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SHORT COMMUNICATIONS

Effective Biosynthesis of Poly(3-hydroxybutyrate) from Plant Oils by *Chromobacterium* sp.

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A wide variety of microorganisms accumulate an optically active polymer of (*R*)-3-hydroxybutyric acid [P(3HB)] within cells as an intracellular storage material of carbon and energy.[1-5] Recently, some microorganisms have been found to biosynthesize poly(3-hydroxyalkanoates) other than P(3HB) from various carbon sources such as alcohols, alkanoic acids, and carbohydrates.[6-10] Since these microbial polyesters are biodegradable thermoplastics, they have attracted much attention as new environmentally compatible materials.[11-13] Biosynthesis of polyesters from plant oils is interesting, for they are moderate price and recyclable raw materials. However only *Alcaligenes* sp. and *Aeromonas* sp. as strain have been used on this study to date. Akiyama *et al.* reported the production of P(3HB) from plant oils, especially palm oil by using *Alcaligenes* sp. AK201[14,15] isolated by colonization on agar plates of a subculture of *Pseudomonas oleovolans*. Shiotani *et al.* have found that *Aeromonas caviae* isolated from soil produced a copolymer of 3HB and 3-hydroxyhexanoate (3HH) from olive oil.[16] The conversion ratio of plant oil into polyester by these strains was up to 0.62 (g polyester/g plant oil) at the most. Therefore, microorganisms producing more efficiently polyesters from plant oils and animals fats as renewable carbon sources are in great demand.

In this study, we report the effective biosynthesis of P(3HB) from different plant oils, especially rice oil, safflower oil and rice bran as the readily available, inexpensive renewable carbon sources by a new *Chromobacterium* species. This bacterial strain that was isolated from a soil at Hottate river (Yonezawa city), possessed purple pigment, and was a Gram-negative, aerobic and motile rod. Further, this strain revealed the ability reducing nitrate, oxidase- and catalase-positive, and was confirmed to be fermentative species by Hugh–Leifson method.[17] The identification tests of the isolated strain by using API 20NE test kit[18] exhibited some comparable behaviors to *Chromobacterium violaceum*, but this strain was different from it in several phenotypic properties examined. Base on these tests, the isolated strain was identified as a *Chromobacterium* sp. and was designated as strain YH709. The microbial polyester synthesis from several plant oils was carried out by two-stage cultivation. YH 709 strain was first grown in the nutrient-rich medium containing yeast extract, polypeptone, meat extract and ammonium sulfate at 30°C for 24 h. The cells were harvested by centrifugation, and transferred into nitrogen-free media containing different plant oils. The cells were incubated for prescribed time at 30°C, harvested, washed and lyophilized. The polyesters were extracted from the lyophilized cells with hot chloroform and purified by reprecipitation with *n*-hexane and by washing with methanol. Table I shows the results of the polyester production by *Chromobacterium* sp. YH709 from different plant oils. Since the plant oils are hydrophobic liquids, they are immiscible with the aqueous mineral medium, resulting in a two phase medium system. YH709 strain produced P(3HB) homopolymer in fairly high amounts on dry cells. At the highest yield of P(3HB) obtained from 10 $g l^{-1}$ of rice oil or safflower oil, the conversion ratio of these plant oils into P(3HB) was as high as 0.72 or 0.88 (g P(3HB)/g plant oil), respectively. In contrast, the conversion of 3 $g l^{-1}$ of palm oil into P(3HB) by *Alcaligenes* sp. AK201 was as high as 0.62 weight ratio.[15] Therefore, *Chromobacterium* sp. YH709 would be considered to be an excellent microorganism for the mass production of P(3HB) homopolymer from plant oils. *Chromobacterium* sp. YH709 was also grown on rice bran containing 14.1 wt% of fatty acid triglycerides, and the polyester content in dry matters consisting of cell and undigested bran was as high as 11.7 wt%. Since P(3HB) was not produced from the rice bran in which fatty acid triglycerides were removed by

Table I. Biosynthesis of poly(3-hydroxybutyrate) from different plant oils (10 $g l^{-1}$) for 48 h at pH 7.0 and 30°C by *Chromobacterium* sp.

Carbon source	Dry cell yield	P(3HB) content[a]	Conversion ratio[b]
	$g l^{-1}$	wt%	$g g^{-1}$
Rice oil	12.9	55.8	0.72
Safflower oil	17.6	49.8	0.88
Soya oil	13.8	42.4	0.59
Olive oil	12.3	45.6	0.56
Linseed oil	9.6	48.7	0.47
Peanut oil	12.6	35.1	0.44
Corn oil	10.1	47.0	0.47
Rice bran	8.1[c]	11.7	0.10

[a] P(3HB) content in dry cells. [b] Conversion ratio of carbon sources into P(3HB). [c] Weight of dry cell and undigested bran.

extraction with *n*-hexane, there was no conversion of the constituents other than fatty acid triglycerides in the rice bran to P(3HB). Hence, the conversion ratio of fatty acid triglycerides contained in the rice bran to P(3HB) results in 0.67. This result should be noted from a viewpoint of effective use of rice bran that was discharged by rice polishing.

Figure 1 shows the P(3HB) yields, the dry cell weights, residual rice oil weights and the conversion ratio of consumed rice oil into P(3HB) when *Chromobacterium* sp. YH709 was cultivated for 48 h in medium containing various amounts of rice oil under a nitrogen-deficient condition. The P(3HB) yields and the dry cell weights increased initially with the amounts of rice oil until they reached constant weights of $10.6\,g\,l^{-1}$ and $20.2\,g\,l^{-1}$ respectively beyond $20\,g\,l^{-1}$ of rice oil concentration. On the other hand, the amounts of residual rice oil (recovered by extraction from cultivation medium with *n*-hexane) increased gradually at the concentration of rice oil of more than $20\,g\,l^{-1}$ and linearly beyond $40\,g\,l^{-1}$, but even $80\,g\,l^{-1}$ of initial rice oil concentration did not exhibit cellular toxicity. Further, although the incubation time to consume $50\,g\,l^{-1}$ of rice oil was needed for about 8 days, the amounts of P(3HB) in the cells were kept at $11\pm0.5\,g\,l^{-1}$ throughout this incubation. These results show that *Chromobacterium* sp. YH709 could be grown in the medium containing a large amount of plant oil to produce a constant amount of P(3HB) by one-step fermentation. Indeed, when this *Chromobacterium* sp. strain was cultivated for 48 h in a mineral medium containing more than $10\,g\,l^{-1}$ of rice oil and $2\,g\,l^{-1}$ of ammonium sulfate, it resulted in accumulation of P(3HB) up to 55% of total cellular dry weight ($7.3\,g\,l^{-1}$). Figure 1 also shows that the conversion of consumed rice oil into P(3HB) resulted in a maximum value of 0.72 weight ratio at $10\,g\,l^{-1}$ of rice oil and remained a constant weight ratio (0.30) beyond $40\,g\,l^{-1}$ of rice oil. At the highest yield of P(3HB) obtained from safflower oil by *Chromobacterium* sp. YH709, the conversion of safflower oil into P(3HB) was as high as 0.88 weight ratio against a theoretical conversion value of 1.38.[19] In a comparative study of *Alcaligenes eutrophus* the conversion of fructose into P(3HB) was found to be as high as 0.35 weight ratio against a theoretical conversion value of 0.48.[8,20] Therefore, this *Chromobacterium* sp. has been found useful for the production of a large quantity of P(3HB) to be able to use plant oils possessing a higher theoretical conversion ratio into P(3HB). The number average molecular weight ($\bar{M}_n$) of P(3HB) was in the range of 2×10^5—6×10^5 depending upon the fermentation conditions. The molecular weight distributions of P(3HB) were unimodal and their dispersities ($\bar{M}_w/\bar{M}_n$) were in the range of 2.1—2.6. The three major fatty acid constituents into rice oil or safflower oil, linoleic acid, oleic acid, and palmitic acid, were also effective for the production of P(3HB) by *Chromobacterium* sp. YH709. Especially the conversion ratio of $10\,g\,l^{-1}$ of linoleic acid to P(3HB) was up to $0.68(g\,g^{-1})$. Further, this strain also exhibited the production of P(3HB) from acetic acid, glucose, and fructose which starts with the condensation reaction of acetyl-coenzyme A (CoA) into acetoacetyl-CoA. Therefore, the pathway of P(3HB) homopolymer biosynthesis from plant oils by *Chromobacterium* sp. could be also linked to acetyl-CoA produced in the β-oxidation cycle from the different fatty acids constituting plant oils.

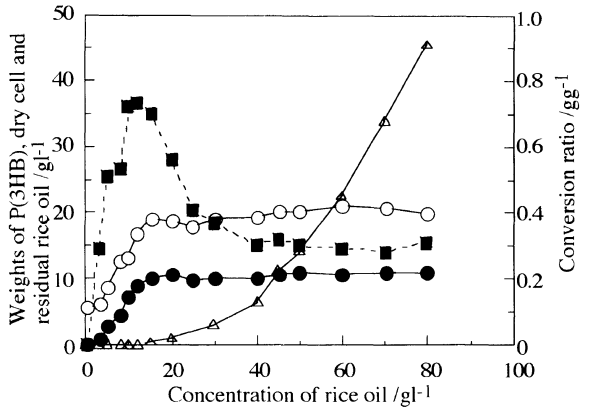

Figure 1. Effect of concentration of rice oil on P(3HB) yields (●), dry cell weights (○), residual rice oil weights (△), and conversion (■) of consumed rice oil into P(3HB) by *Chromobacterium* sp. for 48 h at pH 7.0 and 30°C.

In conclusion, *Chromobacterium* sp. YH709 isolated from soil degraded different plant oils and led to the biosynthesis of P(3HB) homopolymer effectively. When rice oil or safflower oil was used as the sole carbon source, the conversion ratio of these plant oils into P(3HB) was as high as 0.72 or 0.88 (gP(3HB)/g plant oil), respectively. Further, this microorganism was found to be capable of growing in mineral salt medium containing large amounts of plants oils without cellular toxicity and producing a constant amount of P(3HB). Additional studies on the biosynthesis of polyesters from different *n*-alkanoic acids and alcohols by this *Chromobacterium* sp. are in progress.

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