNO_3)	1.0g
Ferric chloride ($FeCl_3$)	0.05g

2.2.1.2 Thermal oxidation ability test

5 mL jet fuel sample was extracted from each Fuel-BH medium and then placed into a hermetically sealed test chamber (Petro Oxy RSSOT Apparatus, Anton Paar, Austria). The chamber is pressurized with oxygen to 700 kPa and possibly heated up to 180°C. The pressure of the chamber would increase to the maximum when the temperature reached the requirement. The high temperature could greatly accelerated the oxidation process resulting in consumption of oxygen and a pressure decrease. The induction period of jet fuel in this test is defined as the time when the pressure drop of 10% comparing to the maximum pressure. The induction period could reflected the oxidation sensitivity of the fuel.

2.2.1.3 Corrosion of A3 steel

Each Fuel-BH medium would be hanging a A3 steel after cultivation. And the center of the steel should close to the oil-water interface. Then the Fuel-BH medium with the A3 steel will be storage at 25ºC for 15d. The corrosion degree of A3 steel in Fuel-BH medium with different cultivate time will be observed after 15d. The composition of A3 steel were shown in Table 3.

Table 3 The composition of A3 steel w%

C	Mn	P	S	Si	Fe
<=0.22	<=1.4	<=0.045	<=0.050	<=0.35	Blance

TABLE 4 THE PRIMERS USED FOR LAMP ASSAY

Primer	Primer designation	Length	Sequence(5'–3')
A-F3	Forward-outer primer	20-mer	GGTGGAGTGATTTGTCTGCT
A-B3	Backward-outer primer	19-mer	CGGCCAAGGTGATGTACTC
A-FIP	Forward-inner primer (F1c +TTTT +F2)	40-mer	GTCCCCCTAAGAAGCCAGCG-TTGCGATAACGAACGAGACC
A-BIP	Backward-inner primer (B1c +TTTT +B2)	39-mer	CGATGGAAGTGCGCGGCAAT-TGACCCTGTCAGTGTAGCG
A-LF	Loop-forward primer (ID24)	21-mer	ACCGGGCTATTTAAGGGACGA
A-LB	Loop-backward primer	22-mer	TGATGCCCTTAGATGTTCTGGG

2.2.2 Develop and evaluate LAMO-LFD method

2.2.2.1 Design of the primers for LAMP reaction

Three sets of primers were designed according to the 18s conserve sequence of A. Penicillioides (GenBank KM582668.1) using the Primer Explorer V4 software, including two outer primer (F3, B3), two inner primer (FIP, BIP) and two loop primers (LF, LB) (Table 4). And the position of the primers in the 18s conserve sequence were shown in Fig.1. The 5' end of A-FIP was labeled with biotin, while, the 5' end of A-LF was labeled with FITC. The outer primer of F3 and B3 were also used to be the primers of PCR method.

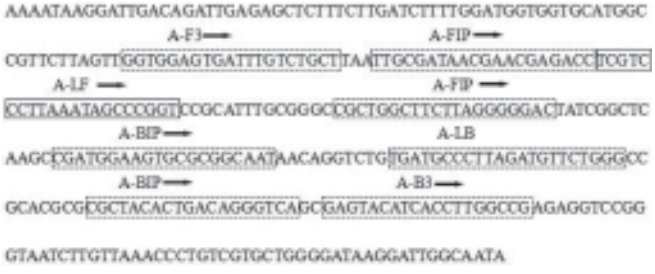

Fig.1 The position of primers at 18s conserve sequence of A. Penicillioides

2.2.2.2 Optimize the LAMP reaction

The LAMP reaction was optimized comparing to Notomi (2000) that the reaction volume was 25 μL, including 0.2 μM of each F3 and B3, 1.6 μM of each FIP and BIP, 0.8 μM of each LF and LB, 0.8 M betaine, 10 mM KCl, 20 mM Tris-HCl (PH 8.8), 0.1% Tween20, 10 mM $(NH_4)_2 SO_4$, 8mM $MgSO_4$, 1.4mM dNTPs, 8U Bst DNA and 2 μL of template DNA. The negative control did not contain the template DNA which was replaced by ultrapure water. In order to determine the best reaction condition, the LAMP reaction was carried out at different temperature of 61, 63 and 65□ for 60 min. The total LAMP reaction were monitored by Real-time Turbidimeter (Loopamp LA-320, Japan).

2.2.2.3 Sensitivity of LAMP-LFD

Ten-fold serial dilutions (10^0–10^{-8}) of DNA extracted from Aspergillus penicillioides were used as the template for LAMP reaction with the optimized condition described above. The LAMP reaction product was respectively detected and visualized by Real-time Turbidimeter (Loopamp LA-320, Japan) and LFD. In addition, the PCR method was also used as a control for LAMP-LFD. The PCR reaction were conducted in 50 μlreaction mixture containing 2.5 μl10×buffer, 20 μM of each F3 and B3, 5U Ex Taq DNA polymerase (TaKaRa), 2.5 mM dNTPs and 3 μl template of DNA. And the PCR reaction condition was as follows: 2 min at 94ºC, 1 min at 94°C, 1 min at 52°C, 2.5 min at 72°C, 35 cycles; 7.5 min at 72°C. The PCR reaction product was detected by electrophoresis on a 1% agarose gel.

2.2.2.4 Specificity of LAMP-LFD

The specificity of LAMP-LFD compared to PCR was evaluated using the template DNA abstracted from the 6 fungi strains at the condition described above. The LAMP reaction product was respectively detected and visualized by Real-time Turbidimeter (Loopamp LA-320, Japan) and LFD, while, the PCR reaction product was analyzed by 1% agarose gel electrophoresis.

2.2.2.5 Application of LAMP-LFD for field detection

11 jet fuel samples were collected from one army oil depot, and numbered Jet-1, Jet-2, Jet-3, Jet-4, Jet-5, Jet-6, Jet-7, Jet-8, Jet-9, Jet-10 and Jet-11. All samples were respectively filtered by 0.22 μm membrane, then the membrane was repeatedly flashed by 1.5 ml ultrapure water. At last, the ultrapure water were re-collected to the 2 ml EP for the abstraction of DNA template. The genomic DNA abstracted from the samples were used as template for the LAMP reaction, and the results were analyzed and visualized by LFD.

3. Results

3.1 THERMAL OXIDATION ABILITY OF JET FUEL

According the requirements and steps of JFTOT Procedure (ASTM D3241[24]/IP 323), we measures the induction period of jet fuel which has different contamination degree by A. Penicillioides and the result was shown in Fig.2. With the extending of cultivation time, the induction period of jet fuel decreased gradually. And it indicated that the growth of A. Penicillioides had weakened the thermal oxidation ability of jet fuel.

3.2 CORROSION OF A3 STEEL

Took out the hanging steel after 15d from the Fuel- BH

medium, and corrosion degree of each A3steel was shown in Fig.3. With the extending of cultivation time, the corrosion degree of steel got deeper. In other words, the growth of A. penicillioides in jet fuel resulting in the enhancing corrosion of Fuel-BH mediun. In addition, with the growth of A. penicillioides in jet fuel, many insoluble has been produced. And that is the main reason of the fouling in fuel system.

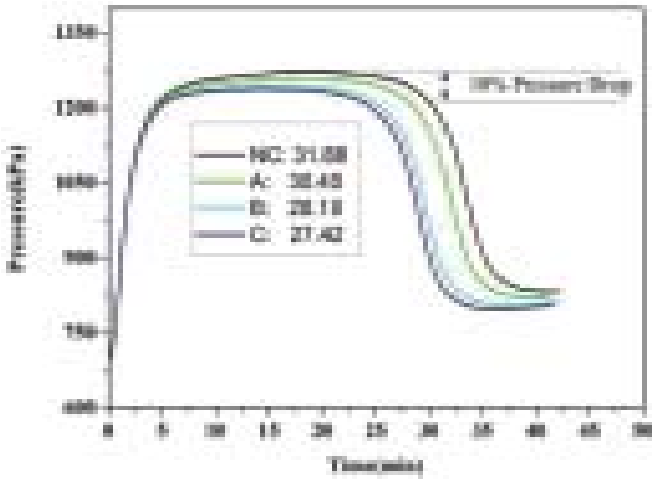

Fig.2 Induction period of different jet fuel samples

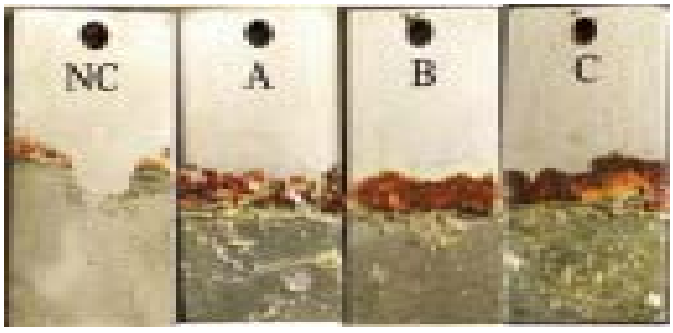

Fig.3 Corrosion of A3steel in different Fuel-BH medium

3.3 The best reaction condition of LAMP assay

The LAMP reaction was carried out with the template of genomic DNA abstracted from A. penicillioides at the temperature range from 61 to 65□. Comparing the three sets of real-time amplification carve of LAMP (Fig.4), we could find that the LAMP reaction at 65□ has the earliest threshold time and the highest amplification efficiency. Therefore, the best reaction condition of LAMP assay was 65□ for 60 min.

3.4 Sensitivity of LAMP-LFD assay compared to PCR and LAMP methods.

The sensitivity of LAMP-LFD was compared to the PCR and LAMP methods using the 10-fold serial diluted (8.2 ng/μl-0.082 fg/μl) DNA of A. penicillioides. The detection limit of LAMP-LFD assay (Fig.5B) was 0.82 pg/μl, while, the LAMP (Fig.5A) and PCR (Fig.5C) methods were 8.2 pg/μl. This indicated that the sensitivity of LAMP-LFD assay was 10 times higher than PCR and LAMP methods.

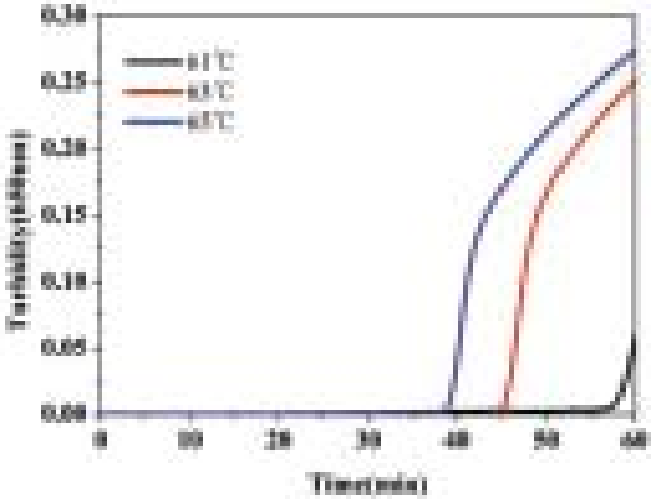

Fig.4 The real-time amplification carve of LAMP at different Temperature

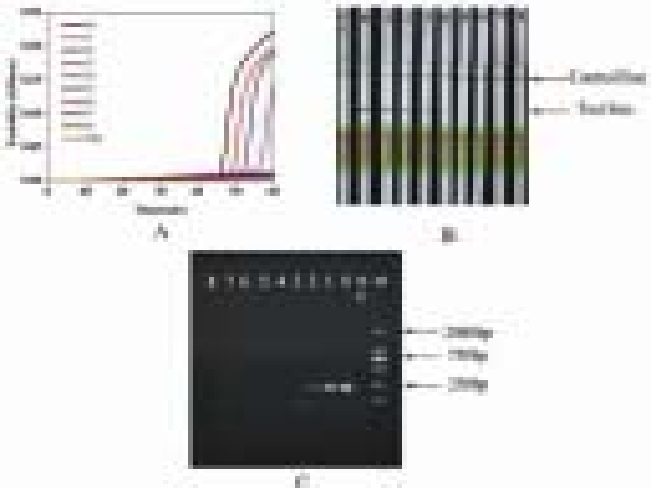

Fig.5 Comparing the sensitivity of PCR(C), LAMP(A)and LAMP-LFD(B) assay

Mark: DL2000 DNA Marker; NC: negative control; 0-8:10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}

3.5 Specificity of LAMP-LFD compared to PCR and LAMP methods

In order to determine whether the LAMP-LFD assay was more specific than PCR method, the genomic DNA abstracted from 6 fungi strains were used as template. The results shown that the LAMP (Fig.6A) and LMAP-LFD (Fig.6B) assay which only using genomic DNA of A. Penicillioides as template resulting in a positive reaction. While the PCR reaction (Fig.6C) was positive not only with A. penicillioides, but also with Cladosporium resinae, Trichodermaviride and Aureobasidium pullulans.

M: DL2000 DNA Marker; NC: negative control;1-6: Cladosporium resinae, Penicllium restrictum, Khuskia oryzae, A. penicillioides, Trichodermaviride, Aureobasidium pullulans

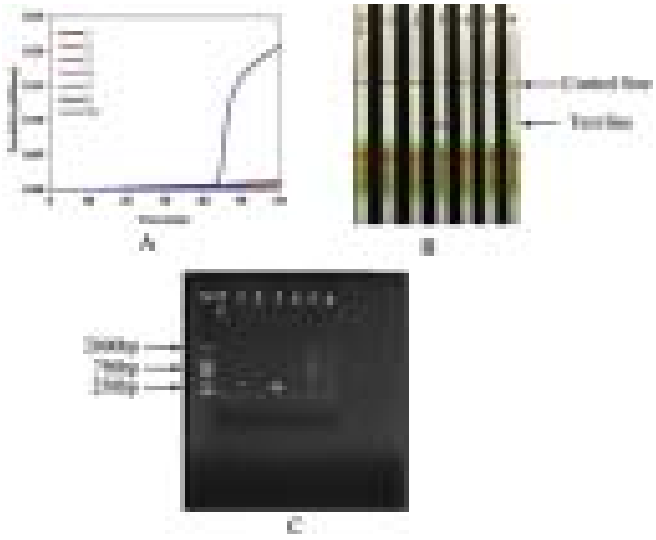

Fig.6 The specificity of PCR(C),LAMP(A) and LAMP-LFD(B) assay for detection of A. Penicillioides

3.6 Detection of A. Penicillioides in jet fuel samples

The usefulness of the LAMP-LFD for field condition was further evaluated by testing 11 jet fuel sample collected from one army oil depot. Five of them were found positive, while, other six samples and the negative control have no amplification. This preliminarily verified that the LAMP-LFD developed by this study has the applicability for detection A. Penicillioides in jet fuel with the field condition.

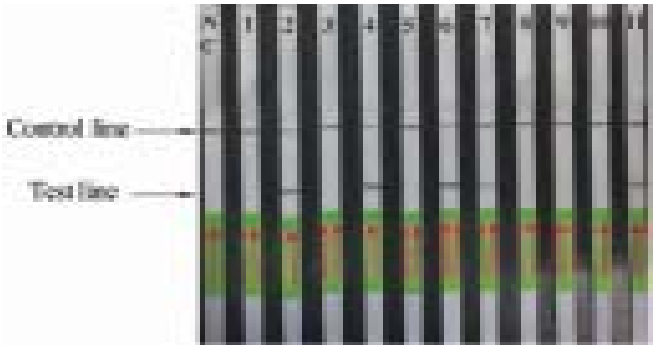

Fig.7 Detection of A. penicillioides in jet fuel samples with the LAMP-LFD assay

NC: negative control; 1-11: Jet-1, Jet-2, Jet-3, Jet-4, Jet-5, Jet-6, Jet-7, Jet-8, Jet-9, Jet-10, Jet-11

4. Discussion

A. penicillioide as one of the main contaminating fungi in jet fuel has never been reported before. According to the corrosion test, the growth of A. penicillioides in jet fuel will produce surfactant which could not only emulsify the oil/water, but also could inclusion other suspended substance to form biofilm. Even though Alahmer (2009) has indicted that emulsified diesel fuel will improves the engine performance, the emulsified diesel fuel is fatal to the aircraft engine. Becky G (1999) has indicated that oxy-organics existence in oil could accelerate the oxidation process. Surfactant as oxy-organics that produced by A. penicillioides in jet fuel could also reduce the thermal oxidation stability of jet fuel and that has been proved by the thermal oxidation stability test. And it will be worse with the growth of A. penicillioides in jet fuel. As we can see in Fig.1, the induction period of jet fuel from A to C has been reduced 14%, which indicated that the jet fuel of C has lost the stored value. However, it is impossible to remove all water which is the essential factor for microbial growth from the tank, especially the semi-buried tank and the buried tank. Therefore, it is crucial importance for storage jet fuel to early detection of A. penicillioides apart from good daily management.

Hill (1993) and Christine (1999) have summarize that the consequence of microbial contamination of jet fuel. One of the main damage is MIC which resulting in infrastructure problems and air crash. In this study we investigate the A. penicillioides contaminations' influence on the corrosivity of jet fuel by the steel corrosion test. And the results indicated that the growth of A. penicillioidesin jet fuel result in serious steel corrosion. In order to maximize simulate the effect of A. penicillioideson the corrosion behavior of tank and pip,we choose 45# steel which is the main component materials of them as the template. Once one part occur corrosion that will accelerate the corrosion of the whole oil tank and finally it will be a serious threat to storage safe (Hill, 2000). In addition, the biofilm formed by the growth of A. penicillioides has strong adsorption shown in Fig.2. However, Andrykovitch (1987) has indicated that biofilm and the sludge that formed by biofilm and fungi would accelerate the corrosion. Therefore, in order to protect the tank from corrosion by A. penicillioides, a rapid and special method for detection of A. penicillioides is urgent need.

In order to satisfy the need of above, we have developed and evaluated a new method for repaid and special detection of A. penicillioides by LAMP combined with LFD. LAMP-LFD method comparing to PCR methods do not rely on special and expensive equipment, easy operation and time-saving. In addition, LAMP amplification performed at isothermal condition, therefore, a thermostat water bath could satisfy the reaction need. So, LAMP-LFD will be a better choice for filed applied. Sensitivity and specificity are the two key evaluation criteria for a detection method. The genome DNA detection limit of A. Penicillioides by LAMP-LFD assay developed in this study is 0.82 pg/μl which 100-times more sensitive than the PCR method. In addition, the LAMP-LFD method could accurately detect A. penicillioides without nonspecific application. Meanwhile, the whole detection process lasting 90min including DNA extracted which saving almost 2h comparing to PCR method. In order to avoid the false positive reactions, Real-time turbidimeter (Loopamp LA-320, Japan) substituted gel electrophoresis was used as a detector for screening the most suitable condition of LAMP reaction

5. Conclusion

In conclusion, it is the first report that A. penicillioides as one of the main contaminating fungus could weaken the thermal oxidation stability of jet fuel and result in steel corrosion. To our knowledge, this is also the report that the development of LAMP method combined with LFD for repaid and accurate detection of A. penicillioides in jet fuel. Meanwhile, it is a sensitive, time-saving and easy operation. Thus, the LAMP-LFD assay has broad application foreground in the detection of contaminating fungus in jet fuel.

Acknowledgment

We will thank to the Key Laboratory of Applied Marine Biotechnology of Ningbo University for the support during the experiments. This study was fully funded by a grant from General Logistics Department of PLA (yx214L048).

References

[1] Rosenberg E., Perry A., Gibson D. T., Gutnick D. L. (1979): Emulsifier of Arthrobacter RAG-1: specificity of hydrocarbon substrate. Applied & Environmental Microbiology, 37(3): 409-413.

[2] Morton L. H. G., Surman S. B. (1994): Biofilms in biodeterioration e a review. International Biodeterioration & Biodegradation, 34(3-4): 203-221.

[3] Passman F. J. (2013): Microbial contamination and its control in fuels and fuel systems since 1980 – a review.International Biodeterioration & Biodegradation, 81(5): 88-104.

[4] Gaylarde C.C., Bento F. M., Kelley J. (1999): Microbial contamination of stored hydrocarbon fuels and its control. Revista De Microbiologia, 30(1): 01-10.

[5] Nazareth S., Gonsalves V. (2013): Aspergillus penicillioides - A true halophile existing in hypersaline and polyhalineeconiches. Ann Microbiol, 64(1): 397-402.

[6] Samson R. A., Lustgraaf B. V. D. (1978): Aspergillus penicilloides and Eurotiumhalophilicum in association with house- dust mites. Mycopathologia, 64(1): 13-6.

[7] ASTM Standard Test Method for Thermal Oxidation Stability of Aviation Turbine Fuel,ASTM standard D3241,2011.

[8] Sicard M., Boulicault J., Coulon K., Thomasset C., J. Ancelle, Raepsaet B., Ser F. (2013): Oxidation stability of jet fuel model molecules evaluated by rapid small scale oxidation tests. In The 13th International Conference on Stability, Handling and Use of Liquid Fuels, 2013 IASH.

[9] Tao Z.P., Liang X., Liu J.H., Wang X. Q. (2006): Effect of Cladosporium on corrosion behaviors of A3 steel in oil-water medium. ActaPetroleiSinica, 22(2): 98-102.

[10] Ferrari M.D., Neirotti E., Albornoz C. (1998): Occurrence of Heterotrophic bacteria and fungi in an aviation fuel handling system and its relationship with fuel fouling.Revista Argentina De Microbiología, 30(3): 105-114.

[11] Redondo C., Cubero J., Melgarejo P.. (2009). Characterization of Penicillium species by ribosomal DNA sequencing and Box, ERIC and REP-PCR analysis. Mycopathologia, 168(1): 11-22.

[12] Nazareth S., Gonsalves V., Nayak S. (2012): A first record of obligate halophilic aspergilli from the Dead Sea. Indian Journal of Microbiology, 52(1): 22-27.

[13] Asad S., Amoozegar M.A., Pourbabaee A., Sarbolouki M.N., Dastgheib S.M.M. (2007): Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. BioresourceTechnol, 98(11): 2082-2088.

[14] Sharkey F.H., Banat I.M., Marchant R. (2004): Detection and Quantification and Gene Expression in Environmental Bacteriology. Applied & Environmental Microbiology, 70(7):3795-3806.

[15] Xu X.W., Li T., Li Y., Li Z.X. (2015): Identification and Analysis of C. annuum micro RNAs by High-throughput Sequencing and Their Association with High Temperature and High Air Humidity Stress. International Journal Bioautomotion, 19(4):459-472.

[16] Notomi T., Okayama H., Masubuchi H., Yonekawa T., Watanabe K., Amino N. (2000): Loop-mediated isothermal amplification of DNA. Nucleic Acids Research, 28(12): E63-E63.

[17] Deng J., Pei J., Gou H., Ye Z., Liu C., Chen J. (2015): Rapid and simple detection of Japanese encephalitis virus by reverse transcription loop-mediated isothermal amplification combined with a lateral flow dipstick.Journal of Virological Methods, 213: 98-105.

[18] Thongkao K., Longyant S., Silprasit K., Sithigorngul P., Chaivisuthangkura P. (2015): Rapid and sensitive detection of Vibrio harveyi by loop mediated isothermal amplification combined with lateral flow dipstick targeted to vhhP2 gene. Aquaculture Research, 46(5): 1122-1131.

[19] Alahmer, A.,Yamin, J.A., Sakhrieh, A.,Hamdan, M.A. (2009). Experimental Study of Using Emulsified Diesel Fuel on the Performance and Pollutants Emitted from Four Stroke Water Cooled Diesel Engine.International Journal of Heat and Technology, 27(1):67-71.

[20] Grinstead B., Zabarnick S. (1999): Studies of jet fuel thermal stability, oxidation, and additives using an isothermal oxidation apparatus equipped with an oxygen sensor. Energy fuels, 13(3): 756-760.

[21] Hill E.C. (2000): Detection and remediation of microbial spoilage and corrosion in aviation kerosene–from refinery to wing. InProc. 7th International Conference on Stability and Handling of Liquid Fuels, 24-29.

[22] Andrykovitch G., Neihof R.A. (1987): Fuel-soluble biocides for control of Cladosporiumresinae in hydrocarbon fuels. Journal of Industrial Microbiology & Biotechnology, 2(1), 35-40.