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Preliminary Study of Biotransformation of Aldehydes and Ketones by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564)

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Abstract: The application of *Clostridium* species as a whole cell biocatalysis represents an attractive alternative to conventional chemical synthesis. Solvent-producing cultures of *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564) might act as a promising biocatalyst toreduce a variety of the carbonyl groups to the corresponding alcohols through whole cell biotransformation process. The main objective of this work was to investigate the ability of *C.saccharoperbutylacetonicum* N1-4to biotransform some aldehydes and ketones into the corresponding alcohols. A screening of 13 substrates belonging to aldehydes and ketones group was performed to illustrate the ability on the production of alcohol via biotransformation using the growing cells of *C. saccharoperbutylacetonicum* N1-4. The experiment was carried out with initial substrates of concentration 2.5 mM, pH 6.8±0.2 and temperature 30°C. Enzyme that catalyse this biotransformation might be dehydrogenase enzyme since it catalyses the reduction reaction of aldehydes and ketones to the corresponding alcohols. Gas chromatography analysis indicated that microbial biotransformation definitely represents a new attractive approach of *C. saccharoperbutylacetonicum* N1-4 since it revealed that butanal, pentanal andcyclohexanone were biotransformed into butanol, pentanol and cyclohexanol, respectively.

Key words: *Clostridium saccharoperbutylacetonicum* N1-4, whole cell biotransformation, reduction of ketone and aldehyde and alcohol production

INTRODUCTION

Biocatalysts become advance as an alternative to traditional organic synthesis that offer greener environment to eliminate or reduce hazardous chemical substances for the industrial transformation of natural or synthetic materials through biotransformation process whereby a conversion of one chemical to another (transformed) by a chemical reaction performed by microorganisms (Faber, 2000). Recently, whole-cell biocatalysis is used to perform biotransformation which attracts interest for the production of chiral compounds such as alcohol (Goldberg *et al*., 2007). Application of this white biotechnology by whole cell biocatalytic synthesize important chiral compounds such as aldehydes and ketones for producing alcohols as green chemistry products in industries such as pharmaceuticals, agrochemicals and natural products (Goldberg *et al*., 2007).

Enzyme alcohol dehydrogenases (ADHs) catalyse the interconversion for the reduction of these carbonyl groups (aldehydes and ketones) into alcohols Chakraborty *et al*. (2005), Nicolaou *et al*. (2010), reported among Gram-positive (Gram+) bacteria, the list of species that importance to biocatalysis in performing biotransformation, include organisms in the genera Clostridia which code for ADHs. Tashiro and Sonomoto (2010) has stated that, methabolic pathway of *Clostridium acetebutylicum* shows the biotransformation of butyraldehyde to butanol thus, *C. saccharoperbutylacetonicum* N1-4 has a potential to transform aldehyde and ketone into alcohol since it code for aad/adhE gene (aldehid/alcohol dehydrogenase). Biotransformation of clostridia species must be able to resist a variety of adverse conditions to a number of toxic chemicals to grow under a low redox potential, enabling a variety of stereospecific reductions yielding chiral products that are difficult to synthesize (Ezeji *et al*., 2007).*C. saccharoperbutylacetonicum* N1-4 containing enzyme ADHs mightreact ideally as whole cell biocatalysis (instead of purified enzyme preparations) that practically do not produce waste, works in water, room temperature and near neutral pH (Ezeji *et al*., 2007).

The vast majority of dehydrogenases and reductases used for aldehyde/ketone reduction and alcohol oxidation require nicotinamide cofactors, such as NADH and NADPH. Thus, biotransformation of aldehyde or ketone by enzyme alcohol dehydrogenase requires NADPH as a

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cofactor which should be regenerated. Hence, the functional overexpression of alcohol dehydrogenase was sufficient to enable and sustain the desired reduction reaction via the relatively low specific activity of alcohol dehydrogenase with NADPH. In addition it is less expensive and recycling of the cofactor is most easily achieved in metabolically active whole cells biocatalysis because enzyme purification are tedious, significant production cost and may lead to a rapid loss of enzyme activity (Ohtake *et al.*, 2006).

Therefore, by using whole cells of *C. saccharoperbutylacetonicum* N1-4, reductive reactions result in the production of an alcohol with the possible cofactor regeneration *in vitro* for NADPH, thus its regeneration in metabolically active cells are not only reduce the cost, convenient and in some cases enzymes are more stable within the cells thus extending the life of the biocatalyst in producing alcohols (Faber, 2000). Nevertheless, no detailed description has been proposed for the biotransformation of aldehyde and ketones under anaerobic conditions of *C. saccharoperbutylacetonicum* N1-4. Therefore, more works are required to concentrate on the ability of this *Clostridium* species, to catalyse several aldehydes and ketones into respective alcohols.

MATERIALS AND METHODS

Microorganism and inoculum preparation: *C. saccharoperbutylacetonicum* N1-4 (CSN14) was provided by the Biotechnology Lab, Chemical Engineering and Bioprocess Department at Universiti Kebangsaan Malaysia. It was kept at 4°C as a suspension of spores in a 15% Potato Glucose Medium (PGM) as a stock culture. The PG medium contained the following substances per litre of distilled water; 150 g fresh mesh potato, 10 g glucose, 0.5 g $(NH_4)_2SO_4$ and 3 g $CaCO_3$. After mixing the above substances the medium was incubated in boiling water for 1 h and stirred at 10 min intervals. After that, the medium was filtered through gauze and sterilized at 121°C for 15 min. The PG inoculum was prepared by transferring 1 mL of this suspension sporesinto 10 mL of a solution of 15% PG medium per litre of distilled water as a growth medium, heat shock for 1 min in boiling water and thereafter, cooled in iced water and incubated at 30°C for 1-2 days under anaerobic conditions.

Culture media and experimental procedure: The culture was then transferred to Tryptone Yeast Extract Acetate medium (TYA medium components per litre of deionized water constitute; 20 g glucose, 2 g yeast extract, 6 g tryptone, 3 g CH_3COONH_4, 0.3 g $MgSO_4{\bullet}7H_2O$, 0.5 g KH_2PO_4 and 10 mg $FeSO_4{\bullet}7H_2O$. The initial pH of the medium was maintained to 6.8±0.2 and sterilized at 121°C for 15 min) and was incubated anaerobically at 30°C for 15-18 h and used as apre-culture. The optimum growth culture of pre-culture was used 20% for main culture in 200 mL working volume. Aldehydes and ketones obtained from Sigma-Aldrich as substrates were measured at concentration of 2.5 mM each before transferred into TYA main culture for incubation at 30°C. Since *C. saccharoperbutylacetonicum* N1-4 is anaerobe bacteria, precaution was controlled to minimize the introduction of oxygen into the culture after medium sterilization and during inoculation.

Analytical procedures: Samples were analysed for Optical Density (OD) by spectrophotometer at 680 nm and pH were measured by using pH meter. All samples were centrifuged at 12,000 rpm for 15 min for measuring the transformation product. Then, the supernatant was mixed with dichloromethane (DCM) and centrifuged again for 10 min. The extraction (bottom layer) was filtered by syringe and used for determining the concentration of solvent (aldehydes and ketones) using gas chromatography, GCFID (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with flame ionization detector and a 30 m capillary column (Equity1; 30×0.32 mm× 1.0 μm film thickness Supelco Co, Bellefonate, PA, USA). The injector and detector temperatures were set at 250 and 280°C, respectively. Helium as the carrier gas was set at a flow rate of 1.5 mL min^{-1}.

RESULTS AND DISCUSSION

C. saccharoperbutylacetonicum N1-4 was able to perform biotransformation of chiral aldehydes and ketones in a batch process which was carried out in a 250 mL Schott bottle with 200 mL working volume for the production of chiral alcohols. The biotransformation process was studied using growing cultures of CSN14 grown in TYA media with the presence of aldehydes or ketones. The Optical Density (OD) was measured for control growth and also for maximum Optical Density butyraldehyde to butanol thus the transformation of the pentanal butanal and cyclohexanone to pentanol butanol and cyclohexanol (Green *et al.*, 1994).

It has been observed that final pH of pentanal butanal and cyclohexanone range between pH 4-5 for most solvatogenic clostridia. The culture pH drop as a low medium pH has been implicated as essential requirement (OD) of biotransformation. Table 1 shows that three substrates out of thirteen tested were transformed to the corresponding alcohols. Results indicated that butanal,

Table 1: Biotransformation of different aldehydes and ketones by CSN14

Substrate	Product	Maximum OD	Control OD	Availability of biotransformation	% of Inhibition	% of Alcohol production
Aldehyde:	**Alcohol:**					
Pentanal	Pentanol	2.702	2.331	Can transform		1.6
Butanal	Butanol	2.734	2.331	Can transform		6.4
Ketone:	**Alcohol:**					
2-butanone	-	2.850	2.331	Cannot transform	22.3	
3-pentanone	-	2.030	2.331	Cannot transform	12.9	
3-heptanone	-	0.371	2.331	Cannot transform	84.1	
2-hexanone	-	1.680	2.331	Cannot transform	27.9	
2-heptanone	-	2.125	2.331	Cannot transform	8.8	
3-metil-2-butanone	-	2.040	2.331	Cannot transform	12.4	
2-pentanone	-	1.187	2.331	Cannot transform	49.1	
3-hexanone	-	2.641	2.331	Cannot transform	13.3	
2,3-butanedione	-	2.010	2.331	Cannot transform	13.7	
2,4-pentanedione	-	2.100	2.331	Cannot transform	9.9	
Cyclohexanone	Cyclohexanol	2.541	2.331	Can transform		26

*OD: optical density

Table 2: Relations of the butanol concentration and transformation product using *C. saccharoperbutylacetonicum* N1-4 growing cell

Substrat	Concentration (mM)	Final pH	(Butanol) g L^{-1}	Transformation product (%)
Pentanal	2.5	5.05	0.749	1.6
Butanal	2.5	5.11	2.366	6.4
Cyclohexanone	2.5	4.89	3.346	26.0

pentanal and cyclohexanone were reduced to the corresponding alcohols (Table 1). All the selected aldehydes and ketones as a substrate for the screening were repeated three times to determine the availability of biotransformation.

Butanol and pentanol production byusing 2.5 mM concentration were lower than produced by 2.5 mM of cyclohexanol. The highest transformation was observed in the culturewith cyclohexanone which is 26% transformation, while cultures with substrates of pentanal and butanal showed 1.6 and 6.4% transformation. This is due to cyclohexanone compound that is more soluble in water but butanal and pentanal may not be soluble in all proportions which are slightly soluble (Hardinger, 2006). The lower members of aldehydes and ketones are miscible with water in all proportions, because they form hydrogen bond with water. However, the solubility of aldehydes and ketones decreases rapidly on increasing the length of alkyl chain. Aldehyde is less stable and more reactive compared to ketone which is more stable but less reactive (Hardinger, 2006).

C. saccharoperbutylacetonicum N1-4 shows the ability to biotransform varioussubstrates including aliphatic and cyclic ketones. Based on the result, cyclic ketone which is cyclohexanone has the highest ability to undergo biotransformation into alcohol as shown in Table 1. On the other hand, CSN14 can grow in the presenceo f 2-butanone, 3-pentanone, 2-hexanone, 2-heptanone, 3-metyl-2 butanone, 2-pentanone, 3-hexanone, 2, 3-butanedione and 2, 4-pentanedione with or without inhibition but cannot perform biotransformation process. Previous report by Zanotti-Gerosa *et al.* (2005) indicated that aromatic, heteroaromatic and unsaturated ketones could be reduced with excellent productivity and enantio selectivity but aliphatic ketones were less transformed. Other report stated that aromatic aldehydes are less reactive than aliphatic aldehydes. Thus, from both aldehyde substrates butanal and pentanal successfully transform into alcohol product.

It was also found that, *C. saccharoperbutylacetonicum* N1-4 was stimulated by the addition of 2-butanone (22.3%) and 3-hexanone (13.3%) because there is no inhibitionoccurred. However, 3-pentanone, 2-hexanone, 2-heptanone, 3-metil-2-butanone, 2-pentanone, 2, 3-butanedione and 2, 4-pentanedione were not suppressed by *C. saccharoperbutylacetonicum* N1-4 growth but cannot perform biotransformationduring the growth due to the inhibition in Table 1. The lowest inhibition was 8.8% of 2-heptanone and the highest inhibition was 3-heptanone with 84.1 %.

In Table 2, the result shows three successful substrates that can perform biotransformation which are pentanal butanal and cyclohexanone tends to have relation between the production of transformation product and butanol concentration and the relationship is linear. Solvent production occurred which resulted in an increase in the butanol production which is 0.749 g L^{-1} (pentanal), 2.366 g L^{-1} (butanal) and the highest is 3.346 g L^{-1} (cyclohexanone) (Table 2). Cell has to synthesize butanol dehydrogenase enzyme to catalyse the reaction of for

good solvent production due to acidogenic phase characterised by rapid growth, production of hydrogen and carbon dioxide, acetic and butyric acid solvent (Hipolito *et al*., 2008). Previous study has stated that the most suitable pH in solvent production is ph 4.8 (Bahl and Durre, 2001).

The capabilities of dehydrogenase enzyme of *C. saccharoperbutylacetonicum* N1-4 to biotransform aldehyde and ketone synthesized in the laboratory were investigated and measured by using Gas Chromatography Flame Ionization Detector (GCFID).

CONCLUSION

In conclusion, the capabilities of *C. saccharoperbutylacetonicum* N1-4 (ATCC 13564) to biotransform aldehydes or ketones were studied. The biocatalytic process by *C. saccharoperbutylacetonicum* N1-4 represents suitable substrates for thepreparation of a wide range of alcohols using substrates none previously reported in the literature. The results obtained shows that *C. saccharoperbutylacetonicum* N1-4 could be responsible for the biotransformation process of butanal, pentanal, cyclohexanone into butanol, pentanol, cyclohexanol, respectively.

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REFERENCES

Bahl, H. and P. Durre, 2001. Clostridia-Biotechnology and Medical Applications. 1st Edn., Wiley-VCH, Weinheim, Germany, ISBN-13: 978-3527301751, Pages: 279.

Chakraborty, A.A., R.P. Phadke, F.A. Chaudhary, P.S. Shete, B.S. Rao and K.D. Jasani, 2005. Optimization of redox reactions employing whole cell biocatalysis. World J. Microbiol. Biotechnol., 21: 221-227.

Ezeji, T.C., N. Qureshi and H.P. Blaschek, 2007. Bioproduction of butanol from biomass: From genes to Bioreactors. Curr. Opin. Biotechnol., 18: 220-227.

Faber, K., 2000. Biotransformations in Organic Chemistry. 4th Edn., Springer, USA.

Goldberg, K., K. Schroer, S. Lutz and A. Liese, 2007. Biocatalytic ketone reduction-a powerful tool for the production of chiral alcohols-part I: Processes with isolated enzymes. Applied Microbiol. Biotechnol., 76: 237-248.

Green, E.M., M.S. Kalil, P. Williams and G.M. Stephens 1994. Screening for reduction of aldehydes and ketones by solventogenic cultures of the strict anaerobe, *Clostridium acetobutylicum*. Biotechnol. Tech., 8: 733-738.

Hardinger, S., 2006. Chemistry 14D. Hayden-McNeil Publication, USA.

Hipolito, C.N., E. Crabbe, C.M. Badillo, O.C. Zarrabal and M.A.M. Mora *et al*., 2008. Bioconversion of industrial wastewater from palm oil processing to butanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). J. Cleaner Prod., 16: 632-638.

Nicolaou, S.A., S.M. Gaida and E.T. Papoutsakis, 2010. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: From biofuels and chemicals, to biocatalysis and bioremediation. Metab. Eng. J., 12: 307-331.

Ohtake, H., S. Yamashita and J. Kato, 2006. Development of a new biotechnological basis for improving industrial sustainability in Japan. Eng. Life Sci., 6: 278-284.

Tashiro, Y. and and K. Sonomoto, 2010. Advances in Butanol Production by Clostridia. In: Current Research Technology Education Topics Applied Microbiology Microbial Biotechnology, Mendez-Vilas, A. (Ed.). Formatex, Spain, pp: 1383-1394.

Zanotti-Gerosa, A., W. Hems, M. Groarke and F. Hancock, 2005. Ruthenium-catalysed asymmetric reduction of ketones. Platinum Metals Rev., 49: 158-165.