DETERMINATION OF THE ANTIMICROBIAL PROPERTIES OF OLIGO-2-HYDROXY-1-NAPHTHALDEHYDE

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SUMMARY

Oligo-2-hydroxy-1-naphthaldehyde (OHNA) was synthesized by oxidative polycondensation using H_2O_2 (35%, aqueous solution), air O_2 and NaOCl (34%, aqueous solution) by Kaya and Şenol and the products were characterized by spectral techniques /3/. Antimicrobial activities of the first and second fractions of OHNA were tested against *Corynobacterium xerosis* CCM 2824, *Proteus vulgaris* ATCC 6897, *Staphylococcus epidermidis* NRRL B-4877, *S. aureus* ATCC 6538, *Enterobacter aerogenes* ATCC 13048, *Salmonella thyphi-murium* CCM 5445, *Pseudomonas aeroginosa* ATCC 27853, *Escherichia coli* ATCC 11230, *E. coli* ATCC 23998, *Bacillus cereus* ATCC 7064, *B. cereus* ATCC 99, *B. subtilis* ATCC 6633, *Yersinia* spp., *Neisseria canis*, *Rhodotorula rubra*, *Kluyveromyces fragilis* NRRL 2415, *Saccharomyces cerevisiae* ATCC 9763, *S. ovarum*, *Debaryomyces hensenii*, *Hansenula anamola*, *Candida albicans*, *C. utilis*, *Aspergillus niger*, *A. fumigates*, *A. versicolor*, *A. flavus*, *A. parasiticus*, *Penicillium granulatum*, *P. chrysogenum*, and *P. herque*. OHNA demonstrated antimicrobial activity against various bacteria and yeast, but did not affect filamentous fungi.

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KEY WORDS

oligo-2-hydroxy-1-naphthaldehyde, antimicrobial activities

INTRODUCTION

The growth of microorganisms affects more than our health. Processes used to control microorganisms are either physical or chemical, though a combination of both may be employed. Chemical methods use any one of a variety of antimicrobial chemicals. The method chosen depends on the circumstances and the required degree of control /1/. Antimicrobial agents may be a synthetic chemical or a natural product. Agents that do not kill but only inhibit growth are called static agents /2/. Antimicrobial agents can vary in their selective toxicity. Some act in a rather non-selective manner and have similar effects on all types of cells; others are far more selective and are more toxic to microorganisms than to animal tissue. Antimicrobial agents with selective toxicity are especially useful as chemotherapeutic agents in treating infectious disease-causing microorganisms *in vivo* without harming the host /2/.

Producers of a wide variety of goods recognize that, unless microbial growth is controlled, the quality of their products can be compromised. This ranges from undesirable changes in the safety, appearance, taste, or odor of food products, to the decay of untreated lumber /1/.

The aim of the present study was to determine the antimicrobial activity of the first fraction (insoluble in water) (OHNA-I) and the second fraction (soluble in water) (OHNA-II) of oligo-2-hydroxy-1-naphthaldehyde (OHNA). These solutions were tested for antimicrobial activity against various bacteria, yeast and filamentous fungi.

MATERIALS AND METHODS

The first and second fractions of OHNA were synthesized by oxidative polycondensation using H_2O_2 (35%, aqueous solution), air O_2 and NaOCl (34%, aqueous solution) by Kaya and Şenol, and its structures have been characterized /3/. The number average molecular weight (M_n), mass average molecular weight (M_w) and polydispersity

index (PDI) values of OHNA were found to be 500 $g.mol^{-1}$, 1880 $g.mol^{-1}$ and 3.75, respectively /3/.

Test microorganisms were obtained from the culture collection of Ege University Faculty of Science, Basic and Industrial Microbiology Department.

In vitro antimicrobial studies were carried out by the agar-disc diffusion method against the test bacteria and yeasts (see Table 1) and filamentous fungi (*Aspergillus niger*, *A. fumigates*, *A. versicolor*, *A. flavus*, *A. parasiticus*, *Penicillium granulatum*, *P. chrysogenum*, and *P. herque*). Because the agar-disc diffusion method is generally not suitable for filamentous fungi, this method was used after modification.

Antimicrobial activity studies against test bacteria and yeasts

Mueller Hinton agar (Oxoid) was used as the most suitable medium for antimicrobial activity studies. The sterilized medium at 45-50°C was poured into 90 mm diameter Petri dishes. Bacteria and yeast cultures were suspended in 7-8 ml brain heart infusion broth (Oxoid). The bacteria were incubated at 37 ± 0.1°C for 24 hours. Yeast cultures were incubated at 27 ± 0.1°C for 24 hours. Prepared bacterial suspensions were inoculated at 10 µl, and yeast cultures were inoculated as 10^2 cfu/ml into Muller Hilton agar. Microbial cultures were separated with L bags and all Petri dishes after inoculation were allowed to dry for 15-20 minutes at room temperature. The concentration of OHNA was 200 ppm in DMF solvent. These chemicals were impregnated into 6 mm diameter discs at 10 µl. All discs were dried at 50°C and placed into the Petri dishes containing the bacteria and yeast. Inhibition zone diameters were measured after 24-48 hours using the agar-disc diffusion method /4,5/. Experiments were repeated three times and results were expressed as mean values.

Antimicrobial activity studies against filamentous fungi

Because the agar-disc diffusion method is generally not suitable for filamentous fungi, this method was used after modification. Malt extract agar (OXOID) was used for antimicrobial activity studies against filamentous fungi. The solutions of OHNA were added to Petri dishes to 100 µl. The sterilized medium at 45-50°C was poured into 90 mm diameter Petri dishes. All Petri dishes were allowed to dry for 15-

20 minutes at room temperature. Spore suspensions of filamentous fungi were inoculated onto malt extract agar at 10^5 cfu/ml by plate dilution techniques using Thomas slides. The evaluation of filamentous fungi was carried out by means of reproduction on the medium and reduction of colony numbers at the end of a 7-day period /6,7/. Experiments were repeated three times.

RESULTS AND DISCUSSION

Fungicidal and bactericidal activities of OHNA against photogenic fungi and bacteria are recorded in Table 1. It has been suggested that OHNA with the O donor system might inhibit enzyme production. Enzymes which require free hydroxyl groups for their activity appear to the especially susceptible to deactivation by the -OH and -COOH groups of OHNA /3/. OHNA facilitates their diffusion through the lipid layer of spore membranes to the site of action ultimately killing them by combining with -OH groups of certain cell enzymes. Variation in the efficacy of different biradical agents against different organisms depends on the impermeability of the cell /4,5/. The hydrocarbon acts as a lipophilic group /5/ to drive the compound through the semipermeable membrane of the cell. Chelation reduces the polarity of the central ion mainly because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalization within the whole chelate ring. Chelation increases the lipophilic nature of the central atom, which favors its permeation through the lipid layer of the membrane. In this study, OHNA was demonstrated to have high biological activity. According to the antimicrobial activity studies, the first fraction and the second fraction of OHNA demonstrated high activity against *Corynobacterium xerosis* CCM 2824, *Proteus vulgaris* ATCC 6897, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeroginosa* ATCC 27853, *Escherichia coli* ATCC 11230, *E. coli* ATCC 23998 and *Neisseria canis,* and weak activity against *Staphylococcus epidermidis* NRRL B-4877, *S. aureus* ATCC 6538, *Bacillus cereus* ATCC 7064 and *B. cereus* ATCC 99. OHNA-I and OHNA-II demonstrated a good degree of anti-yeast activity against *Rhodotorula rubra*, *Kluyveromyces fragilis* NRRL 2415, *Saccharomyces cerevisiae* ATCC 9763, *S. ovarum* and *Debaryomyces hensenii,* but weak activity against *Hansenula anamola*

TABLE 1

Antimicrobial activity of oligo-2-hydroxy-1-naphthaldehyde

Test organism	Zones of inhibition* (mm)						
	1	2	3	4	5	6	7
Corynobacterium xerosis CCM 2824	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proteus vulgaris ATCC 6897	20.0	25.0	20.0	15.0	20.0	20.0	15.0
Staphylococcus epidermidis NRRL B-4877 10.0	6.0	6.0	6.0	6.0	6.0	6.0	
Staphylococcus aureus ATCC 6538 P	8.0	8.0	6.0	6.0	6.0	6.0	6.0
Enterobacter aerogenes ATCC 13048	10.0	11.0	10.0	17.0	13.0	14.0	13.0
Salmonella thyphimurium CCM 5445	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Pseudomonas aeroginosa ATCC 27853	10.0	10.0	10.0	12.0	14.0	10.0	12.0
Escherichia coli ATCC 11230	6.0	6.0	6.0	14.0	11.0	15.0	12.0
Escherichia coli ATCC 23998	6.0	6.0	6.0	13.0	14.0	14.0	14.0
Bacillus cereus ATCC 7064	8.0	8.0	9.0	8.0	7.0	9.0	10.0
Bacillus cereus ATCC 99	6.0	6.0	7.0	8.0	8.0	10.0	8.0
Bacillus subtilis ATCC 6633	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Yersininia spp.	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Neisseria canis	10.0	18.0	12.0	15.0	10.0	12.0	10.0
Rhodotorula rubra	15.0	13.0	15.0	17.0	10.0	18.0	12.0
Kluyveromyces fragilis NRRL 2415	10.0	12.0	15.0	6.0	6.0	14.0	13.0
Saccharomyces cerevisiae ATCC 9763	20.0	18.0	20.0	17.0	18.0	20.0	17.0
Saccharomyces ovarum	8.0	10.0	12.0	10.0	10.0	10.0	12.0
Debaryomyces hensenii	8.0	6.0	7.0	6.0	10.0	12.0	10.0
Hansenula anamola	6.0	6.0	6.0	6.0	10.0	6.0	6.0
Candida albicans	7.0	8.0	6.0	6.0	6.0	6.0	10.0
Candida utilis	6.0	6.0	6.0	6.0	6.0	6.0	6.0

* **2** and **5** are OHNA-I and OHNA-II (for air O_2 oxidant); **1** and **3** are OHNA-I and OHNA-II (for NaOCl oxidant); **4** (reaction conditions 75°C and 4 h), **7** (reaction conditions 90°C and 15 h) and **6** are OHNA-I, OHNA-II and OHNA-I (for H_2O_2 oxidant) /3/.

and *Candida albicans* (Table 1). OHNA did not show any activity against filamentous fungi (results not shown).

Microorganisms which produce visible growth at 0-7°C within 7-10 days, with an optimum temperature of 20-30°C, and which might not grow above 35°C, are called psychrotrophs. These microorganisms are most commonly associated with refrigerated foods, and cause food spoilage. They are widely distributed in nature. The major psychrotropic bacteria, found in dairy foods, meat, poultry and seafood, include species of *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas*, *Staphylococcus*, *Yersinia*, *Clostridium, Flavobacterium*, and *Micrococcus*. Psychrotrophic yeast genera include *Candida*, *Debaryomyces*, *Hansenula*, *Pichia*, *Saccharomyces*, *Rhodotorula*, *Torulopsis*, and *Trichosporan*; mold genera include *Aspergillus*, *Alternaria*, *Cladosporium*, *Mucor*, *Penicillium*, and *Rhizopus* /8/.

Generally the presence of saprophytic species, or 'antracoid bacilli', in cultures of clinical specimens is regarded as contamination and of no clinical significance. Rarely, however, *B. cereus*, *B. subtilis,* or some other species may act as an opportunist pathogen in a debilitated or injured person, causing, e.g., bacteriaemia, meningitis, endocarditis or pneumonia in cases of drug abuse, leukaemia, renal disease or immunosuppression; endophthalmitis after trauma or surgery of the eye; and meningitis after spinal anesthesia. Food poisoning with *B. cereus* is most often caused by contaminated rice and rice dishes /9/. Rope spoilage was found to occur at *B. subtilis* counts about 10^8 cfu/g, and infective doses for food poisoning have been reported /10,11/.

The intestinal commensal strains of *E. coli* commonly cause opportunist infections in other parts of the body where there is some abnormality or impairment of defense. They are the commonest cause of urinary tract infections (cystitis, pyelitis and pyelonephritis) and are commonly present in appendix abscesses, peritonitis, cholecystitis, septic wounds and bedsores. They may infect the lower respiratory passages or cause bacteriaemia and endotoxic shock, particularly in surgical or otherwise debilitated patients being treated with antibiotics to which they are resistant, and they occasionally cause meningitis in neonates /9/. There are pathogenic strains that cause distinct syndromes of diarrhea and that have been associated with food-borne illnesses /8/.

Proteus species are free-living saprophytes in soil, vegetation, water and sewage, and are found in the intestine in many healthy persons. They occur in infections of the urinary tract, wounds and other sites /9/. Since *Proteus* spp. have high proteolytic activity, they cause spoilage of meat and fish products /12/. It flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators, humidifiers and disinfectant solutions. Isolation of *P. aeroginosa* from healthy carriers or environmental sites is significant only when there is a risk of transfer to compromised patients, e.g. by nurses' hands or respirators. Normally, human faucal carriage of *P. aeroginosa* is low, around 3%; however, carriage increases with the length of stay in the hospital, reaching 30% after 3 weeks, and thus can present a distinct risk of endogenous infection. Infections due to *P. aeroginosa* are seldom encountered in healthy adults, but in the last two decades the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients with impaired immune defenses. Susceptibility to infection with *P. aeroginosa* may also be occupational, e.g. ear infections in divers, or recreational, e.g. whirlpool (Jacuzzi)-associated rash. *P. aeroginosa* has high intrinsic resistance to many antibiotics at levels attainable in body tissues. *Corynebacterium xerosis* is commonly present in man as a commensal of the mouth, throat, nose, skin and conjunctiva, but there have been very few reports of cases, even in compromised hosts, in which they may have had a pathogenic role. *Enterobacter aerogenes* is a saprophyte in water, soil and vegetation, but sometimes found also in human feces and infections /9/.

Human infection with *N. canis* was published by Hoke and Vedros in 1982. This isolate came from a cat bite wound on a child. No other clinical details were described. In 1989, *N. canis* was reported in a mixed culture that included *Pasturella multocida* and *Eiknella corrodens* from a cat bite wound on the arm of a previously healthy 36 year-old woman /13,14/.

The authors hope the present study makes a small contribution towards the search for new or stronger antimicrobial agents in the ongoing struggle against pathogenic microorganisms.

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