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Dedicated to Professor Wolfgang BABEL on the occasion of his 65th birthday

Synthesis of Poly-β-hydroxybutyrate by *Agrobacterium radiobacter* after Growth on *D*-Carnitine

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Summary

Agrobacterium radiobacter, isolated from soil, was cultivated on a minimal medium containing different trimethylammonium compounds. In contrast to other bacteria, *A. radiobacter* is able to utilise *D*(+)-carnitine, but not the physiologically *L*(–)-carnitine, as the sole source of carbon and nitrogen. *D*(+)-Carnitine, which does not exist in nature, is produced as a waste product in chemical procedures for *L*(–)-carnitine production. During growth on *D*(+)-carnitine, a NAD^+-specific *D*(+)-carnitine dehydrogenase (specific activity 0.159 U) as an initial enzyme of degradation and poly-β-hydroxybutyrate (PHB) was formed as storage material. The highest PHB content was achieved at growth on a culture medium, which contains 0.5% *D*(+)-carnitine during the stationary growth phase (60% PHB of cell dry weight). Whereas oxygen limitation has no significant effect on the PHB production, phosphate limitation leads to an increase in PHB formation of up to 71% PHB of cell dry weight after growth on 1% *D*(+)-carnitine. *A. radiobacter* seems to be attractive for the conversion of the waste product *D*(+)-carnitine to PHB valuable as biodegradable and biocompatible plastic.

Introduction

Poly-β-hydroxybutyrate (PHB) is an intracellular storage polymer, whose biological function is to provide a reserve of carbon and energy. This compound belonging to the polyhydroxyalkanoates (PHAs), which are synthesised by several bacterial species, is accumulated intracellularly in large quantities due to the limitation of one or more environmental or nutritional factors, for example, nitrogen, phosphorus, potassium or oxy-

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gen in the presence of excess carbon [1, 2]. PHB is completely biodegradable. Since the physical properties of PHB resemble those of polypropylene [3], the commercial production of PHB is of considerable interest.

High costs for production are a disadvantage compared to those of petrochemical plastics. In PHB production about 40% of the total production cost is for raw material [4]. Using a cheap carbon source is one possibility to decrease the production cost of PHB. Therefore, the formation of PHB was investigated at a variety of carbon sources cheaper than glucose, for example, lactose [5], xylose [5, 6, 7], molasses [8], starch [9–11] and whey [11, 12]. PHB production using by- or waste products is especially attractive.

The ubiquitously occurring *L*(–)-carnitine (*R*(–)-3-hydroxy-4-trimethylaminobutyrate) is essential for the transport of long-chain fatty acids through the inner mitochondrial membrane [13]. From the consideration of the function of *L*(–)-carnitine in eukaryotic cell metabolism, a series of clinical applications, e.g. in the treatment of patients with carnitine deficiency syndromes [14–16], in the prophylaxis and therapy of various heart diseases [17] and in the replacement therapy of haemodialysis patients [18, 19], have followed.

In contrast to *L*(–)-carnitine, the *D*(+)-enantiomer (*S*(+)-3-hydroxy-4-trimethylaminobutyrate) does not exist in nature. *D*(+)-Carnitine is not only physiologically inactive, but it is also bound and transported by the active *L*(–)-carnitine transport system of cell membranes, thereby diminishing *L*(–)-carnitine within the cells and inhibiting *L*-specific reactions [20]. Although different chemical and biotechnological procedures for the stereospecific *L*(–)-carnitine production have been proposed [21], the main amount of *L*(–)-carnitine is produced by resolution of racemic carnitine or its precursors via their diastereomeres by means of optically active acids [e.g. 22, 23]. The latter method provides *D*(+)-carnitine as a waste product in equimolar amounts to the *L*-enantiomer. For the first time, this paper presents the possibility of the application of *D*(+)-carnitine in the production of PHB by *Agrobacterium radiobacter*.

Materials and Methods

Cultivation of Bacteria and Cell Disruption

Agrobacterium radiobacter was used in all experiments. The strain was isolated from soil by screening agar-agar plates containing minimal medium with *D*(+)-carnitine as the sole carbon and nitrogen source and was identified based on the Api-system of BIOMÉRIEUX [24]. The strain was maintained on minimal medium [25] at 30 °C, pH 7.5, supplemented with *D*(+)-carnitine or *D,L*-carnitine and other trimethylammonium compounds as the sole source of carbon and nitrogen. Cultivation was carried out in 500 ml ERLENMEYER flasks each containing 100 ml of culture medium on a rotary shaker (120 rpm). By measuring the apparent absorbance of the culture at 600 nm, the growth of the cells was observed. Bacteria were harvested at intervals of 24 h.

To achieve oxygen limitation, bacteria were cultivated in airtight closed flasks. Experiments under phosphate limitation were carried out with $^1/_{10}$ of phosphate, which is normally used for this minimal medium (20 mM).

Cell disruption was achieved by ultrasonication (Bandelin Sonoplus GM 70, Bandelin MS 73 microtip; 5 min under cooling with 1 min disruption equally). The insoluble cell matter was sedimented at 15,000 × g for 20 min.

Determination of Carnitine Dehydrogenase Activities and of D(+)-Carnitine

Carnitine dehydrogenase activities were assayed in a reaction mixture (2 ml) containing 0.1 M Tris/HCl-buffer (pH 9.0), 2 mM NAD^+, 50 mM *L*(–)-carnitine or *D*(+)-carnitine and an appropriate amount of enzyme solution [26]. The reaction was started by addition of protein. The increase of NADH absorbance was recorded at $\lambda = 340$ nm and 25 °C.
D(+)-Carnitine concentration in the culture medium was determined using purified *D*-carnitine dehydrogenase from *Agrobacterium* sp. described by OBÒN *et al*. (1999) [27]. The assay contained 50 mM Tris/HCl buffer, (pH 9.0), 300 µM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), 30 µM phenazine methosulphate (PMS), 2 mM NAD^+ and 3.25 mU *D*-carnitine dehydrogenase (isolated from *Agrobacterium* sp. (DSM 8888)). The reaction was started by the addition of medium supernatant. The reaction was followed for 5 min by measuring the absorbance at 525 nm. The concentration of *D*(+)-carnitine was determined by means of a calibration curve, which was linear in a range from 0.5 to 10 mM *D*(+)-carnitine.

Lyophilisation and PHB Determination

Lyophilisation of cells was achieved at –50 °C and a pressure of 0.2 bar for a minimum of 24 h (Lyophilisator CHRIST alpha1-4). The dry cell material was weighed into special pressure-tight flasks for PHB determination, which was carried out by gas chromatography using the method of RIIS and MAY [28]. Therefore, a gaschromatograph from HEWLETT PACKARD 5890 series II (Germany) and a column HP ultra 2 crosslinked 5% ph Me silicone, 25 m × 0.3 mm × 0.33 µm film thickness (USA) were used. Approximately 40 mg of dry cell material, 2 ml dichlorethane (DCE), 2 ml HCl/propanol (conc. HCl : propanol 1 : 4) and 200 µl of an intern reference substance (2 g benzoic acid in 50 ml propanol) were heated at 100 °C in a pressure-tight flask for 2 h. After cooling, the acid was extracted. 1 µl of the heavy DCE/propanol phase containing the 3-hydroxybutylester was injected for analysis. A calibration curve was used to determine the PHB amount of the dry cell material.

Results and Discussion

Utilisation of Trimethylammonium Compounds

A. radiobacter isolated from soil is able to utilise *D*(+)-carnitine and *D,L*-carnitine as the sole source of carbon and nitrogen under aerobic conditions. During growth on these compounds, a NAD^+-specific *D*(+)-carnitine dehydrogenase (EC 1.1.1.108) is formed (0.159 U/mg). In contrast to other bacteria [e.g. 29, 30], *L*(–)-carnitine and some other quaternary ammonium compounds (γ-butyrobetaine, crotonobetaine) are not used by *A. radiobacter* as a carbon and nitrogen source. No NAD^+ specific *L*(–)-carnitine dehydrogenase was detected. This is contradictory to that described for different bacteria [30]. A further *Agrobacterium* sp. isolated from soil is able to utilise *D*(+)- and *L*(–)-carnitine as the sole source of carbon and nitrogen under aerobic growth conditions. During growth on *D*(+)-carnitine, two carnitine dehydrogenases with different stereospecifities are induced in this strain [30]. *Pseudomonas* sp. AK I can also use *D*(+)-carnitine and *L*(–)-carnitine as the sole source of carbon and nitrogen, and forms a NAD^+-specific *L*(–)-carnitine dehydrogenase on both carbon sources [29]. A NAD^+- or $NADP^+$-dependent *D*(+)-carnitine dehydrogenase could not be found after growth of *Pseudomonas* sp. AK I on *D*(+)-carnitine or other trimethylammonium compounds. Therefore, the utilisation of *D*(+)-carnitine and the occurrence of *L*(–)-carnitine

dehydrogenase could be explained by the existence of a carnitine racemase [29]. Such an activity was detectable in cell-free extracts of *D*(+)-carnitine-grown cells [29]. 3-Dehydrocarnitine formed by the *L*(–)-carnitine dehydrogenase could be degraded to glycine betaine and a C_2-compound like acetate or acetyl-CoA [31]. Utilisation of the non-physiological *D*(+)-carnitine by *A. radiobacter* represents a peculiarity, because thus far, only the degradation of *L*(–)-and *D*(+)-carnitine as the sole source of carbon and nitrogen had been found in bacteria.

The degradation of *D*(+)-carnitine by *A. radiobacter* is dependent on the concentration, the time and the growth phase, respectively. The strain is able to utilise up to 85% of *D*(+)-carnitine of the culture medium, which contains 31.05 mM *D*(+)-carnitine. This is adequate to a 0.5% *D*(+)-carnitine solution. When the medium contains 1% *D*(+)-carnitine (62.1 mM), only 62% are metabolised. So, the efficiency of *D*(+)-carnitine degradation is higher at lower *D*(+)-carnitine concentrations in the medium.

PHB Production

Influence of Carnitine

Fig. 1 shows the growth and PHB production of *A. radiobacter* with 0.5% *D*(+)-carnitine as a carbon and nitrogen source. The accumulation of PHB ensues during the growth. The PHB content increased during the first 72 h of cultivation in relation to the bacterial growth. The highest PHB level (60% PHB of cell dry weight) was achieved in the stationary growth phase (after 96 h). After this time, the PHB content decreases. If the culture broth contains a higher *D*(+)-carnitine concentration (1 or 2%), the PHB content is constant up to 168 h.

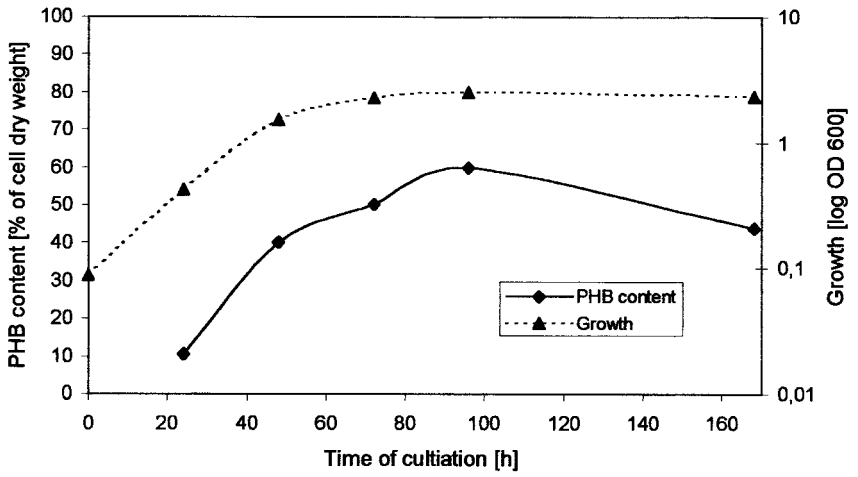

Fig. 1. Growth and PHB production by *A. radiobacter* with 0.5% *D*(+)-carnitine as the sole carbon and nitrogen source

Most bacteria produce PHB during the stationary growth phase only if nutrients are limited or growth conditions are imbalanced. Only some microorganisms are able to produce this storage material during growth, for example, a variety of lactic acid bacteria [32]. The PHB production of *A. radiobacter* ensues during the growth as well.
The PHB content is dependent on the kind of carnitine (*D* (+)- or *D,L*-carnitine) used and the concentration of carnitine in the culture broth. Tab. 1 shows the PHB amounts obtained when using different concentrations of *D* (+) and *D,L*-carnitine in the culture medium. By cultivation on 0.5% *D* (+)-carnitine, the highest PHB content (60% PHB of cell dry weight) was achieved, whereas the lowest PHB content was obtained after cultivation on 3% *D,L*-carnitine. It is shown that there are differences in PHB production between growth on *D* (+)-and *D,L*-carnitine. PHB amounts reached on *D,L*-carnitine are lower than such on *D* (+)-carnitine. The *D*(+)-carnitine dehydrogenase is inhibited by *L*(–)-carnitine [33]. Thus, the first step in *D* (+)-carnitine degradation is inhibited, and less metabolites are available. There does not seem to be a direct correlation between carnitine concentration and the amount of PHB. Cultivation with higher concentrations of carnitine was found to be unsuccessful. If the culture broth contains 5% of *D,L*-carnitine, the growth of the bacteria is increasingly hindered over time. No bacterial growth was obtained after a cultivation time of 7 days on 10% *D,L*-carnitine.

Tab. 1. Influence of carnitine concentration on PHB production by *A. radiobacter*

Carnitine [%]	PHB content after 96 h [% of cell dry weight]
1.0 *D,L*	35
3.0 *D,L*	32
0.5 *D*	60
1.0 *D*	44
2.0 *D*	49

With the exception of glycine betaine, no other trimethylammonium compounds have been used as a carbon and nitrogen source by *A. radiobacter* as mentioned before. Glycine betaine seems to be a metabolite of carnitine degradation. In *Pseudomonas* sp., 3-dehydrocarnitine formed by the *L* (–)-carnitine dehydrogenase could be degraded to glycine betaine and a C_2 compound like acetate or acetyl-CoA [31]. Glycine betaine is metabolised by stepwise demethylation to glycine [34], whereas the C_2 compound provides the initial substance for the synthesis of PHB. The PHB content reached at growth on glycine betaine accounts for 28%. This is comparable to the PHB amount produced by cultivation on 1% *D,L*-carnitine at same conditions. But compared to the cultivation with 1% *D*(+)-carnitine, it is clearly lower.

Influence of Limited Growth Conditions

It is well known that limited growth conditions can promote an increase of PHB production in microorganisms. Therefore, the influence of phosphate and oxygen limita-

tion on PHB production was investigated. Tab. 2 shows the influence of oxygen and phosphate limitation on the PHB production by *A. radiobacter*. The highest PHB amounts, which were reached by phosphate or oxygen limitation and at unlimited growth conditions, are illustrated.

Oxygen limitation was tested by cultivation on different *D* (+)-carnitine concentrations in the culture medium. Under oxygen limitation, the bacterial growth is lower than under unlimited conditions after a cultivation time of 72 h. The highest PHB content was measured after 96 h with 58% PHB of cell dry weight with 1% *D* (+)-carnitine in the culture medium. After 168 h, the PHB content decreased to 22% of cell dry weight. Because of the unfavourable growth conditions under oxygen limitation, PHB as a carbon and energy source was metabolised during this short time. In the cultivation of *A. radiobacter* on 0.5% *D* (+)-carnitine, PHB production could not be obtained. Under such unfavourable conditions, 0.5% *D* (+)-carnitine solution seems not to provide enough energy for secondary metabolism. Oxygen limitation does not result in a significant rise in PHB production. Only the cultivation on 1% *D*(+)-carnitine allows a little increase to be reached.

Compared to oxygen limitation, bacterial growth and PHB amount are different at phosphate limitation. Growth and PHB amount do not decrease after a cultivation time of 96 h. It remains constant or increases up to 168 h. Fig. 2 shows the progress of PHB production at unlimited conditions and with phosphate and oxygen limitation. In contrast to oxygen limitation, phosphate limitation has a significant effect on PHB accumulation. In the cultivation on 0.5% *D* (+)-carnitine, the highest PHB content was measured with 66% of cell dry weight after 72 h. The PHB amount produced remains nearly constant up to 168 h. Cultivation on this concentration of *D*(+)-carnitine does not lead to a significant rise in PHB formation. But in the cultivation of *A. radiobacter* on 1% *D* (+)-carnitine, a significant increase in PHB production was detectable. The highest PHB content was achieved after 168 h with a PHB content of 71% cell dry weight. This is a raise in PHB content up to 27% compared to the PHB amount produced under unlimited growth conditions.

Tab. 2. Effect of limited growth conditions on PHB production by *A. radiobacter*

Limitation	*D* (+)-Carnitine content [%]	Growth OD (600 nm)	PHB content [% of cell dry weight]	Time of cultivation [h]
Oxygen	0.5	0.9	Not detectable	96
	1.0	1.1	58	96
Phosphate	0.5	2.2	65	168
	1.0	2.0	71	168
Unlimited	0.5	2.5	60	96
	1.0	2.4	44	96

In some other bacteria, phosphate limitation has an increasing effect on PHB production. In *M. rhodesianum* MB126, the amount of PHB produced could be increased up to

57.5% [35], whereas *Pseudomonas* 135 accumulated 34.5% PHB under phosphate limitation [36].

Different limitation conditions lead to different effects in PHB synthesis. In some other bacteria, phosphate limitation was more effective for PHB production than oxygen limitation, for example in *M. rhodesianum* MB126 when grown on methanol (phosphate limitation: 57.5%, oxygen limitation: 29.3%) [35].

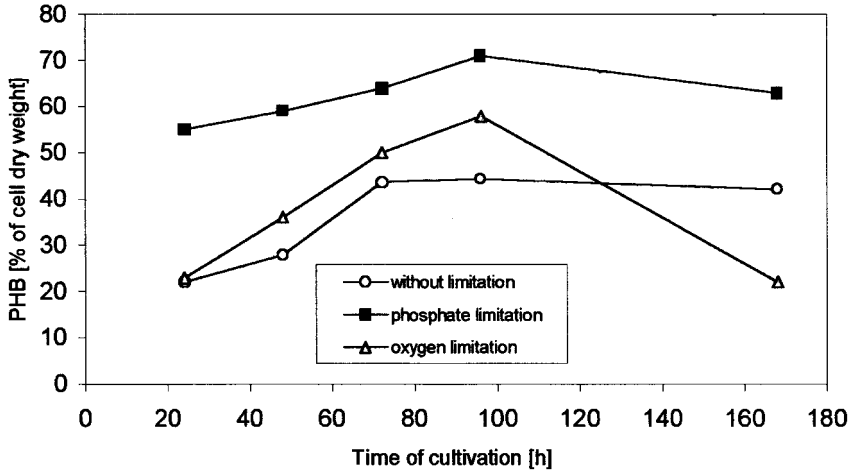

Fig. 2. Production of PHB by *A. radiobacter* cultivated on 1% *D*(+)-carnitine under phosphate and oxygen limitation as well as unlimited growth conditions

The PHB amount obtained using phosphate limitation after growth on 1% *D* (+)-carnitine was rather high compared to PHB production on other inexpensive cultivation substrates. Only *Azotobacter chroococcum* strains achieved a similar PHB content (74%) when cultivated on starch in batch culture [10, 11]. A recombinant strain of *Escherichia coli* is able to produce 80% PHB by cultivation on whey in fed-batch culture [11].

Because of the high PHB amount produced by *A. radiobacter* after cultivation on the waste product *D*(+)-carnitine as the sole carbon and nitrogen source, the strain seems to be a good candidate to lower the cost of PHB production.

Some more investigations for the complete degradation of *D*(+)-carnitine of the *D,L*-racemate are necessary. If it would be possible to purify the *L*(–)-carnitine from the racemic mixture and to further increase the PHB amount, this procedure would be an efficient and economically interesting method for the production of PHB and *L*(–)-carnitine.

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References

[1] ANDERSON, A.J., DAWES, E.A.: Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates. Microbiol. Rev. **54** (1990), 450–472.

[2] LEE, S.Y: Bacterial polyhydroxyalkanoates. Biotechnol. Bioeng. **49** (1996), 1–14.

[3] EVANS, J.D., SKIDAR, S.K.: Biodegradable plastics: An idea whose time has come? Chem. Tech. **20** (1990), 38–42.

[4] CHOI, J., LEE, S.Y.: Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. Appl. Microbiol. Biotechnol. **51** (1999), 13–21.

[5] YOUNG, F.K., KASTNER, J.R., MAY, S.W.: Microbial production of poly-β-hydroxybutyric acid from *D*-xylose and lactose by *Pseudomonas cepacia*. Appl. Environ. Microbiol. **60** (1994), 4195–4208.

[6] RAMSAY, J. A., ALY HASSAN, M.-C., RAMSAY, B. A.: Hemicellulose as a potential substrate for production of poly(β-hydroxyalkanoates). Can. J. Microbiol. **41** (1995), (Suppl1), 262–266.

[7] BERTRAND, J.-L., RAMSAY, B.A., RAMSAY, J.A., CHAVARIE, C.: Biosynthesis of poly-β-hydroxyalkanoates from pentoses by *Pseudomonas pseudoflava*. Appl. Environ. Microbiol. **56** (1990), 3133–3188.

[8] PAGE, W.J. CORNISH, A. Growth of *Azotobacter vinelandii* UWD in fish peptone medium and simplified extraction of poly-β-hydroxybutyrate. Appl. Environ. Microbiol. **59** (1993), 4236–4244.

[9] LILLO, J.G. RODRIGUEZ-VALERA, F.: Effects of culture conditions on poly(β-hydroxybutyric acid) production by *Haloferax mediterranei*. Appl. Environ. Microbiol. **56** (1990), 2517–2521.

[10] MARTINEZ-TOLEDO, M.V., GONZALES-LOPEZ, J., RODELAS, B., POZO, C., SALMERON, V.: Production of poly-β-hydroxybutyrate by *Azotobacter chroococcum* H 23 in chemically defined and alpechin medium. J. Appl. Bacteriol. **78** (1995), 413–418.

[11] KIM, B.S.: Production of poly(3-hydroxybutyrate) from inexpensive substrates. Enzyme Microbiol. Technol. (2000), 774–777.

[12] YELLORE, V., DESAI, A.: Production of poly-3-hydroxybutyrate from lactose and whey by Methylobacterium sp. ZP24. Lett. Appl. Microbiol. **26** (1998), 391–394.

[13] BREMER, J.: Carnitine – metabolism and functions. Physiol. Rev. **63** (1983), 1420–1480.

[14] CEDERBAUM, S.D., AUENSTAD, N., BERNAR, J.: Four-year treatment of systemic carnitine deficiency. New Engl. J. Med. **310** (1984), 1395–1396.

[15] GILBERT, E.F.: Carnitine deficiency. Pathology **17** (1985), 161–169.

[16] BRENINGSTALL, G.N.: Carnitine deficiency syndromes. Pediatr. Neurol. **6** (1990), 75–81.

[17] REBOUCHE, C.J., PAULSEN, D.J.: Carnitine metabolism and function in human. Annu. Rev. Nutr. **6** (1986), 41–66.

[18] ACHENBACH, H., KÜHN, H.-I., LÖSSNER, J., SEIM, H.: Klinische Anwendung des Carnitins unter besonderer Berücksichtigung der Carnitinmangelsydrome. Wiss. Z. KMU Leipzig, Math-Nat. R. **34** (1985), 259–272.

[19] SCHÄRER, K.: Carnitinverlust durch Hämodialyse. In: GITZELMANN, R., BAER-LOCHER, K., STEINMANN, B. (eds.). Carnitin in der Medizin. Stuttgart: Schattauer, 1987, 114–124.

[20] MEIER, P.J.: *D*-Carnitin harmlos? In: GITZELMANN, R., BAERLOCHER, K., STEINMANN, B. (eds.). Carnitin in der Medizin. Stuttgart: Schattauer, 1987, 101–104.

[21] JUNG, H., JUNG, K., KLEBER, H.-P.: Synthesis of *L*-carnitine by microorganisms and isolated enzymes. Adv. Biochem. Eng. Biotechnol. **50** (1993), 21–44.

[22] CAVAZZA, C.: Verfahren zur Herstellung von *L*-Carnitinamid und des *D*-Kampferats von *D*-Carnitinamid. DE 2927672. (1979).

[23] MÜLLER, D. M, STRACK, E.: Razematspaltung von *D,L*-Carnitin. HOPPE-SEGLER'S Z. Physiol. Chem. **353** (1972), 618–622.

[24] EZSLINGER, M.: *L*-Carnitinbildung aus *D,L*-Carnitin mit *D*-Carnitin metabolisierenden Mikroorganismen. Diplomarbeit, Fakultät für Biowissenschaften, Pharmazie und Psychologie, Universiät Leipzig (1997).

[25] MILLER, J.H.: Experiments in molecular genetics. New York: Cold Spring Habor, Cold Spring Habor Laboratory, 1972, 431–432.
[26] AURICH, H., KLEBER, H.-P., SCHÖPP, W.-D.: An inducible carnitine dehydrogenase from *Pseudomonas aerugenosa*. Biochim. Biophys. Acta. **139** (1967), 505–507.
[27] OBÒN, J.M., BUENDIA, B., CANOVAS, M., IBORRA, J.L.: Enzymatic cyclic assay for *D*-carnitine determination. Anal. Brioche. **274** (1999) 1, 34–39.
[28] RIIS, V., MAI, V.: Gaschromatic determination of poly-β-hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis. J. Chromatogr. **445** (1986), 285–289.
[29] MÖNNICH, K., HANSCHMANN, H., KLEBER, H.-P.: Utilization of *D*-carnitine by *Pseudomonas* sp. AKI. FEMS Microbiol. Lett. **132** (1995), 51–55.
[30] HANSCHMANN, H., DOß, A., KLEBER, H.-P.: Occurrence of carnitine dehydrogenases with different stereospecifity in *Agrobacterium* sp. FEMS Microbiol. Lett. **119** (1994), 371–376.
[31] KLEBER, H.-P.: Bacterial carnitine metabolism. FEMS Microbiol. Lett. **147** (1997), 1–9.
[32] ASLIM, B., FIKRET, C., BEYATH, Y., GÜNDÜZ, U.: Poly-β-hydroxybutyrate production by lactic acid bacteria. FEMS Microbiol. Lett. **159** (1998), 293–297.
[33] HANSCHMANN, H., KLEBER, H.-P.: Purification and characterization of *D*(+)-carnitine dehydrogenase from *Agrobacterium* sp. – a new enzyme of carnitine metabolism. Brioche. Biophys. Acta **1337** (1997), 133–142.
[34] LINDSTEDT, G., LINDSTEDT, S., MIDTREDT, T., TOFFT, M.: Inducible γ-butyrobetaine-degrading enzymes in *Pseudomonas* sp. AKI J. Bacteriol. **101** (1970), 1094–1095.
[35] BREUER, U.: *Methylobacterium rhodesianum* als Produzent von Poly-3-hydroxybuttersäure und Exopolysacchariden. Dissertation. Universität Leipzig (1997).
[36] DANIEL, M., LEBEAULT, J.M., CHOI, J.H., KIM, L.: Effect of nutrient deficiency on accumulation and relative molecular weight of poly-β-hydroxybutyric acid produced by methylotrophic bacterium *Pseudomonas* 135. Appl. Microbiol. Biotechnol. **37** (1992), 702–706.