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RESEARCH PAPER

Microcalorimetric Studies of the Action on Four Organic Acids in *Radix Isatidis* on the Growth of Microorganisms

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Abstract: The actions of four organic acids in *Radix Isatidis*, a traditional Chinese medicinal (TCM) herb, on *Escherichia coli*, *Staphylococcus aureus*, and *Shigella dysenteriae* growth were investigated by microcalorimetry. The power-time curves of *Escherichia coli*, *Staphylococcus aureus*, and *Shigella dysenteriae* with and without organic acids were acquired. Meanwhile, the extent and duration of the inhibitory effects on the metabolism were evaluated by growth rate constants (k_1, k_2), maximum heat output (P_m), and peak time (t_p). The inhibitory activity varied with different drugs. The order of anti-microbial activity of the four organic acids on *Escherichia coli*, *Staphylococcus aureus,* and *Shigella dysenteriae* was: syringic acid > 2-amino-benzoic acid > salicylic acid > benzoic acid. Benzoic acid promoted the growth of *Staphylococcus aureus* and *Shigella dysenteriae*. This study provided a basis for further researches on *Radix Isatidis*.

Keywords: *Radix Isatidis*; microcalorimetry; organic acids; microorganism; anti-microbial activity

Introduction

Radix Isatidis (Banlangen in Chinese), the root of *Isatidis indigotica* Fort, is a traditional Chinese medicinal (TCM) herb, which is officially listed in the Chinese Pharmacopoeia[1]. It has heat clearing, detoxicating, cooling the blood, and anti-inflammatory activity[2]. The chemical constituents of *Radix Isatidis* with various pharmacologic actions are very complicated. The four organic acids (OAs): syringic acid, 2-amino-benzoic acid, salicylic acid, and benzoic acid were segregated and obtained from *Radix Isatidis*[3−5], and their structures were shown in Fig. 1. The four OAs had strong anti-endotoxic effects[6].

To investigate the anti-microbial effect, the four OAs in *Radix Isatidis* were tested against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Shigella dysenteriae* (*S. dysenteriae*) growth.

E. coli, *S. aureus*, and *S. dysenteriae* are perhaps the human pathogenic microorganisms that have been studied extensively. It is a good choice for studying the effects of four OAs in *Radix Isatidis* on their growth *in vitro*. This may help us to understand the general effects that these OAs may have on other microorganisms[7].

Microcalorimetry provides a general analytical tool for the characterization of the microbial growth process. It has been used extensively to investigate drug and the microbial cell interaction and has furnished considerable useful information[7−9]. One of the most prominent features of the microbial growth process is the production of heat. If the heat is monitored by a microcalorimeter, considerable useful information, both qualitative and quantitative, may be obtained. Each type of microbial has a unique power-time trace, as recorded by the microcalorimeter, under a defined set of growth conditions.

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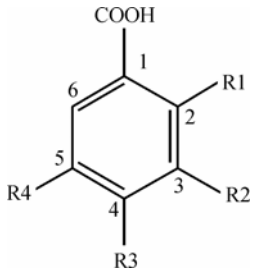

	R1	R2	R3	R4
Benzoic acid	H	H	H	H
Salicylic acid	OH	H	H	H
2-amino-benzoic acid	NH_2	H	H	H
Syringic acid	H	OCH_3	OH	OCH_3

Fig. 1 Chemical structures of investigated OAs from *Radix Isatidis*

Any substance that can modify the metabolic growth processes involved in cell will change the power-time curve obtained from the microcalorimeter. From the power-time curves, not only thermodynamic but also thermo-kinetic information can be obtained.

Microcalorimetry has been previously used to investigate the interaction between drugs and microbial cells[10]. This technique has also been used to investigate the inhibitory effects of selenomorpholine compounds on *E. coli*[11], *S. aureus*[12], and *S. dysenteriae*[13]. In this study, the power-time curves produced by *E. coli, S. aureus*, and *S. dysenteriae* under the action of four OAs at the same concentration (200 μg/mL) were obtained with a Thermal Activity Monitor (TAM) Air Isothermal Calorimeter. From these power-time curves, the growth rate constant k and the generation time t_G of micro-organism growth were calculated.

1 Materials and methods

1.1 Instruments

A TAM Air Isothermal Calorimeter (Thermometric AB, Sweden, Fig. 2) was used to determine the metabolism of *E. coli, S. aureus*, and *S. dysenteriae*. An eight-channel heat conduction calorimeter, for heat flow measurements in the milliwatt range under isothermal conditions, was held together in a single removable block. The system is very sensitive, the detection limit is 2 μW, and the baseline stability (over a period of 24 h) is 6 μW. All calorimetric channels were of twin type, consisting of a sample and a reference vessel. Each vessel was connected to the surrounding heat sink by a Peltier module, and when heat was produced or consumed owing to any process, the temperature of the sample vessel changed. The surrounding temperature was constant and thus a temperature gradient across the Peltier module was developed. This will generate

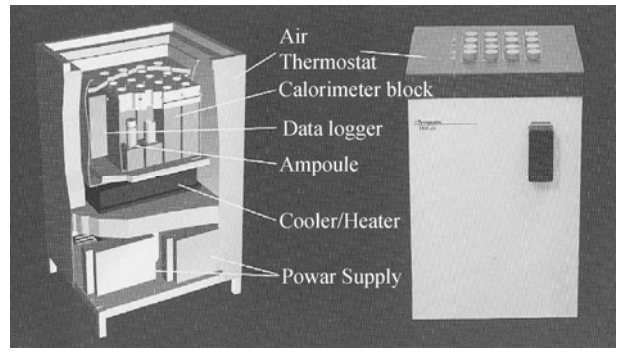

Fig. 2 TAM air isothermal calorimeter
(Thermometric AB, Sweden)

a measurable voltage, which is proportional to the heat flow across the Peltier module and to the rate of the processes taking place in the sample vessel. Such voltage signal was recorded continuously and in real-time through an eight-channel data logger. The software supplied to the TAM air was used to monitor and record the heat flow over the Peltier module. For details of the performance and structure of the instrument, see ref. [14].

1.2 Materials

Lactose Broth (LB) medium, consisting of 5 g NaCl, 10 g tryptone, 5 g yeast extract per liter, pH 7.0, was sterilized by autoclaving for 30 min at 121°C.

E. coli (*Escherichia coli* CMCCB 44103), *S. aureus* (*Staphylococcus aureus* AB 910393), and *S. dysenteriae* (*Shigella dysenteriae* AB 210562) were provided by the National Institute for the Control of Pharmaceutical and Biological Products. The three strains were grown in a peptone culture medium, which contained 10 g peptone, 6 g beef extract, and 5 g NaCl. The medium pH was adjusted to 7.0–7.2 with 1 mM NaOH before autoclaving; these suspensions were used as the inocula for the microbiological assay.

Radix Isatidis was the dried root of *Isatis indigotica* Fort, which was accredited by Xiaohe Xiao, one of the authors, of Institute of Chinese Materia Medica in 302 Hospital of PLA, Beijing 100039, China. The four OAs were extracted from *Radix Isatidis* with the purity > 98%[3] and their structures were shown in Fig. 1.

1.3 Batch experiments of bacteriostatic activity by microcalorimetry

The inocula were homogeneously distributed into 50 mL of LB medium by gentle shaking. Aliquots of 5 mL of the suspensions were added into 20 mL sterilized ampoules containing test samples and sealed tightly. The ampoules were placed in the calorimeter and signals obtained during growth were detected. The experiments were run at 37°C for bacteria and the thermogenic curves were recorded until the recorder returned to the baseline. Since the bacterial metabolic process was monitored under the isothermal and isochoric conditions, the nutrient and oxygen consumed by