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# Beta-Indoleethanol and Beta-Indolelactic Acid Production by *Candida* Species: Their Antibacterial and Autoantibiotic Action

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*Candida* spp. grown in synthetic medium supplemented with L-tryptophan as sole nitrogen source produced β-indoleethanol (β-IEA) and β-indolelactic acid (β-ILA). These compounds isolated from the culture filtrates were characterized by ultraviolet, infrared, and nuclear magnetic resonance spectral studies. Using DL-[$^3$H]trypotophan in the medium, labeled β-IEA and β-ILA were isolated. Further, β-IEA was produced as a result incubating log-phase cells of *C. albicans* with β-ILA. Both β-IEA and β-ILA inhibited the growth of gram-positive and -negative bacteria. Autoantibiotic action of these compounds on *Candida* spp. and the reversal of this inhibition were studied.

*Candida* spp. produced β-phenethylalcohol (5, 7) and its corresponding hydroxy acid, β-phenyllactic acid (7). The autoantibiotic action of β-phenethylalcohol has also been reported (5, 6). Production of β-indoleethanol (tryptophol; β-IEA) and β-indolelactic acid (β-ILA) by *Agrobacterium tumefaciens* (3), *Diplodia natalensis* (1), *Acetobacter xylinum* (4), *Aspergillus niger* (2), and *Rhizobium* spp. (8, 9) has been described. In this paper we report the production and quantitation of β-IEA and β-ILA by both pathogenic and nonpathogenic species of *Candida* grown in the presence of L-tryptophan as the sole source of nitrogen. These compounds have also been examined for their antibacterial and autoantibiotic action.

## MATERIALS AND METHODS

**Organisms.** *Candida albicans* Z248, *C. guilliermondii* Z55, *C. krusei* Z70, and *C. tropicalis* Z56 were obtained from the London School of Hygiene and Tropical Medicine, London, and *C. intermedia* was obtained from the V. P. Chest Institute, New Delhi, India. Stock cultures were maintained on Sabouraud glucose agar. *Escherichia coli, Proteus vulgaris, Paracolobactrum aerogenoides, Aerobacter aerogenes, Bacillus subtilis, B. megaterium,* and *B. cereus* were from the culture collection of this laboratory and were maintained on nutrient agar slants.

**Isolation of compounds.** The growth of organisms and the procedure for the isolation of compounds and their purification were similar to those described earlier (7).

**Spectra.** The infrared spectra were taken in Nujol in a Carl Zeiss model W10 spectrophotometer and the nuclear magnetic resonance spectra of the compounds (10 to 15%) were taken in a Varian model T60MHz. The ultraviolet spectra of the compounds in aqueous solution (10 mg/100 ml) were obtained in a Unicam SP 700A recording spectrophotometer.

**Reversibility of growth inhibition caused by β-IEA and β-ILA.** Side-arm Erlenmeyer flasks (250 ml), each containing 100 ml of medium (described in Table 5), were inoculated with 1 ml of 24-h broth culture in the same medium. After 8 h of incubation on a rotary shaker (200 rpm) at 30 C, the compounds were added aseptically to two flasks and incubated further for another 8 h. The cells thus exposed to the compounds were centrifuged and washed four times with sterile medium, suspended in the same volume of fresh medium, and incubated. The culture filtrate from the flask to which β-ILA was added was extracted using the isolation procedure previously described, and the product was identified by chromatography.

## RESULTS AND DISCUSSION

The physicochemical properties of β-IEA and β-ILA are shown in Table 1.

The infrared spectrum of β-IEA is shown in Fig. 1. The assignments of the peaks are as follows: (i) a sharp peak at 3,400 $cm^{-1}$ corresponds to  of indole nucleus; (ii) a broad peak at 3,200 $cm^{-1}$ corresponds to $C_1$—OH, which is mostly hydrogen bonded; (iii) peaks at 1,430, 1,470, and 1,620 $cm^{-1}$ show that it is an aromatic compound; (iv) peaks at 1,050, 1,100, and 1,190 $cm^{-1}$ further support the presence of the —OH group.

The infrared spectrum of β-ILA is shown in Fig. 2. The assignments of the various peaks are as follows: (i) a sharp peak at 3,400 $cm^{-1}$ corresponds to  ; (ii) a peak at 3,500 $cm^{-1}$

TABLE 1. *Physicochemical properties of β-IEA and β-ILA isolated from a culture filtrate of Candida species*

| Property | β-IEA | β-ILA |
|---|---|---|
| Liquid/solid | Crystalline leaflet | Microcrystalline powder |
| Odor | Fecal odor | Fecal ordor |
| Melting point (C) | 58–59 | 100 ± 1 |
| Solubility in: | | |
| Acetone | Highly soluble | Highly soluble |
| Chloroform | Soluble | Sparingly soluble |
| Ether | Soluble | Soluble |
| Water | Sparingly soluble | Soluble |
| Ultraviolet spectrum ($\lambda_{max}$) (nm) | 278 | 278 |
| Optical rotation | | +6° at 30 C (ca. 2.5 in ethanol) |
| $R_f$ value | 0.41[a] | 0.40[b] |

[a] Thin-layer chromatography solvent system: petroleum ether (40 to 60 C)-ether (15:25, vol/vol); identification by iodination.

[b] Paper chromatography solvent system: benzene-acetic acid-water (15:6:3, vol/vol/vol); identification by spraying with 0.1% $KMnO_4$.

corresponds to $C_2$—OH; (iii) sharp peaks at 1,700 and 1,720 $cm^{-1}$ correspond to $\diagdown C{=}O$; (iv) peaks at 1,400, 1,480, and 1,640 $cm^{-1}$ show the aromatic nature of the compound; (v) peaks at 1,080, 1,100, and 1,200 $cm^{-1}$ further support the presence of the —OH group.

The nuclear magnetic resonance spectra of β-IEA are shown in Fig. 3 and 4. A multiplet centered at 7.4δ corresponds to the indole nucleus. The triplets at 3.9δ (J 6Hz) and 2.9δ (J 6Hz) correspond to two $-CH_2-$ groups coupled to each other. A sharp singlet at 2δ and a broad singlet at 8.2δ correspond to $C_1$—OH and $\diagdown N \diagup H$ of indole, respectively, which disappear on $D_2O$ exchange (Fig. 3). The shift of the singlet of $C_1$—OH in Fig. 3A is due to the concentration difference. A singlet at 4.7δ in Fig. 4B is due to HDO. The absence of a methyl group singlet between 1δ and 2δ and the presence of two triplets indicate that the indole nucleus is separated from $C_1$—OH by an ethylene linkage.

The nuclear magnetic resonance spectrum of β-ILA shows a multiplet signal similar to β-IEA centered at 7.4δ, corresponding to an indole nucleus (Fig. 5). The two quartet signals at 4.2δ (J 5Hz) and 3.2δ (J 5Hz) indicate that protons of $-\overset{H}{\underset{|}{C}}-$ and $-\overset{H}{\underset{|}{C}}-$ are coupled to each other. The signal at 2.5δ is due to $Me_2SO$.

The physicochemical and spectral properties of the isolated compounds are identical to authentic β-IEA and β-ILA (Sigma Chemical Co., St. Louis, Mo.). All *Candida* spp. tested produced both β-IEA and β-ILA (Table 2). *C. guilliermondii* and *C. krusei* produced more β-IEA and less β-ILA. The reverse is the case with the other three species. This pattern of producing either alcohol or hydroxy acid in excess is similar to the production of β-phenethylalcohol and its corresponding hydroxy acid, β-phenyllactic acid (7). The total yield of β-IEA and β-ILA ranged from 14 to 24% among *Candida* spp., as

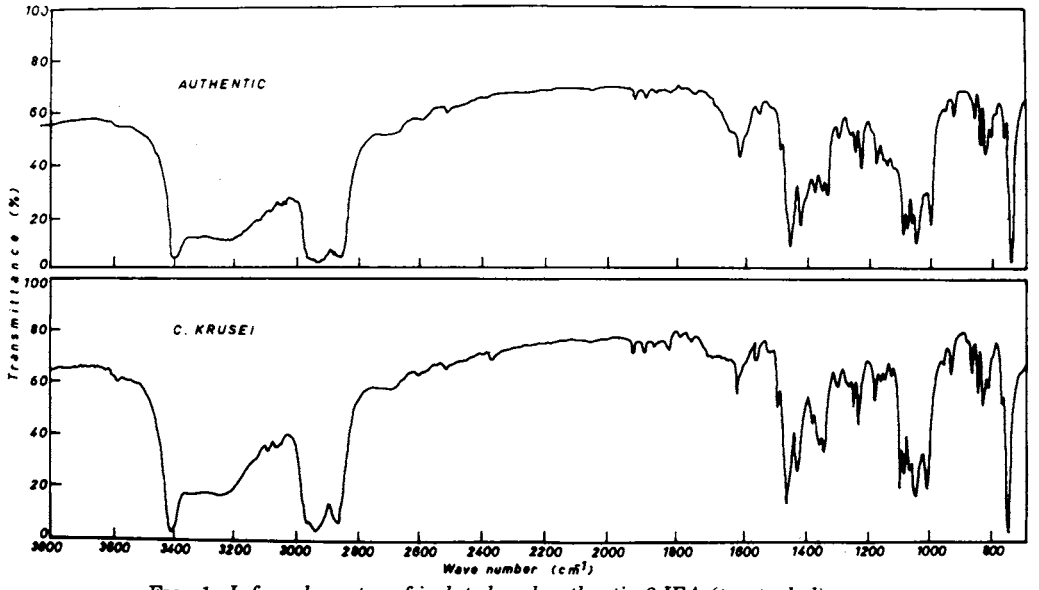


FIG. 1. *Infrared spectra of isolated and authentic β-IEA (tryptophol).*

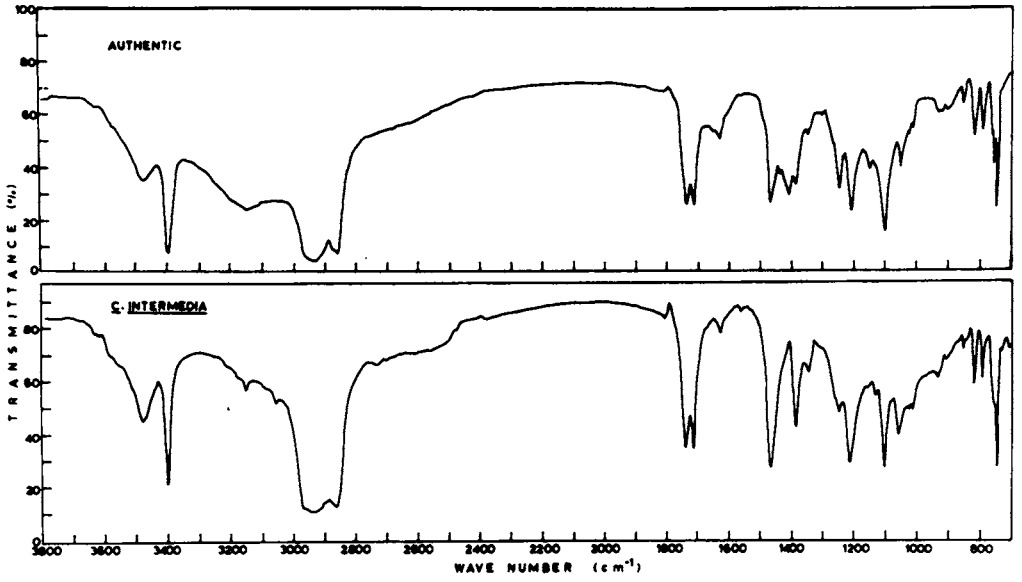


FIG. 2. *Infrared spectra of isolated and authentic β-ILA.*

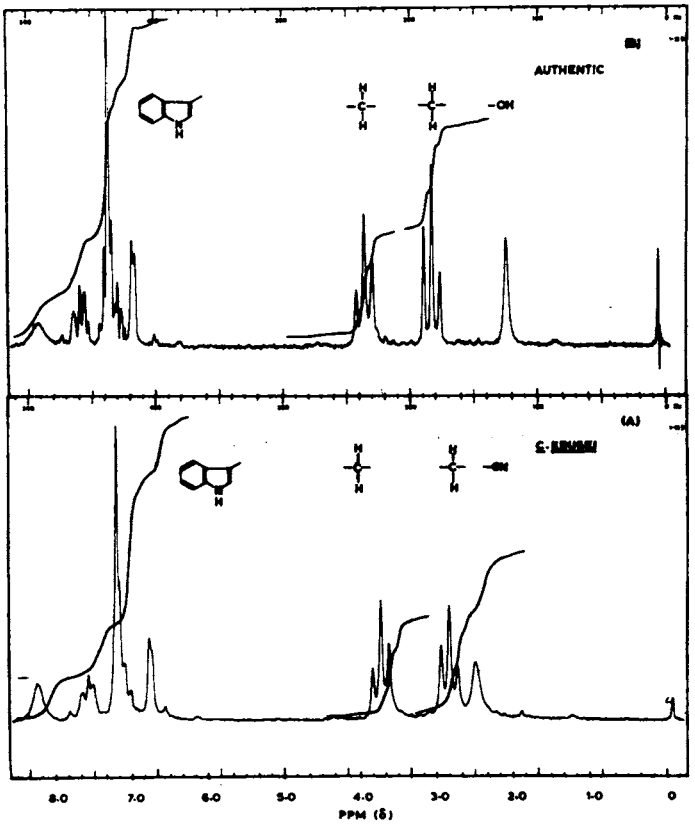


FIG. 3. *Nuclear magnetic resonance spectra of (A) isolated and (B) authentic β-IEA.*

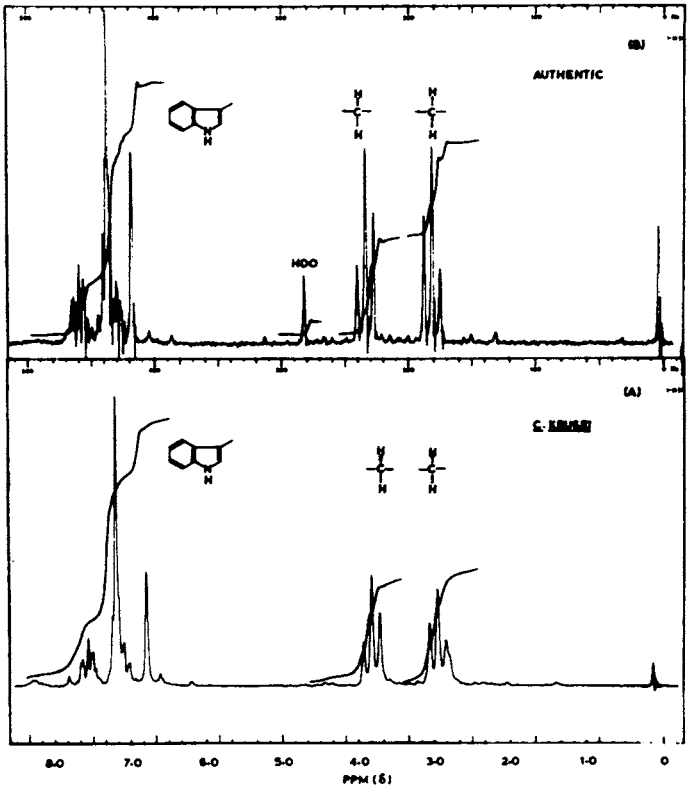


FIG. 4. *Nuclear magnetic resonance spectra of (A) isolated and (B) authentic β-IEA after $D_2O$ exchange.*

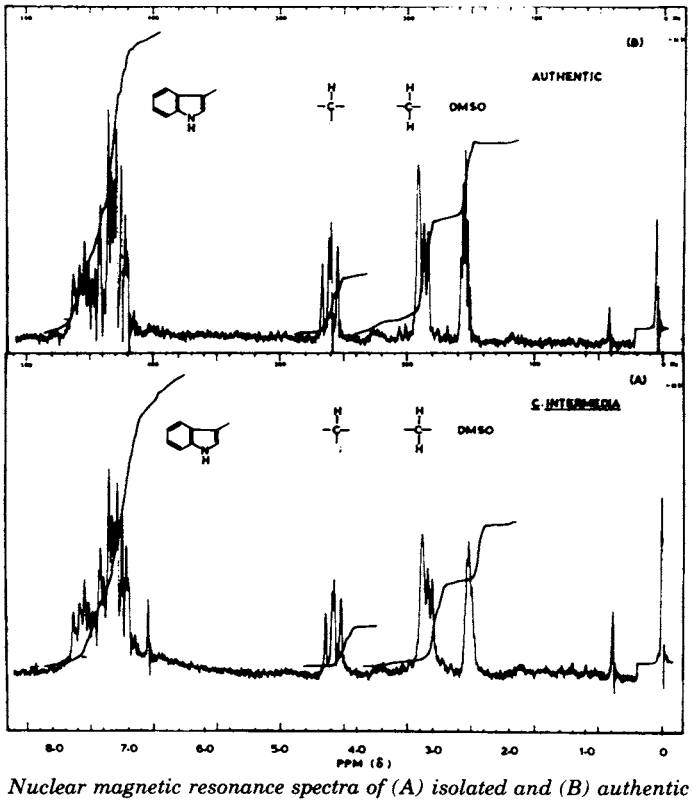


FIG. 5. *Nuclear magnetic resonance spectra of (A) isolated and (B) authentic β-ILA.*

compared to 34 to 67% of β-phenethylalcohol and β-phenyllactic acid together (7).

Labeled β-IEA and β-ILA have been isolated by using DL-[$^3$H]tryptophan (5T) (Table 3). Approximately 17% of the tryptophan taken up by the cells is found converted to β-IEA (specific activity, 0.21 μCi/mmol) and β-ILA (specific activity, 0.165 μCi/mmol). The specific activities of both labeled compounds remained the same after repeated purification.

β-IEA inhibited the growth of both gram-positive and -negative organisms in the concentration range of 6 to 12 mM (Table 4). However, gram-negative organisms in general are more susceptible than gram-positive organisms. Like β-IEA, β-ILA also inhibited the growth of *E. coli* at 6 mM and *B. cereus* at 15 mM.

The autoantibiotic property of β-IEA on *Candida* spp. is revealed by the data in Table 5. All species examined were totally inhibited at concentrations of 6 to 12 mM. However, *C. tropicalis*, which produces the lowest level of β-IEA, is highly susceptible to β-IEA as compared with other species. This was also observed with β-phenethylalcohol (6).

Some data illustrating the nature of inhibition exerted by β-IEA and β-ILA on the growth of *C. albicans* are shown in Fig. 6. When β-IEA was added to log-phase cells, no further growth took place. The inhibition of growth was seen as long as the cells were in contact with β-IEA. After washing the cells free of β-IEA, the cells exhibited a growth pattern similar to the control. This clearly shows the reversible nature of the inhibition by β-IEA. A similar effect is exhibited by β-ILA as well. The inhibition

TABLE 2. *Production of β-IEA and β-ILA by Candida species*

| Yeasts | Yield (mg/liter of medium containing 1 g of L-trypotophan)[a] | | |
|---|---|---|---|
| | β-IEA | β-ILA | % Conversion of L-tryptophan added |
| *C. guilliermondii* | 93 | 62 | 15.5 |
| *C. krusei* | 132 | 50 | 18.2 |
| *C. intermedia* | 28 | 116 | 14.4 |
| *C. albicans* | 25 | 122 | 14.7 |
| *C. tropicalis* | 15 | 193 | 24.3 |

[a] The medium used consisted of: glucose, 20 g; $KH_2PO_4$, 3.5 g; $MgSO_4 \cdot 7H_2O$, 2.5 g; $CaCl_2 \cdot 2H_2O$, 2.5 g; L-tryptophan, 1 g; and biotin, 30 μg; distilled water to 1 liter. Cells were grown at 31 C for 2 days on a rotary shaker.

TABLE 3. *Isolation of [β-$^3$H]IEA and [β-$^3$H]ILA from culture filtrates of C. intermedia*[a]

| Tryptophan | Counts/min |
|---|---|
| Amt added | $3 \times 10^6$ |
| Remaining after the extraction of culture filtrate | $1.27 \times 10^6$ |
| Taken up by the cells | $1.73 \times 10^6$ |
| Converted to: | |
| β-IEA (28.2 mg) $0.80 \times 10^5$ | $2.96 \times 10^5$ |
| β-ILA (122.8 mg) $2.16 \times 10^5$ | |

[a] Cells were grown in the medium described in Table 2, which was supplemented with 10 μCi of DL-[$^3$H]tryptophan (5T) (1,600 mCi/mmol) obtained from BARC, Bombay, India. The compounds were isolated as described in the text, and radioactivity was measured in a Beckman LS-100 liquid scintillation counter. About 17% of the tryptophan taken up by the cells was found to be converted to β-IEA (4.6%) and β-ILA (12.4%).

TABLE 4. *Influence of β-IEA on the growth of gram-positive and gram-negative bacteria*

| Organism | % Inhibition | | | | |
|---|---|---|---|---|---|
| | 3 mM[a] | 6 mM | 9 mM | 12 mM | 15 mM |
| *Escherichia coli* | 12 | 60 | 90 | 100 | 100 |
| *Paracolobactrum aerogenoides* | 60 | 100 | 100 | 100 | 100 |
| *Proteus vulgaris* | 15 | 90 | 100 | 100 | 100 |
| *Aerobacter aerogenes* | 40 | 90 | 100 | 100 | 100 |
| *Bacillus subtilis*[b] | 25 | 50 | 75 | 100 | 100 |
| *Bacillus cereus*[b] | 25 | 25 | 50 | 75 | 100 |
| *Bacillus megaterium*[b] | 25 | 25 | 50 | 75 | 100 |

[a] β-IEA concentration.

[b] Since *Bacillus* spp. form pellicles, the growth inhibition is graded visually. Nutrient broth (5 ml) was inoculated with 0.1 ml of an 18-h culture and incubated at 37 C for 24 h. Growth was measured in a Klett-Summerson colorimeter at 540 nm.

TABLE 5. *Autoantibiotic effect of β-IEA on growth of Candida species*

| Species | % Inhibition | | | |
|---|---|---|---|---|
| | 3 mM[a] | 6 mM | 12 mM | 18 mM |
| *C. albicans* | 60 | 86 | 100 | 100 |
| *C. tropicalis* | 91 | 100 | 100 | 100 |
| *C. krusei* | 57 | 73 | 100 | 100 |
| *C. guilliermondii* | 68 | 88 | 100 | 100 |
| *C. intermedia* | 60 | 85 | 100 | 100 |

[a] β-IEA concentration.

[b] The medium used consisted of glucose, 20 g; $KH_2PO_4$, 3.5 g; $(NH_4)_2SO_4$, 2.5 g; $MgSO_4 \cdot 7H_2O$, 2.5 g; $CaCl_2 \cdot 2H_2O$, 2.5 g; and biotin, 30 μg; in 1 liter of distilled water. A 5-ml amount of medium was inoculated with 0.1 ml of a 24-h culture and incubated at 30 C for 48 h. Growth was measured in a Klett-Summerson colorimeter at 540 nm.

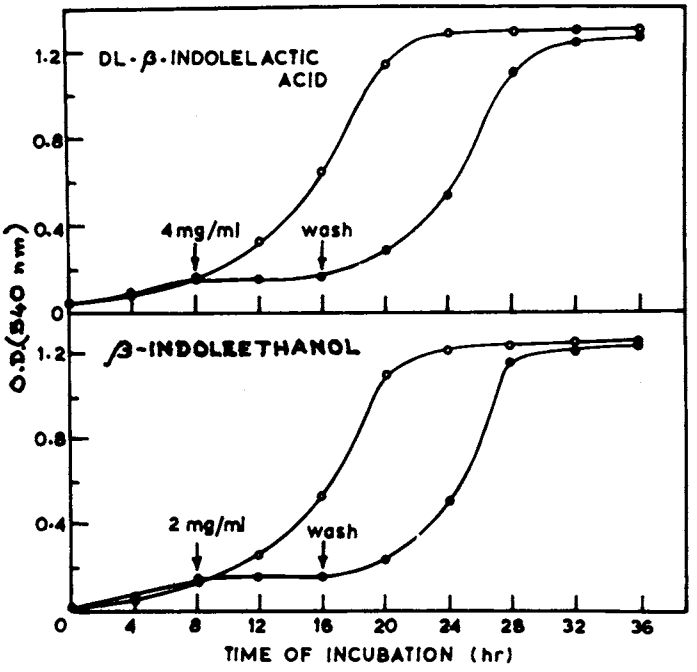


FIG. 6. *Effect of β-IEA and β-ILA on growth of C. albicans.*

caused by β-ILA may be due to its own effect or due to its conversion product. The conversion of β-ILA to β-IEA is revealed by isolating β-IEA from a culture filtrate of *C. albicans* incubated with β-ILA for 8 h. However, the antimicrobial effect of β-ILA by itself is evident from studies on gram-positive and -negative organisms. Thus, the autoantibiotic effect of β-ILA might be due to its conversion product but may also be considered as its own effect. The data presented reveal the ability of *Candida* spp. to convert a normal metabolite such as L-tryptophan to β-IEA and β-ILA. They are possibly biosynthesized via the following pathway:

L-Tryptophan →
indole pyruvic acid ⇄ indolelactic acid
↓
indole acetaldehyde → indoleethanol

The differences in the quantitative production of various alcohols from the corresponding amino acids may be due to the substrate specificity of the enzyme and/or to the autoinhibition exerted by the end products, namely, the alcohols concerned.

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