PRODUCTION OF 2-PHENETHYL ALCOHOL AND 2-PHENYLLACTIC ACID IN CANDIDA SPECIES

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SUMMARY: 2-Phenethyl alcohol (2-PEA) and 2-phenyllactic acid (2-PLA) were isolated from the culture filtrates of Candida species grown in media containing peptone or phenylalanine as nitrogen source. These compounds were characterized by comparing their UV, IR, and NMR spectral properties with authentic samples. Candida species differed markedly in their production of 2-PEA and 2-PLA. Experiments using [^{14}C] -phenylalanine indicated that both 2-PEA and 2-PLA are synthesised from L-phenylalanine. A pathway for the biosynthesis of 2-PEA from L-phenylalanine has been proposed.

INTRODUCTION

The biosynthetic ability of species of Saccharomyces and Candida have been extensively exploited in the production of alcohols (1), vitamins (2) and enzymes (3). 2-Phenethyl alcohol (2-PEA) was isolated from culture filtrates of Candida albicans when grown in Sabouraud's broth (4). Whether the production of this compound is unique to only C. albicans or shared by other species of the genus Candida, as well its physiological role and the enzymology involved in its biosynthesis are some of the aspects about which nothing is known. The purpose of this study is to examine the ability of both pathogenic and nonpathogenic species of Candida to produce 2-PEA and the intermediates involved in its biosynthesis.

MATERIALS AND METHODS

C. albicans Z248, C. guilliermondii Z55, C. krusei Z70 and C. tropicalis Z56 were obtained from London School of Hygiene and Tropical Medicine, London and C. intermedia from V. P. Chest Institute, New Delhi, India. Stock cultures are maintained on Sabouraud's glucose agar.

Erlenmeyer flasks (500 ml) each containing 200 ml of the medium were inoculated with 5 ml of 24 hr broth culture in the same medium. The cells were grown at 31 or 37 C on a rotary shaker for 2 or 7 days. The cells were sedimented by centrifugation at 2000g for 20 min in a Sorvall RC-2B and the supernatant (designated as culture filtrate) was decanted. The cell pellet

Table 1. Physicochemical and spectral properties of 2-PEA and 2-PLA isolated from culture filtrates of Candida species.

Property	2-PEA*	2-PLA**
Liquid/solid	oil (colorless)	leaflet crystals
m.p./b.p.	220 C	120 C
Solubility in acetone	highly soluble	highly soluble
ether	,,	,,
chloroform	,,	slightly soluble
Optical rotation	-	+9°
Rf values	0.72 (TLC)[e]	0.96 (paper chromatography)[f]
U.V. (λ max)[a]	259 nm	258 nm
I.R. absorption[b] range Cm^{-1}	3575 (w) ‖ C_1-OH 3350 (b) ‖	3450 (sh) C_2-OH 1740 (sh) C_1=O
	1460 (sh) ‖ 1508 (sh) ‖ C_6H_5- 1615 (sh) ‖	1460 (sh) ‖ 1500 (sh) ‖ C_6H_5- 1610 (sh) ‖
NMR signals[c] (δ values)	7.40 singlet C_6H_5-	7.40 singlet C_6H_5-
	2.90 triplet $-CH_2$-	4.30 quadruplet HC
	3.90 triplet $-CH_2$-	3.0 quadruplet $-CH_2$-
	1.70 singlet C -OH	2.60 multiplet DMSO
On exchange with D_2O[d]	singlet at δ 1.70 of C_1-OH disappeared	

* From C. guilliermondii ** From C. tropicalis
a Ethyl alcoholic solution (10 mg/100 ml), Unicam SP 700A
b Neat's spectrum (2-PEA) and Nujol spectrum (2-PLA), Carl Zeiss Model W10
c 2-PEA in $CDCl_3$ and 2-PLA in DMSO-D_6 (10-15%), Varian Model T60 MH_2
d 2-PEA was treated with D_2O and kept overnight for exchange
e Solvent system: petroleum ether:ether (1:5)— Identification by iodination
f Solvent system: benzene:acetic acid:water (15:6:3) — Identification by spraying with 0.1% $KMnO_4$
(sh) sharp; (b) broad; (w) wide.

was washed twice with distilled water and the washings were pooled with culture filtrate.

Extraction and purification: Procedure 1 —The filtrate from 2 litres of culture was extracted with chloroform (1 vol for every 10 vol) and the extraction was repeated four times. The chloroform extract was dried over

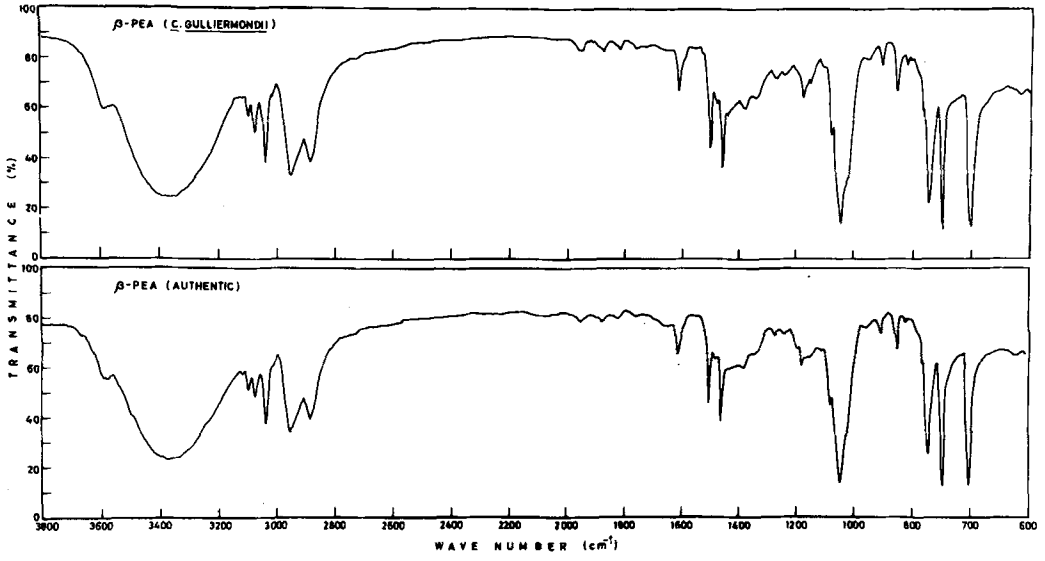

FIG. I. INFRARED SPECTRA OF THE ISOLATED AND AUTHENTIC β-PEA

anhydrous sodium sulphate and evaporated to dryness at 37 C. The oily material thus obtained was treated with 5 ml of acetone and the acetone insoluble material was filtered off. The filtrate was dried and the acetone precipitation was repeated twice. The acetone-free material (250 mg) was applied to 50 X 1.5 cm column of silica gel and eluted with petroleum ether : ether (4:1 v/v) mixture. The first 100-150 ml of the eluate was evaporated at 37 C and the residue (30 mg) was dried in vacuum and used for spectral studies.

Procedure 2 —Step A: One litre of the culture filtrate was extracted with solvent ether (2 vol for every 10 vol) twice and the ether extract was dried at 37 C. The material was taken in 100-150 ml of warm water and the pH was adjusted to 8.0 with sodium bicarbonate and extracted twice with ether. The ether extract was dried over anhydrous sodium sulphate and evaporated at 37 C. The oily material was treated with 5 ml of acetone and the acetone-insoluble material was filtered off and the filtrate was evaporated at 37 C and dried in vacuum and used for spectral analysis. Step B: The alkaline aqueous solution from step A was acidified to pH 3.0 with HCl and the process of ether extraction and acetone treatment was repeated as in step A. The crystalline material obtained was dried in vacuum and used for spectral studies.

RESULTS AND DISCUSSION

From the culture filtrates of C. guilliermondii grown in glucose-peptone broth (Table 2, medium 1) an oily material was isolated by a modified extraction and purification procedure 1 (4). Its physicochemical and UV, IR (Fig.1) and NMR (Figs. 2, 3) spectral properties have been examined (Table 1). This compound was characterised as 2-PEA and confirmed with authentic 2-PEA (BDH sample). Besides C. guilliermondii, other species of Candida like C. albicans,

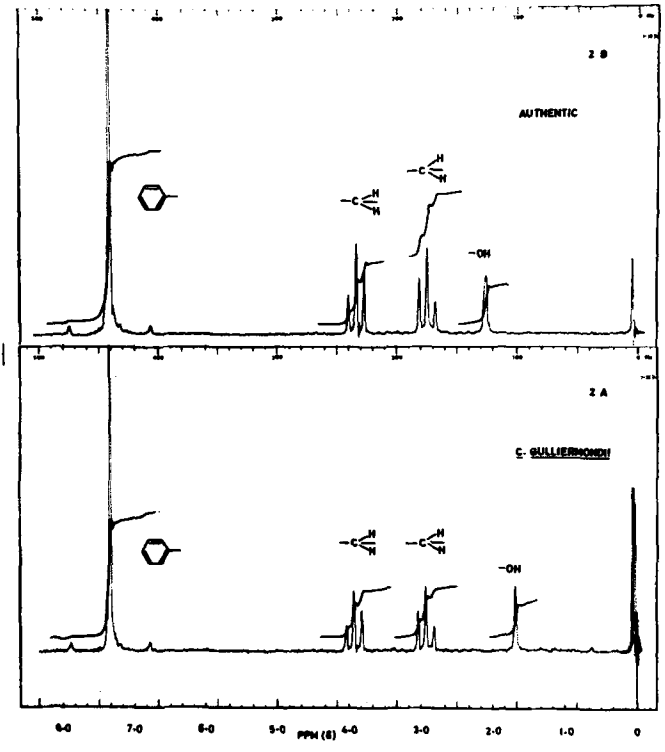

FIG. 2. NUCLEAR MAGNETIC RESONANCE SPECTRA OF ISOLATED (2A) AND AUTHENTIC (2 B) β-PEA

C. krusei and C. intermedia also produced 2-PEA under identical conditions (Table 2). However, only in C. tropicalis no detectable amount was found.

To define the culture medium, peptone was replaced by ammonium sulphate at a concentration of 0.25% (Table 2, medium 2). At this concentration ammonium sulphate supports good growth of all species of Candida* but none of the species produced 2-PEA. This indicated that amino acid(s) present in peptone is required for the biosynthesis of 2-PEA. Hence, the effect of L-phenylalanine, a compound possibly utilized in the biosynthesis of 2-PEA was examined. With L-phenylalanine as nitrogen source all species including C. tropicalis produced 2-PEA (medium 3). The presence of ammonium sulphate along with L-phenylalanine, however, had not interfered in the biosynthesis of 2-PEA (medium 4).

The possible presence of other compounds in culture filtrates was examined by altering the method of extraction (procedure 2). By this procedure, a

* V. P. Chowdary and G. Ramananda Rao, unpublished data.

Table 2. Influence of nitrogen source on the production of 2-PEA by *Candida* species *

Species	Medium 1	Medium 2	Medium 3	Medium 4
C. guilliermondii	produced	not produced	produced	produced
C. albicans				
C. krusei				
C. intermedia				
C. tropicalis	not produced	not produced	produced	traces

* Cells were grown in 2 L of medium at 37 C for 7 days. Isolation was by Procedure 1.

Medium 1 : glucose, 4%; peptone, 2%.
Medium 2 : glucose, 2%; $(NH_4)2SO_4$, 0.25%; KH_2PO_4, 0.35%; $MgSO_4.7H_2O$, 0.25%; $CaCl_2$, 0.25% and biotin, 3 ug per 100 ml.
Medium 3 : same as 2, but $(NH_4)_2SO_4$ is replaced by 1% of L-phenylalanine (Sigma)
Medium 4 : both $(NH_4)_2SO_4$ and L-phenylalanine were present at the same concentrations as in media 2 and 3.

Table 3. Production of2-PEA and 2-PLA by *Candida* species*

Species	2-PEA (mg)	2-PLA (mg)	Total of 2-PEA & 2-PLA (mg)	Conversion
C. guilliermondii	578	87	665	66.5%
C. krusei	308	28	336	33.6%
C. intermedia	158	378	536	53.6%
C. albicans	168	412	580	58 %
C. tropicalis	18	620	638	63.8%

* Cells were grown in 1 L of medium 3 at 31 C for 2 days. Isolation was by procedure 2.

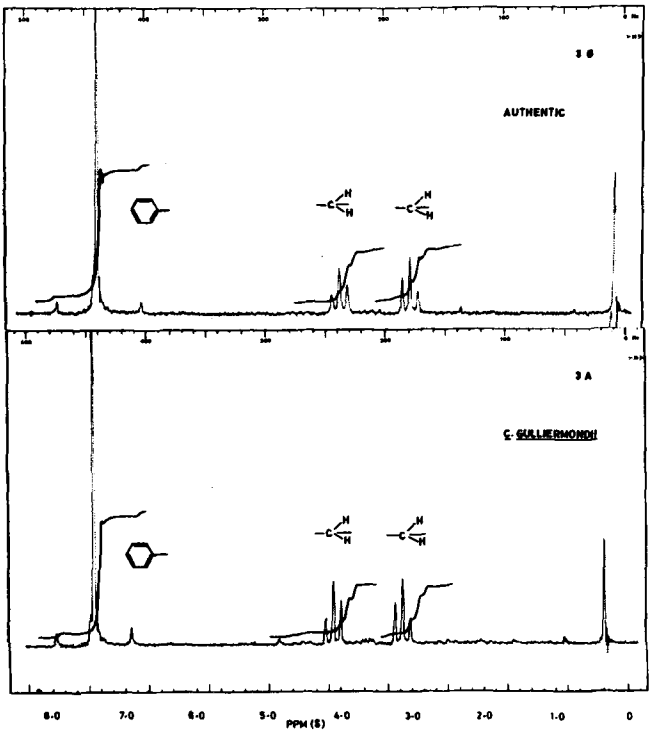

FIG. 3. NUCLEAR MAGNETIC RESONANCE SPECTRA OF ISOLATED (3A) AND AUTHENTIC (3B) β-PEA AFTER D_2O EXCHANGE

crystalline material in high yields was isolated from C. tropicalis and was characterised as 2-phenyllactic acid (2-PLA) by its physicochemical, IR (Fig. 4) and NMR (Fig. 5) spectral properties (Table 1) and it was confirmed with authentic sample of 2-PLA (Sigma).

Employing procedure 2, the production of 2-PEA and 2-PLA in Candida species has been quantitated (Table 3). Candida species differed markedly in their capacity to produce these compounds. C. guilliermondii had produced high amounts of 2-PEA and low amounts of 2-PLA. The same is true with C. krusei while the reverse is the case with C. tropicalis. The conversion of L-phenylalanine into 2-PEA and 2-PLA (together) by various species ranged from 34 to 67%.

Experiments using [^{14}C](U)-phenylalanine were also carried out. Labelled 2-PEA and 2-PLA were isolated from culture filtrates of C. intermedia (Table 4). The total yield of both labelled 2-PEA and 2-PLA was about 52%. This agreed

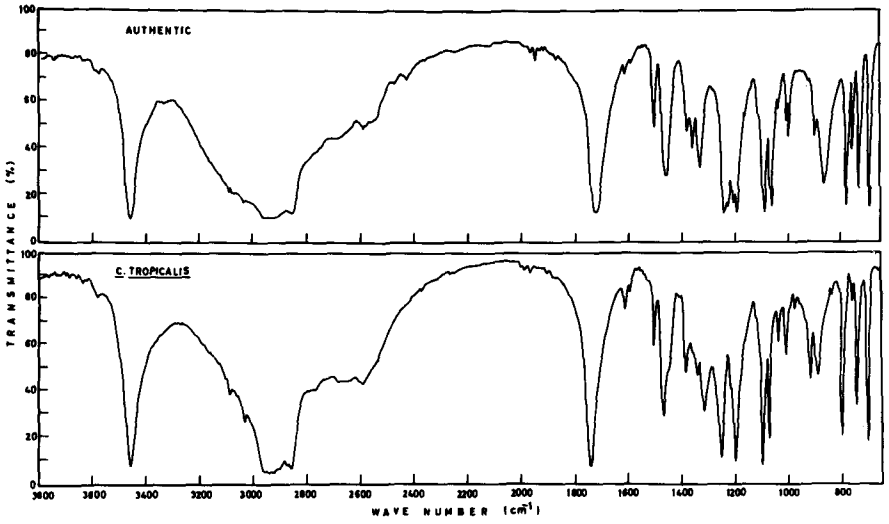

FIG. 4. INFRARED SPECTRA OF ISOLATED AND AUTHENTIC β-PHENYLLACTIC ACID

Table 4. Isolation of [^{14}C]-labelled 2-PEA and 2-PLA from culture filtrates of C. intermedia

Phenylalanine left in the culture filtrate after extraction		1.2 x 10^6 cpm
Phenylalanine utilized		9.8 x 10^6 cpm
Counts in 2-PEA (190 mg)	1.6 x 10^6 cpm	5.1 x 10^6 cpm
Counts in 2-PLA (410 mg)	3.5 x 10^6 cpm	
% Incorporation into 2-PEA	16.3	52.3
% Incorporation into 2-PLA	36.0	

Cells were grown in medium 3 supplemented with 8 uCi of [^{14}C]-(U)-L-phenylalanine (Sp. activity, 153 mCi/mmole, obtained from BARC, Bombay, India)

well with the quantitative data obtained in experiment using cold L-phenylalanine (Table 3).

The data presented reveal that all the five species of Candida are 2-PEA producers and they possess the ability to convert a normal metabolite like L-phenylalanine into 2-PLA and 2-PEA. 2-PEA is possibly biosynthesised by

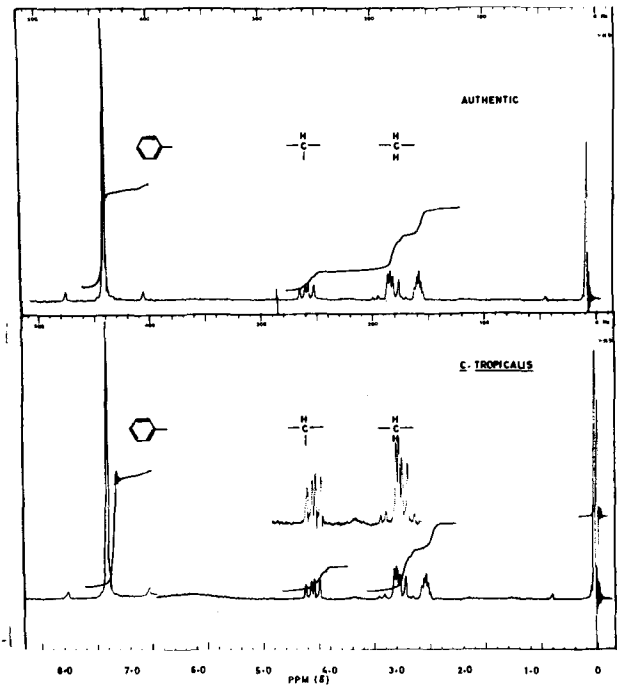

FIG. 5. NUCLEAR MAGNETIC RESONANCE SPECTRA OF ISOLATED AND AUTHENTIC β-PHENYLLACTIC ACID

the following pathway: L-Phenylalanine — Phenylpyruvic acid — Phenyllactic acid — Phenethyl alcohol.

The presence of amino acid pools in C. utilis (5) and their excretion into the medium during fermentation (6) have been reported. The conversion of excess phenylalanine in the mobile intracellular pool into 2-PEA and its excretion is a possibility.

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