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Comparison of the Biosynthetic Pathway of 10-Hydroxy-2-Decenoic Acid between Microorganisms and *Apis mellifera*

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The research of biosynthetic pathway of 10-Hydroxy-2- Decenoic acid(10-HDA) include two aspect: Microorganisms and *Apis mellifera*. BH002[1] has been screened and ferment to produce 10-HDA. Secretion regularity of 10-HDA and protein in mandibular glands of worker *Apis mellifera* were researched. About 25 days, 10-HDA content was the highest. 54kD protein of forager's mandibular glands were different to counterparts of Chinese honeybee. Regardless of microorganisms whether or *Apis mellifera*, the detail biosynthetic pathway 10-HDA had not yet clear nowadays[2].

Key words: 10-hydroxy-2-decenoic acid, Microorganisms, *Apis mellifera*.

It has been proved that 10-HDA has many functions for people which include enhancing the immune function of body, inhibiting and killing cancer cells, antibacterial, anti-inflammatory, fight ulcer, radioresistance, depressing blood-fat[3-11]. In 2010, Xin-Yu Yang *et al.,* has reported two significant findings: 10-HDA has dual inhibitory effects at the activity of matrix metalloproteinases-1(MMP-1), matrix metalloprotein -ases-3(MMP-3) and inhibition of p38 and JNK–AP-1 signaling pathways. Because of its special function, the supply of 10-HDA can not meet the people's urgent demand of health care and pharmaceutical products. In addition, 10-HDA may be a potential medicine for rheumatoid arthritis[6] and inhibits VEGF-induced angiogenesis in human umbilical vein endothelial cells[12].

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In nature ,10-HDA has been only found in worker honey bees which may function as preservatives and larval nutrients in brood food[13]. Several strains yielded this acid through screening from raw royal jelly, soil and honeycomb etc. as well as mutagenesis. And the biosynthetic pathway of 10-HDA remains an unsolved problem. Therefore many scientists has been working on the exploitation and improvement of 10-HDA synthesis pathway.

MATERIALS AND METHODS

The strains producing 10-HDA

Wild stains producing 10-HDA have not yet been report. In earlier work, Strains producing 10-HDA were all screened but low yield. These strains were confirmed to the genera of bacteria or candida. According to the taxonomic theory and morphological characteristics, strain BH002 was identified as genus Candida Berkh, and strain RJ002 and RJ004 were genus Micrococcus[1].

In addition, it was come to light that some bacteria yielded mono unsaturated fatty acid through anaerobic pathway. In progress of this

pathway 2-decenoyl-S-ACP(Fig 1) was found which was extremely similar with 10-HDA and may be transformed into it by oxidation and dethioester[14].

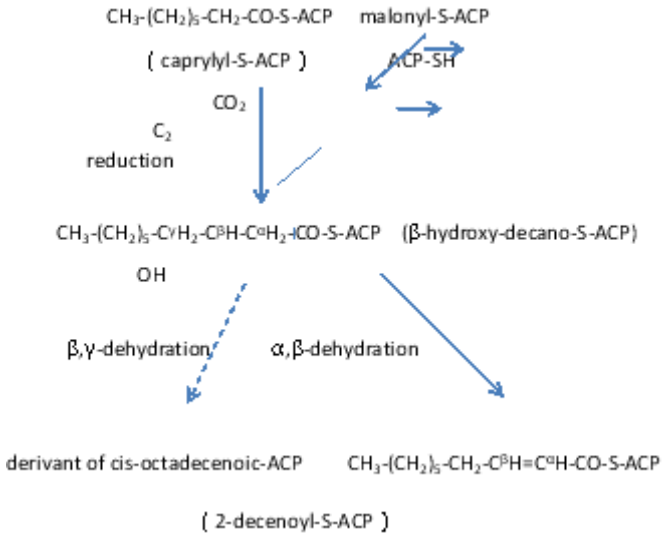

Fig. 1. Anaerobic Pathway of monounsaturated acid synthesis

The fermentation of strains producing 10-HDA

Many parameters were used to screen strains, such as pH value,NH_2-N,residual sugar , media types or fermentation time and product in fermentation broth were separated and purified by centrifuge and adding diethyl ether or chloroform[15]. 10-HDA content in fermentation broth was detected by high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC)[16] or gas chromatography (GC).

Secretion regularity of 10-HDA in the mandibular glands of worker *Apis mellifera*

During 7 days before worker and queen incubating, very small amount 10-HDA remaining in worker body gradually decreasing when food take change from royal jelly into pollen or honey, and 10-HDA content always maintain 1/8 to 1/5 of queen[17].

From 0 day to 30 days, 10-HDA content in mandibular glands of worker *Apis mellifera* trend to rise on the whole (Fig. 2). On 0 day, the content was the lowest; And gradually increasing till 10 days; From 10 days to 20 days, 10-HDA content remained stable; And it got the highest about 25 days and then decreasing.

The research progress of protein in the mandibular glands of worker *Apis mellifera*

Mandibular Gland Protein between Three Castes of *Apis cerana cerana* and *Apis mellifera ligustica* also was taken comparison lately. The

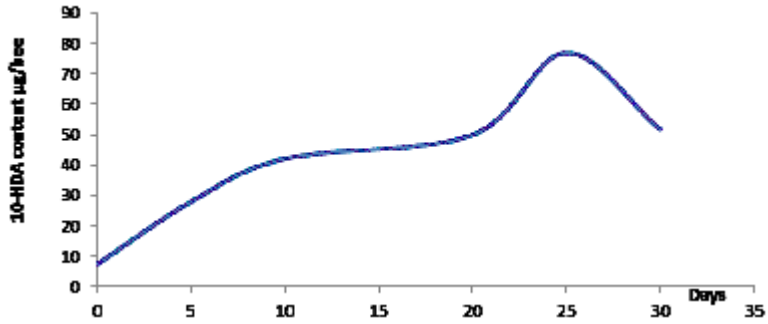

Fig. 2. Trends of 10-HDA content in mandibular glands of worker *Apis mellifera* from 0 to 10 days

proteins extracted from mandibular glands of newly hatched queens, forager workers, and drones of both species, were quantified respectively and applied for SDS- PAGE. The result showed that Many protein bands in SDS- PAGE profiles of different castes of both species were the same, though few proteins were evidently different. As for Italian honeybee, a 76kD protein of queen's mandibular gland, a 54kD protein of forager's, and a 27kD protein of drone's were different to those counterparts of Chinese honeybee, which were 74, 36 and 48kD respectively. These different proteins probably reflect the species discrepancy in gland metabolism and some of them may be key enzymes of expressing 10-HDA[2].

Summary

As mentioned above, microorganism pathway to produce 10-HDA was relatively rapid and light work load. To make clear of metabolic pathways of 10-HDA was to apply in industrial production finally by microorganism. The disadvantages of this pathway was low yield and that the strains were unstable.

The synthetic pathway of 10-HDA in mandibular glands were more ground reported .The detail metabolic pathway of 10-HDA can be taken clear by this pathway. Dimensional electrophoresis, mass spectrometry (MS), over expression and removal of genes can be used to find out relevant key enzymes and genes. For more likely, most scholars lean to this pathway.

Forecation and Looking into the distance

In 1997, A more important fact found by Eeika *et al*. is that the pathway of mandibular acid biosynthesis is one of several characteristics that differ between the female honey bee castes. They also showed that the mandibular acids are synthesized from octadecanoic acid in three steps: (1) hydroxylation at the and -1 position; (2) â-oxidation of the 18-carbon hydroxy acids to the 8 and 10-carbon length respectively[18]. Therefore, it was predicted that the biosynthesis pathway of 10-HDA must include these.

In a word, further studies on the control and key enzymes of this pathway should give insights into the fascinating process of biosynthesis of 10-HDA. This review is based on the metabolic product of biochemical reactions which can be concluded to several of number limited biosynthesis route and biochemical reaction. A possible biosynthesis pathway of 10-HDA can be concluded with the existing results of 10-HDA , other fatty acid and insect biological pheromone biological metabolism.

In biosynthesis pathway of 10-HDA ,cytochrome P450 ,medium chain acyl CoA dehydrogenase and acyl–acyl carrier protein thioesterase may be involed. On the one hand, we can compare by two-dimensional electrophoresis the proteins from honey bees between high-producting and low-producting 10-HDA to identify or affirm them. On the other hand, mRNA of the three key enzymes obtained by PCR is synthesized corresponding DNA under reverse transcription. Therefore, we can acquire the key enzymes and their DNA by this method though a lot of work need to be done in the future.

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