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Microbial Stereo Inversion of (R) 3 Chloro-1,2-Propandiol by *Wickerhamomyces anomalous* MGR6-KY209903

Suresh Kumarasamy [1,*], Vasuki Subramanian [1], Mathiyazhagan Narayanan [1], Muthusamy Ranganathan [1]

[1] PG and Research centre in Biotechnology, MGR College, Hosur, Tamilnadu, 635130, India

* Correspondence: sureshbioteck@yahoo.com;

Scopus Author ID 57195420982

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Abstract: Our interest has been focused on the production of chiral compounds by the method of enantioselective microbial transformations of prochiral starting material with yeast. The preliminary assimilation was exercised with *Wickerhamomyces anomalous MGR6* grown on synthetic medium and observed assimilation by pH change of the medium.The specific rotation of (R-3- Chloro-1,2-propanediol) was -5 (c=2.5, in C_2H_5OH) in conditions with enatiomeric excess of 85.6 % *Wickerhamomyces anomalous* product. The structural and dynamic properties of R-3-Chloro-1,2-propanediol in GCMS spectrum, the most prevailing compound among them retention time of 9.63 was obtained. The IR Spectrum, a shallow broad band at wavelength 3290.56 signifies the presence of O-H stretching, the narrow peak both at 1637.56 indicates the presence of C=O stretching in conjugation with functional group of R- 3 Chloro-1,2-propanediol($C_3H_7ClO_2$).The sharp peaks in HPLC spectrum of R 3 Chloro-1,2-propanediol in the retention time peak were 2.47 min and concentration 100%**.** These results concluded that the strain assimilated and sterio inversion of R-3- Chloro-1,2-propanediol *via RS* 3-chloro-1,2-propanediol. The possibility to produce optically active R-3- Chloro-1,2-propanediol was discussed.

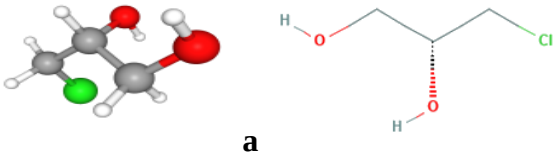
Keywords: MCH-Monochlorohydrin; *Wickerhamomyces anomalous MGR6* KY209903; enatiomeric excess; polarimeter; Biotransformation; Stereo inversion.

1. Introduction

The life science industry is a significant market for the pharmaceutical industry. The total revenue from trading in the pharmaceutical and agrochemical industries for the year 2018 is estimated to be in excess of US 150 billion, of which the greatest share comes from the pharmaceutical industry. Optically active intermediates used as chemical building blocks, auxiliaries, or advanced intermediates have an estimated fraction of 17% of the market. Selling of special intermediates is increasing at about 10–12% annually. Because of the increasing demand in the pharmaceutical industry for optically active intermediates, these compounds have a disproportionate share with 12% annually. About 78% of the active intermediates compounds that pharmaceutical companies have in the pipeline are chiral, and it is estimated that this fraction will increase, as the development of active compounds continues to be improved [1].

The optically active (R)-3-chloro-1,2-propanediol is a useful starting material for synthesizing a variety of 5 drugs and optically active compounds having physiological activity [2]. A process for the preparation of optically active 3-Chloro-1,2-propanediol which

comprises cultivating in a medium containing racemate 3-Chloro-1,2-propanediol a bacterium and yeast, which, when cultivated in a medium containing racemate 3-Chloro-1,2-propanediol (Figure.1) as a sole carbon source, can grow and proliferate, has an ability to assimilate S(-)-3-Chloro-1,2-propanediol preferentially compared to R-3-Chloro-1,2-propanediol and belongs to bacteria and yeast, or its culture cells and recovering R-3-Chloro-1,2-propanediol from the resulting culture broth [3].



a **b**

Figure 1. (R)-3-Chloro-1,2-propanediol **a)** Interactive Chemical Structure Model; b) Chemical Structure Depiction.

Yeast can be used for a number of bioconversions [4].Large numbers of yeast species are currently recognized for their ability to utilize hydrocarbons as sole sources of carbon and energy listed 114 species and varieties of hydrocarbon-assimilating yeasts. Some of these have since been reduced to synonymy with other species. Species are found in the genera *Candida, Debaryomyces* (including *Schwanniomyces* and *Wingea), Lodderomyces, Clavispora, Metschnikowia,Pichia, Stephanoascus,* and *Yarrowia*\ basidiomycetous species are in genera *Leucosporidium, Rhodosporidium,Sporidiobolus, Sporobolomyces, Rhodotorula,* and *Trichosporon.* The discovery that some taxa are basidiomycetes has broadened perception of the nature of yeasts. This group is represented by numerous strains that were chosen and adapted for specific industrial fermentations [5].

Wickerhamomyces anomalus is a frequently found yeast species in natural environments (plants, soil, fruits, animals) and in various fermentations, and there are some beneficial effects that have been previously reported, such as functioning as an intermediate agent and intermediate producer [6-10].

Biotransformations technique is becoming increasingly popular in the pharmaceutical production of enantio and regiopure intermediates for the synthesis of complex organic compounds. Whole microbial cells can be used to carry out such specific chemical reactions that are otherwise difficult to achieve active synthetically [11].The reports an eco-friendly and efficient methodology for biotransformation of halohydrins to 3 Chloro 1, 2-propanediol using microbes. This procedure involves the addition of water avoiding the utilization of additional solvents and avoiding acidic and basic conditions to afford 3-Chloro-1,2-propanediol nearly yield about 68% without any need for any further purification so only a small amount of waste is generated [12].

In this paper, we report the properties of the assimilation and dehalogenation activities in *Wickerhamomyces anomalus MGR 6* extracts and the conversion of (R)-Monochlorohydrin using the microbial resolution and characterization studies by polarimeter, FTIR, NMR, GCMS and HPLC.

2. Materials and Methods

2.1. Culture collection.

Identified yeast cultures were stored in PG and Research centre in Biotechnology, MGR College, Hosur, Tamilnadu, India. The pure culture of the strains was obtained under standard

operating protocols [13]. The following *Wickerhamomyces anomalus* MGR 6 *(*KY209903) strain has been used for further experiments.

2.2. Assimilation of 3 Chloro-1,2-propanediol in solid media.

The isolate was subjected to primary screening for sorting out their ability for the degradation and assimilation for (*R,S*)-3Chloro-1,2-propanediol. *Wickerhamomyces anomalus* MGR 6 strain was streaked on to agar plates containing synthetic medium (Racemic 3-Chloro-1,2-propanediol -0.5g , diammonium sulfate-0.5g, dipotassium hydrogen phosphate-0.1g,diammonium sulphate -0.2g,sodium dihydrogen phosphate-0.05g, Magnesium sulphate-0.05g,ferrous sulphate-0.0001g, copper sulphate-0.0001g ,manganese sulphate-0.0001g , agar-0.5g bromothymol blue, 0.008g - agar / 100ml) (pH-6.8) and incubated at 30^0C for 48hrs. After incubation period, the plates were observed for colour and pH change in the medium, which indicates the assimilation of (*R,S*) 3 Chloro-1, 2-propanediol [14].

2.3. Conversion of optically active Monochlorohydrin.

Wickerhamomyces anomalus MGR 6 seed culture (5 ml) was inoculated into 2.5 L of the synthetic medium containing 2.0% (v/v) (*R,S*)- 3 Chloro-1,2-propanediol as a sole source of carbon source in a 5L fermenter,the culture broth was taken out and the cells were removed by centrifugation and to derive the supernatant alone.The 250ml medium was allowed to condense about 150ml using the rotary evaporator and extracted with 100ml ethyl acetate. The evaporation and extraction process was repeated thrice. Then it was dried on anhydrous magnesium sulphate and the solvent residue was distilled using vacuum evaporation (rotavap) to get a condensed syrup of optically active 3-Chloro1,2-propanediol [15].The optically active condensed syrup was further subjected to characterization studies by polarimeter, FTIR, NMR, GCMS and HPLC.

2.4. Determination of specific rotatory power by polarimeter.

The optical activity and specific rotatory power of condensed syrup were determined using JASCO-181 digital polarimeter at ambient temperature at NCL, Pune for standard procedure. The optical rotation was measured at the wavelength of the D line of sodium (λ = 589.3 nm) at 20°C, on a layer 1 dm length. It was expressed in degrees. The reading, which appeared on the digital panel was noted and the calculated the optical rotation, purity of enantiomers using the formula given below. $\alpha_{\lambda}^{T} = \frac{\alpha}{lXC}$

2.5.GC-MS analysis.

The condensed syrup was separated by Perkin Elmer Clarus 680 GC-MS capillary column model Elite-5MS (30m X 0.25mm). The Gas chromatography was directly interfaced with Clarus 680 mass spectrometer with an interface temperature of 250°C. Sample ionization was electron impact and was analysed by positive mode. Spectrum comparison was carried through NIST library [17].

2.6. HPLC analysis.

50µl of condensed syrup R- 3 Chloro-1, 2-propanediol was taken separately in a clean dry glass vial with septum.The samples were allowed to run for about 25 minutes C18 column with mobile phase comprises of acetonitrile and water in the ratio 60:40v/v. Flow rate Flow rate was adjusted to 1ml/min. Detection-UV detection set to the range of 290nm. Finally,Retention time, peak area, Theo plate, tail factor and graph of the standard were recorded [18].

2.7. FTIR analysis.

The condensed 1 ml syrup sample was mixed with 1ml of KBr. Appropriate pressure was excreted to form a transparent pellet. FTIR spectra of the product were recorded from the spectral range of 400-4000$cm^{-1.}$

2.8. NMR analysis.

Sample concentration was adjusted to 50Mm, instrument type is Bruker, 1H NMR of the and sample R-3 Chloro-1,2-propanediol was analyzed at the frequency of 600MHz and the pH was adjusted to the range of 7.00, the temperature was adjusted to 25^0C and the chemical shift reference used was TMS.1H NMR spectrum was allowed to run now, and the spectrum was acquired from it was recorded.

2.9. Dehalogenation Liberation of chloride ions.

For chloride liberation test, freshly prepared reagents (mercuric thiocynate solution and ferric alum solution) were used. Mercuric thiocyanate solution - adding 0.3g of mercuric thiocyanate in 100ml of 95% ethyl alcohol and ferric alum solution - dissolving 6g of ferric ammonium sulphate in 100ml of 6N nitric acid. The experiment was carried out by transferring 10ml of the sample solution into sterile tubes. 1ml of mercuric thiocynate solution was added into each tubes and mixed well, then 2ml of ferric alum solution was added to it finally and vortexed for 15min. The reaction mixture was kept undisturbed for 15min.Visible colour change was observed, photographed against light background and the optical density was measured immediately after 15min at A_{660nm}. The total amount of chloride ions liberated was calculated [19].

3. Results and Discussion

3.1. Assimilation reaction.

The ability to degrade chloride ions of the isolates was assessed by basic visual identification of the medium in the. Flasks inoculated with, *Wickerhamomyces anomalus* MGR 6 were found dispersed in the medium and involves in changing the colour of the medium from dark green colour to pale greenish yellow coloured solution along with a reduction in the pH {from nearly neutral stage-pH 6.8 to acidic condition pH-5.54} and the same was observed in agar plate, media plate changes its colour from dark green colour to pale greenish yellow colour (Figure.2).In a previous repot of the employing various assimilation tests alone may be reliable as a universal means for identification. Numerous novel species, intraspecies variability of strains and conflicting assimilation profiles might be responsible for screening and identifications [19].

Figure 2. Assimilation efficiency of *Wickerhamomyces anomalus* MGR 6.

3.2. Conversion.

In the production medium incubated at fermentor condition *Wickerhamomyces anomalous* MGR6 exhibited an exponential growth rate of 1.929 N **(**Figure.3). The isolate *Wickerhamomyces anomalous* MGR6 liberated 1.323 ppm of chloride ions from the medium, whereas % yield of *Wickerhamomyces anomalous* was R-3-chloro-1,2-propanediol 66%. In a previous study, the characterization and production of an intermediate by *Wickerhamomyces anomalus* strain PY189 was carried. The highest efficiency production was observed when the isolate was grown in a synthetic medium with chloride ions 1.323 ppm with a statistical micelle concentration of 204 mg/l [20].

Figure 3. Conversion of optically active Monochlorohydrin in fermenter.

3.3. Specific rotation and enatiomeric excess of the product.

The assimilation and degradation of racemic 3-Chloro-1,2-propandiol ceased at 55%, regardless of the concentration of racemic 3-Chloro-1,2-propandiol used 2%.

Table 1. Specific rotation and enatiomeric excess of the product.

S.No	Product Name	Wavelength (nm)	Solvent	Concentration (%)	Specific Rotation Pure (-7^0)	% ee
1	R MCH (3-Chloro-1,2-propanediol std)	589	Ethanol	2.5	0^0	-
2	MGR 6 product	589	Ethanol	2.5	-5^0	85.6

Therefore, the conversion of R-3-Chloro-1,2-propandiol was considered to be stereospecific. The residual R-3-Chloro-1,2-propandiol was extracted with ethanol from the cultivation. The specific rotation of product (R-3-chloro-1,2-propanediol) was -5^0 (c=2.5, in C_2H_5OH) with enatiomeric excess of 85.6 % respectively (Table 1). A previous method for producing optically active 1,2-dio1s by microbial stereoinversion was developed. It was found

that some microorganisms like yeast could convert only (R)-I,2-pentanediol in the racemate to the (S)-enantiomer, molar yield 93%, enantiomeric excess 60% [21].

3.4. GC-MS spectrum (R) 3 Chloro-1, 2-propanediol.

GC-MS was used for determining the production recovery of 3 Chloro-1,2-propanediol. Components in GC-MS were recognized rapidly by its molecular ion, due to the higher specificity in SIM mode. The fragmentation patterns of detected components were given in table 2. The microbial extract yielded symmetrical peaks and excellent separation of components [22-23].

Table 2. Characteristic peaks of R-3 Chloro 1,2-propanediol MGR6 by GC-MS.

Isolate	Line no.	Hit no.	Compound Name	Molecular weight	CAS No.	Scan no.	Retention time	Molecular formula
MGR6	4	1	R-3 Chloro1,2 propanediol	110.539g/mol	96-24-2	750	11.750	$C_3H_7ClO_2$

The characteristic peaks were shown in Figure 4 and the retention time of (R) 3 Chloro-1,2-propanediol was 9.693(scan number 750) obtained from MGR6 respectively. From the extracted product from the production medium inoculated with MGR6, total number of six components were detected the most prevailing compounds were 1,2-benzenediol(39%), 4-(Chloromethyl)-2-heptyl (53%), 3-bromo-1,2-propanediol(64%), propane,2-bromo-1-chloro-methane (92%), and the most prevailing compound detected was R-3 Chloro-1,2-propanediol.CAS number, molecular weight and molecular formula for R-3 Chloro 1, 2-propanediol were 96-24-2,110.539g/mol and $C_3H_7ClO_2$ respectively.

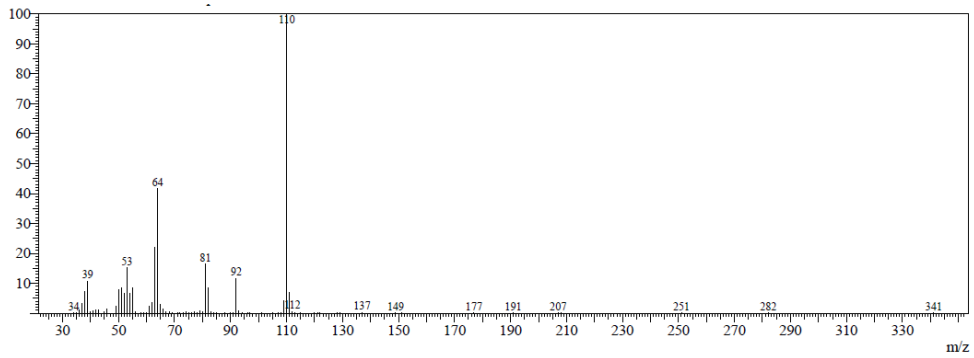


Figure 4. GCMS spectrum of optically active (R) 3 Chloro-1,2-propanediol (MGR 6).

3.5. High Performance Liquid Chromatogrpahy spectrum of (R) 3 Chloro-1,2-propanediol

The chromatographic separation was achieved using gradient separation methodology with the use of hexane and 2-propanol as mobile phase at the ratio of 60:40.The elution was carried out at the flow rate of 1.0ml/min. The sharp peaks obtained indicates the presence of R 3 Chloro-1,2-propanediol.The retention time peak are, theoretical plates were given in figure 5 and table 3.The sharp peaks in HPLC indicates the presence of 3 Chloro-1,2-propanediol.The retention time peak was (MGR 6) 2.47min, concentration was 100%. Therefore, the optimum chromatographic conditions for resolving with a retention time of 3.81, 11.17, and 27.42, respectively. Obviously, the R 3 Chloro-1,2-propanediol was separated from the derivatization agent and its dimer product by HPLC [24].

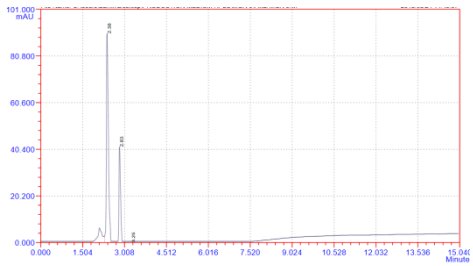


Figure 5. HPLC chromatogram of (R) 3 Chloro-1,2-propanediol obtained from MGR6.

Table 3. Characteristic feature of HPLC chromatogram of standard and (R) 3 Chloro-1,2-proanediol

Index No.	Compound	Retention Time(min)	Peak area (%)	Concentration	Theo. plate	Tail factor
1.	Standard 3 Chloro-1,2-propanediol	2.472	36457.5	100.00	295	0.01
3.	MGR 6	2.47	19547.6	100	110	0.83

3.6. FT-IR Spectrum of (R) 3 Chloro-1, 2-propanediol.

The IR Spectrum obtained from MGR6 produced a broad band at 3290.56 shows the presence of O-H stretching,1637.56 shows the presence of C=O group, the bands at 1087.85 and 1045.42 signifies the presence of C-O stretching. The functional group present in the sample solution was thus identified using the IR-spectral datas, that supports the presence of R-3 Chloro-1,2-propanediol($C_3H_7ClO_2$) (Figure 6). In an earlier study, it has been observed that IR Spectrum obtained from yeast showed a shallow broad band at wavelength 3309.8 signifies the presence of O-H stretching, the narrow peak at 1637.56 indicates the presence of C=O stretching in conjugation with aryl ketones, the band at 1087.85 and 1045.42 indicates the C-O stretching that signifies the presence of alcoholic group [25-27].

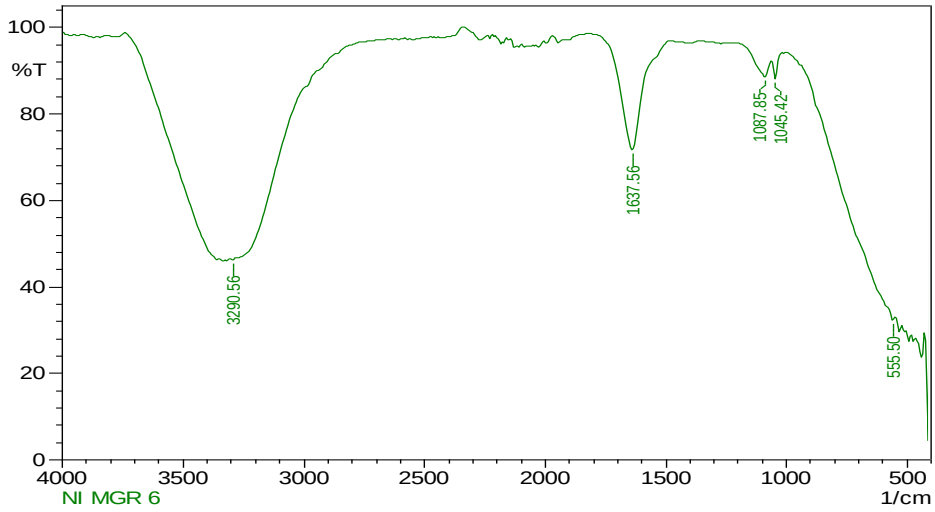


Figure 6. FT-IR Spectrum of (R) 3 Chloro-1, 2-propanediol obtained from isolates MGR6.

3.7. NMR spectrum of (R) 3 Chloro-1,2-propanediol.

R-3-Chloro-1,2-propanediol, colorless oil.1H NMR(400 MHz, DMSO-d_6): δ 5.12 (1H,d, CHOH,exchangeable with D_2O); 4.73(1H, t,CH_2OH,exchangeable with D_2O);3.67-3.62 (2H, m, CH_2Cl);3.53-3.50 (1H, m,CHOH); 3.39-3.35(2H, m,CH_2OH) (Figure 7).

Previously reported the establish the position of the chlorine released from DCP, proton NMR was performed, and chemical shifts were read and recorded for reaction mixtures

containing enzyme, ferricyanide and DCP. NMR spectra three signals an intermediate product was assigned to 2-chloroacrolein.The time course of its aldehyde proton measure (9.31 ppm) displayed the same trend as the two protons (6.72 and 6.57 ppm) [28].

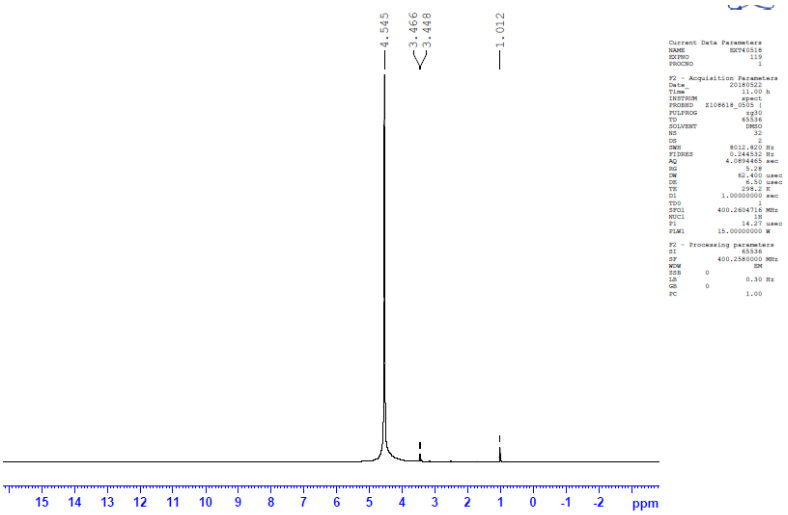


Figure 7. NMR Spectrum of (R) 3 Chloro-1, 2-propanediol obtained from isolates MGR6.

4. Conclusions

It was found that an effective method for the preparation of pure optically active (R)-3-Chloro 1,2-propanidol was established based on assimilation and degradation with *Wickerhamomyces anomalus* MGR6 (KY209903). It can be concluded that subjecting low priced (RS) -3-Chloro 1,2-propanidol to the action of *Wickerhamomyces anomalus* MGR6 (KY209903) to selectively metabolizing (R)-3-Chloro 1,2-propanidol. The specific rotation of the R-MCH obtained was -5^0 that the confirmation of (R) form and its optical purity was found to be 85.6 % enantiomeric excess and produced (R) form 16.2g of Monochlorohydrin.

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Conflicts of Interest

The authors declare no conflict of interest.

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