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MICROBIAL TRANSFORMATION OF THE ANALGESIC PHENACETIN AND RELATED COMPOUNDS

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SUMMARY:

In the present work, the microbial transformation of various analgesics vere studied. It was found that Phenacetin was metabolized to Acetanilide by aspergillus niger and to p-Ethoxy aniline by Aspergillus flavus. Other compounds namely, Acetanilide, Acetaminophen and Acetozolamide were not affected under same conditions.

FENASETİN VE DİĞER BAZI ANALJEZİK BİLEŞİKLERİN MİKROBİYAL TRANSFORMASYONU

ÖZET:

Bu çalışmada, çeşitli analjezik bileşiklerin mikrobiyal transformasyonu üzerine çalışmıştır. Fenasetin'in Aspergillus niger tarafından Asetanilid'e ve Aspergillus flavus tarafından p- Etoksi Anilin'e metabolize olduğu bulunmuştur. Asetanilid, Asetaminofen ve Asetozolamid aynı koşullar altında bu mikroorganizmalar tarafından etkilenmemektedir.

INTRODUCTION

The chemical activites of fungal spores were first discovered in 1958 by Getrig and Knight. It was shown that selected microorganisms can mimic mammalian metabolism and are easier to use (1,2). A number of investigation (3-10) estabilished that non- germinating spores of fungi and actinomycetes can accomplish a wide range of conversion of steroid molecules. Microbial conversion of phenylbutazone studied by winternitz (11). A few microorganisms have also ability to convert tertiary amines to N- oxides and some primary amines to nitro compounds via hydroxylamine intermediate (12). The formation N-oxygenated metabolites when Cunninghamella echinulata cultures were incubated with (±) - N - (n-propyl) - amphetamine was described by Coults et al. (13). Four oxygenated products were isolated and shown to be identical with mammalian liver metabolites. The soil microorganism Mycobacterium smegmatis has the ability to N- oxidize amino acids and incorporate the resulting N- hydroxyamino acid unit into larger molecules (14,15,16). Amphetamine and five alkylated homologues were also readily metabolized by Mycobacterium smegmatis (13).

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Anisoquinoline alkaloid was O- alkylated by C. blakesleena, while a natural antitumor agent transformed by C.echinulata (17,18). p- Aminobenzene was degraded by B. subtilis to Aniline (19).

The aim of the present work was to use some representative organism to study the conversion of analgesic Phenacetin and related compounds.

MATERIALS AND METHODS

a- Chemicals

All chemicals are obtained from Aldrich Chem.Comp., Wisconsin, USA.

b. Instrumentation

NMR spectra's were recorded on Varian XL- 200 (200 MHz). Mass spectra's were taken by Shimadzu LKB-9000 instrument.

c. Microorganisms

All test organisms used in this work are listed in TABLE I. Bacrerial strains were maintained on Brain Heart infusion Agar (OXOID) while fungal strains were maintained on Sabouraud Dextrose Agar (OXOID) slants were stored in a refrigerator at 4°C prior to use. All organisms mentioned in the present work were laboratory strains of the culture collection in the College of Pharmacy, King Saud University. Aspergillus flavus collected in our Microbiology laboratory was identified by common wealth Mycological Institute, Ferry Lane, Kew Surrey, England. However, Aspergillus niger (2022) was provided by Mycological Reference Laboratories, London, England.

d. Fermentation Medium and Inoculum

Cultures were grown in Nutrient Broth (OXOID) supplemented with 1% glucose. The medium sterilised by autoclaving 121°C for 15 minutes before use.

The surface growth from slant of test organism was suspended in 5 ml of sterile supplemented nutrient broth. one ml of this suspension was used to inoculate 50 ml of supplemented nutrient broth held in 250 ml cotton - plugged Erlenmayer flaks (STAGE-1)- The stage 1 flaks were incubated at 27°C in shaker - bath operating 140 osci/min. for 24-72 hrs for fungi. The stage 1 flaks were also carried out under the same condition, however incubated at 37°C for bacteria. From the actively growing stage 1 culture, one ml was transferred to 50 ml of fresh medium held in a 250 ml Erlenmayer flask (STAGE-2). After 24 hrs of incubation on shaker- bath, 50 mg of substrate dissolved in 0.2 ml DMSO was added to stoge 2 flask. The substrate - containing flaks were incubated for an adiditional 24-72 hrs. The culture was then extracted with an equal volume of chloroform for three times. The chloroform solution was dried over anhydrous sodium sulfate. The chloroform was evaporated to dryness under vacuum. then samples were analysed by MS and NMR spectrometry.

Controls were used in this work to ensure the metabolites were not artifacts.

e. Control Studies

Culture controls consisted of fermentation blanks in which each organism was grown under identical conditions, as biotransformation cultures, but without sub

Table I: Microorganisms Used for the Transformation of the Analgesic Phenacetin and Related Compounds

No	Test Organisms	Source
1	Staphylococcus aureus	Laboratory strain
2	Escherichia coli	Laboratory strain
3	Proteus vulgaris	Laboratory strain
4	Pseudomonas aeruginosa	Laboratory strain
5	Salmonella Sp.	Laboratory strain
6	Candida albicans	Laboratory strain
7	Aspergillus flavus	Isolated strain confirmed by Commonwealth Mycological Institue, England.
8	Aspergillus niger (2022)	Mycological Reference Laboratories, London England

strate and substrate without microorganism. The cultures were extracted and analyzed as described for the biotransformation cultures (TABLE II).

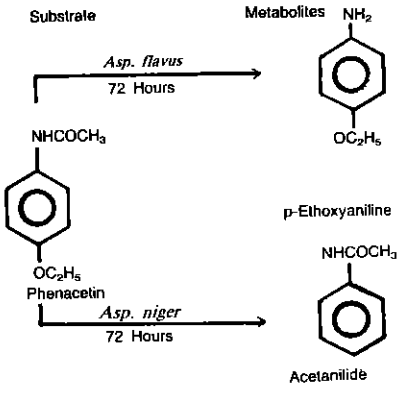
Table II: Activities of Microorganisms on the Substrates Tested

Test Organisms	Acetaminophen	Acetanilide	Acetazolamide	Phenacetin	Control
S.aureus	-	-	-	-	-
E.coli	-	-	-	-	-
P.vulgaris	-	-	-	-	-
Ps.aeruginosa	-	-	-	-	-
Salmonella sp.	-	-	-	-	-
C.albicans	-	-	-	-	-
Asp.flavus	-	-	-	p-ethoxy aniline	-
Asp.niger	-	-	-	Acetanilide	-

RESULTS AND DISCUSSION

As it is evident from Table II, Acetaminophen, Acetanilide and Acetazolamide remained uneffected by the test organisms. However, it has been reported previously that Acetanilide was converted to 2- hydroxyacetanilide by Aspergillus ochraceous and to Aniline by other different microoganisms (1). The pattern of hydroxylation of Acetanilide by Streptemyces species was found to be in 2- and 4- positions. It is also reported that Basidiomycete hydroxylates Acetanilide mainly in the 2- position.

Surprisingly enough, Phenacetin has undergone biotransformation and was metabolized to Acetanilide by Aspergillis niger and to p- Ethoxy Aniline by Aspergillus flavus (SCHEME 1).



SCHEME I: Biotransformation of Phenacetin by Asp. flavus and Asp. niger

The presence of Phenacetin metabolites by A. niger and A. flavus were confirmed by MS/NMR analysis.

1. METABOLITE OF A. niger

The mass spectrum showed an M^+ at m/e 135 and a base peak (100%) at m/e 93. The mass spectrum was identical with that of authentic Acetanilide. The proton - NMR spectrum displayed the following signals; a singlet at 2.1 ppm (-$NHCOCH_3$) and multiplet at 7.21 ppm (Five aromatic protons). These are in agreement with that found for Acetanilide.

2. METABOLITE OF A. flavus

The mass spectrum showed an M^+ at 137 and a base peak (100%) at m/e 108. The mass spectrum was identical with that of authentic p- Ethoxy aniline. The proton - NMR srectrum displays the following signals; a triplet centered at 1.3 ppm (-CH_2Me) a singlet at 3.4 ppm (-NH_2), quarted centered at 6.56 ppm (four aromatic pro-

tons). These are in agreement with that found for p- Ethoxyaniline by A. flavus was an expected metabolic pathway as it has been reported earlier that Acetanilide undergoes deacetylation to aniline by Penicillium chrysogenum (1). However, the biotransformation of Phenacetin to Acetanilide by A. niger was unusual reaction since no previous reports have indicated deetherification as a metabolic pathway by microoganisms. This finding should be substantiated by more studies using other similar substrates.

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