
Xing LIU 1,b, Li-Zhi DANG 1,c, Xiu-Ming LIU 1,d, Yuan-Dong LI 1,e, Yi-Peng ZHANG 1,f, Gang DU 2,g and Yan-Qing DUAN 1,a, *

1 Technology Center, China Tobacco Yunnan Industrial Co., LTD KunMing, 650231, People’s Republic of China
2 Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Kunming, 650000, People’s Republic of China

a 18372454@qq.com, b 88084159@qq.com, c 2966200808@qq.com, d 2966093755@qq.com,
e 18987172437@126.com, zhanguqq790129@126.com, g 2966200256@qq.com
*Corresponding author

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Abstract. The fungal strain which could degrade β-carotene was isolated and screened from the samples of tobacco collected from Yunnan Honghe. The strain was classified and identified with morphology of strain and ITS sequence analysis. The preliminary identification showed that the strain was Penicillium Sp.. The fermenting product with fruit fragrance was obtained by the degradation of β-carotene with the strain Penicillium Sp.. The samples smell good and the degradation of β-carotene was measured.

Introduction

Carotenoids are a kind of isoprenoid pigments widely distributed in nature[1, 2]. The degradation of carotenoids has been subject of intense research for several decades[3]. A wealth of different apocarotenoids is derived from the excentric cleavage of the carotenoids polyene chain, and many of these apocarotenoids act as potent flavour compounds such as β-ionone and β-damascenone which have low flavor thresholds[4, 5]. Biotransformation catalysis can be carried out under mild conditions which effectively decreases energy demands and costs [6-9].

Aroma compounds derived from carotenoid breakdown from microorganisms were reported in the cyanobacterium Microcystis aeruginosa[10]. In a previous work [11-13], numerous filamentous fungi and yeasts, which were known for de novo synthesis or biotransformation of mono-, sesqui-, tri- or tetraterpenes, were screened for their capability to degrade β-carotene. Some strains discolored a β-carotene-containing growth agar, indicating an efficient carotenoid degradation. Microorganisms could add stereospecificity to bioprocesses, and reduce complicated separation and purification steps[14]. This feature is especially applicable to flavor compounds, once different isomers of the same compound possess their own unique aromatic properties[15]. Because biotechnology presents a cost-effective and renewable source of bioactive products, many flavor compounds obtained by this means are likely to become substitutes for their synthetic counterparts [16, 17]. The research performed by Del Toro-Sánchez and coworkers constituted the first report on microbial carotenoid cleavage resulting in safranal production, when carotenoid breakdown at the 7,8 double bond has been described in cyanobacteria[18]. Microorganisms are capable to produce an amazingly broad array of flavor compounds, by de novo synthesis, such as monoterpens and esters by Saccharomyces cerevisiae [19-21]. On the other hand, researchers have focused on bioconversion processes that offer more economic advantages. Different classes of microorganisms have been described by relating their individual biosynthetic pathways to the production of specific flavor compounds. Series of results about the bioconversion of lutein to tobacco aroma were obtained by Sanchez-Contreras and collaborators[22]. Two strains isolated from residual mud of marigold flowers (Tagetes erecta) could degrade lutein into compounds with tobacco aroma which were identified as 7,8-dihydro-β-ionol, β-ionone, 7,8-dihydro-β-ionone, and 3-hydroxy-β-ionone[23].
In this paper, the *Penicillium* Sp. was isolated from the samples of tobacco collected from Yunnan Honghe and used to degrade β-carotene for aroma.

**Materials and Methods**

**Samples and Reagents**

β-Carotene power (purity 1%) was purchased from Zhejiang pharmaceutical Limited by Share Ltd Xinchang pharmaceutical. β-Carotene (purity 30%) were purchased from BASF. All other reagents used in this study were analytical grade chemicals.

**β-Carotene stock Solution Preparation**

β-Carotene (5g) was dissolved in dichloromethane (20 mL) and mixed with Tween-80 (1 g). The solvent was distilled off under reduced pressure and the residue was dispersed in distilled water (100 mL).

**Isolation of β-carotene Degrading Strain**

The fungal strain *Penicillium* Sp. was isolated from the leaf of tobacco collected from Honghe, Yunnan province, PRC. The strain of *Penicillium* Sp. was grown at 28°C and stored at 4°C on agar slopes composed of Glucose (30.0g), potato (100.0g), agar (20g) mixed into distilled H₂O (1L).

**Identification of the Isolated Strain**

The bacterial strain was initially identified based on its morphological and biochemical properties. Further identification was conducted by DNA sequencing analysis. Polymerase chain reaction (PCR) was carried out using a ITS sequence. The PCR program was 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with a final 10 min extension at 72°C. The PCR product was cooled and analyzed by electrophoresis in 2% agarose gel. The desired band was excised with a sterile scalpel, eluted in sterile water overnight at 4°C, and then sent to Sangon Company (Shanghai, China) for sequencing. The obtained nucleotide sequence was used for sequence similarity analysis through BLAST (GenBank). Sequence alignments were performed with the program ClustalW. A phylogenetic tree was constructed using the N-J method.

**Culture Conditions and Fermentation**

*Penicillium* Sp. broth media were transferred into 250ml conical flask (100mL each). It was prepared with medium (1L): Glucose (30.0g), K₂HPO₄ (1g), MgSO₄·7H₂O (0.5g), KCl (0.5g), FeSO₄ (0.01g), and pH was maintained at 5.8. 10ml stock solution was mixed into broth media (1L). Seed flasks without β-Carotene were prepared from three-day old slants and allowed for one day on a shaker at 28°C. The remaining flasks within β-Carotene were inoculated from the seed flasks and placed on a rotatory shaker (220rpm) at 28°C for fermentation for 96h.

**Results and Discussion**

Cultivation of the strains on a β-carotene-containing medium proved to be an adequate test system as ‘positive’ strains could fade the color of medium after 3 d, which indicated efficient degradation of β-carotene. Therefore the fermentation broth smell sweet-scented.

**The Fungal Strain Screened for Degradating B-Carotene**

![Figure 1. The morphological properties of the strain *Penicillium* Sp.](image)
The Fungal Strain Penicillium Sp.

CCGATGCGACTGGGAGGAATACCAGAGTGAGGGCCCTCTGGGTCCAACCTCCGACCC
CGTGGTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGCAT
CTGCCCCCGGGCCCGCGCCCGCAAGACACCTGTGAACTCTGTCTGAAGATTGCAGT
CTGAGCAGATAGCTAAAATCAGTAAAATCTTCTAACAACCGGATCTTTGCTGCTCCGC
CATGAAAGAACGAGCAAAAGTCGATAAGTAATGTGAAATTGAGATAATTCAGTAG
CTGACGTTTTTGAACGCACATTGGCCGCCCCTGTTATTTGGGCGGATGCTGGTCCAGC
GTCAATTGCTGCCCTCAAGCCACCGTTGTGGTGTGGGCCCCCCCACCCCCCCCCGCC
CGTCGCGCGGGGCAGGGCCGGGCGACCGCGCGCGGGCCATTCATTTTGTTG
TCACCCGCTCTGTAAGGCGCGCCGGCCGGCCGGGACGACCCAAATCATTCTTTGC
AGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGG
AGGAA

Figure 2. Nucleotide sequence of Penicillium Sp. ITS sequence.

Figure 3. Phylogenetic analysis of Penicillium Sp. based on ITS sequence with N-J tree.

The morphological character of strain Penicillium Sp. was obtained using microscope with
imaging system. The sequence (544 bp) was shown in Fig. 2. The phylogenetic tree was generated
using the MEGA software as shown in Fig. 3. The results revealed that the ITS sequence displayed
highest similarity (identity 100%) with Fusarium verticillioides, therefore the strain was identified as
ITS sequence as Penicillium Sp..

Conclusion

β-carotene could be degraded by the strain isolated from the leaf of tobacco and the tobacco flavor
with fruity flavor was produced. The strain was identified as Penicillium Sp. by its morphological,
biochemical properties and ITS sequence.

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Literature References


