# STUDIES ON NICOTINIC ACID AND SOME OF ITS DERIVATIVES ON GROWTH AND SPORULATION OF BACILLI

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## ABSTRACT

**Nicotinic acid derivatives were used to study their effects on growth and sporulation of bacilli. Nicotinic acid neither inhibits the growth nor the sporulation while methyl and ethyl nicotinates inhibited sporulation, utilization of organic acids and polybetahydroxybutyrate synthesis/ utilization without affecting growth. Nicotinamide treatment produces more than 95% heat labile spores with less dipicolinic acid levels and its effects could be reversed with the addition of exogenous dipicolinic acid. A model has been proposed depicting possible sites of action of nicotinic acid derivatives during growth and sporulation of bacilli.**

## INTRODUCTION

THE process of sporogenesis is an unique event in the life cycle of bacilli which has been studied in detail to understand the mechanism of differentiation. Dormant spore and its corresponding vegetative cell of a *bacillus* species is completely different in many respects, such as water content[1], low molecular weight proteins[2], dipicolinic acid (DPA), intracellular[3] pH and resistance to various physical and chemical factors[4]. Use of various chemicals has been reported to understand some of the spore-specific events during sporogenesis such as inhibitor of sporulation and/or heat resistance[5–8]. The present communication reports studies to elucidate the effects of some of the nicotinic acid derivatives on the growth and sporulation of bacilli.

## MATERIALS AND METHODS

The work was carried out with *Bacillus cereus* T and *Bacillus megatarium* QM B1551 obtained from Prof. P. Setlow, University of Connecticut, Health Center, Farmington, Conn., USA. The organisms were grown in a liquid medium containing (g/l of distilled water):- D-glucose, 0.1; yeast extract, 0.2; $K_2HPO_4$, 0.05; $CaCl_2$, 0.01 and a mixture of mineral solution whose composition was described elsewhere[9]. Solutions of D-glucose, $K_2HPO_4$, $CaCl_2$ and yeast extract were prepared separately, autoclaved and added before inoculation. The pH of the medium was adjusted to $7.0 \pm 0.1$ before sterilization. The organisms were grown by following an active culture procedure[10] and an appropriate amount of test compound was added at zero time ($t_0$) followed by incubation at $30 \pm 1$°C on a rotary shaker. Growth was monitored spectrophotometrically at 600 nm. The total viable *counts* (TVC) and heat stable counts (HSC) were determined[10] by plating an appropriate dilution on nutrient agar followed by incubation of petri plates at 37°C for 24 hr. The DPA, polybetahydroxybutyrate (PHB) and volatile organic acids were extracted and determined[11–13]. The cell-free extracts were prepared from 7 hr cultures and the citrate synthase and the aconitase were assayed[14,15].

## RESULTS AND DISCUSSION

Data presented in table 1 show that nicotinic acid failed to inhibit either growth or sporulation. This observation is quite interesting because α-picolinic acid (structurally related compound) specifically inhibits sporulation in bacilli without affecting growth[5,6]. This is also intriguing and difficult to explain on the basis of the present observations. Methyl and ethyl nicotinate specifically inhibit sporulation (table 1), utilization of organic acids and PHB synthesis/accumulation (table 2) without affecting the growth. Similar observations have been reported with ethyl picolinate[6]. The effect of ethyl picolinate was completely reversed with the addition of ferrous ions[6]. Ethyl nicotinate inhibition, on the other hand, could not be reversed even with various metals, amino acids, vitamins, and TCA cycle intermediates either alone or in combination.

Ethyl nicotinate treated cultures accumulate organic acids in the medium which are not utilized by the

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**Table 1** *Effects of nicotinic acid derivatives on growth and sporulation of bacilli*

| Treatment | Counts/ml | | Per cent of maximum | |
|---|---|---|---|---|
| | TVC ($\times 10^8$) | HSC | HSS | DPA |
| Control | 2.10 | $2.10 \times 10^8$ | 100 | 100 |
| Nicotinic acid (1 mg/ml) | 2.11 | $2.10 \times 10^8$ | 100 | 100 |
| Methyl nicotinate ($3.0 \times 10^{-2}$M) | 2.00 | $<10^4$ | <2 | <2 |
| Ethylnicotinate ($3.0 \times 10^{-2}$M) | 2.10 | $<10^4$ | <2 | <2 |
| Nicotinamide (1 mg/ml) | 2.10 | $3.5 \times 10^6$ | <5 | 10–15 |
| NA + DPA (400 $\mu$g/ml) | 2.0 | $2.1 \times 10^8$ | 100 | 105 |

The data represent average of duplicates using *B. megaterium* and similar data were obtained with *B. cereus* under the same conditions. Data collected after 30 hr of growth.

**Table 2** *Determination of volatile organic acids and polybeta-hydroxy-butyric acid*

| Treatment | Per cent of maximum | |
|---|---|---|
| | Volatile organic acids in the medium | Polybeta-hydroxy butyrate in the cells |
| Control | 2–4 | 100 |
| Nicotinic acid | 3–5 | 100 |
| Methyl nicotinate | 100 | 5 |
| Ethyl nicotinate | 100 | 3 |
| Nicotinamide | 3 | 100 |

Organic acids and PHB were determined from 7 hr cultures by following published procedures[12,13]. These data represent the average of five experiments.

**Table 3** *Estimation of TCA cycle enzymes in the cell-free extracts of B. cereus*

| Treatment | Per cent of maximum activity | |
|---|---|---|
| | Citrate synthase | Aconitase |
| Control | 100 | 100 |
| Methyl nicotinate | <5 | <5 |
| Ethyl nicotinate | <5 | <5 |

The cell-free extracts were prepared from 7 hr cultures and represent the average of five experiments. The enzymes were assayed as mentioned[14] in the text.

cell suggesting a possible block in TCA cycle. Based on this assumption, *in vitro* studies were carried out on TCA cycle enzymes using cell-free extracts. Data presented in table 3 suggest inhibition of citrate synthase and aconitase by ethyl nicotinate treatments. These enzymes are expressed during stationary phase in bacilli for energy generation and providing intermediates for spore constituents. Our data suggest a possible repression of these enzymes by ethyl nicotinate and therefore, organic acids are not utilized further for energy purposes and cell constituents synthesis.

Another nicotinic acid derivative, nicotinamide treatment exhibited completely different effects on sporulation. It did not affect either the growth, sporulation, organic acid utilization or PHB accumulation/synthesis (tables 2 and 3) but produces more than 95% heat labile spores (table 1) with less amount of DPA (about 10–15% of the maximum). These spores germinate poorly (15–20%) and germination ability was completely lost on storage at 4°C for 2 months (data not given). Preliminary reversal studies showed that nicotinamide effects could be restored with the addition of exogenous DPA along with nicotinamide (table 1). This observation shows that nicotinamide possibly interferes with the intermediate(s) of DPA synthesis which highlights the significance of DPA[16] in the development and/or maintenance of thermoresistance and germination of dormant spores.

Based on these studies, a model is proposed (figure 1) depicting the possible site(s) of action of

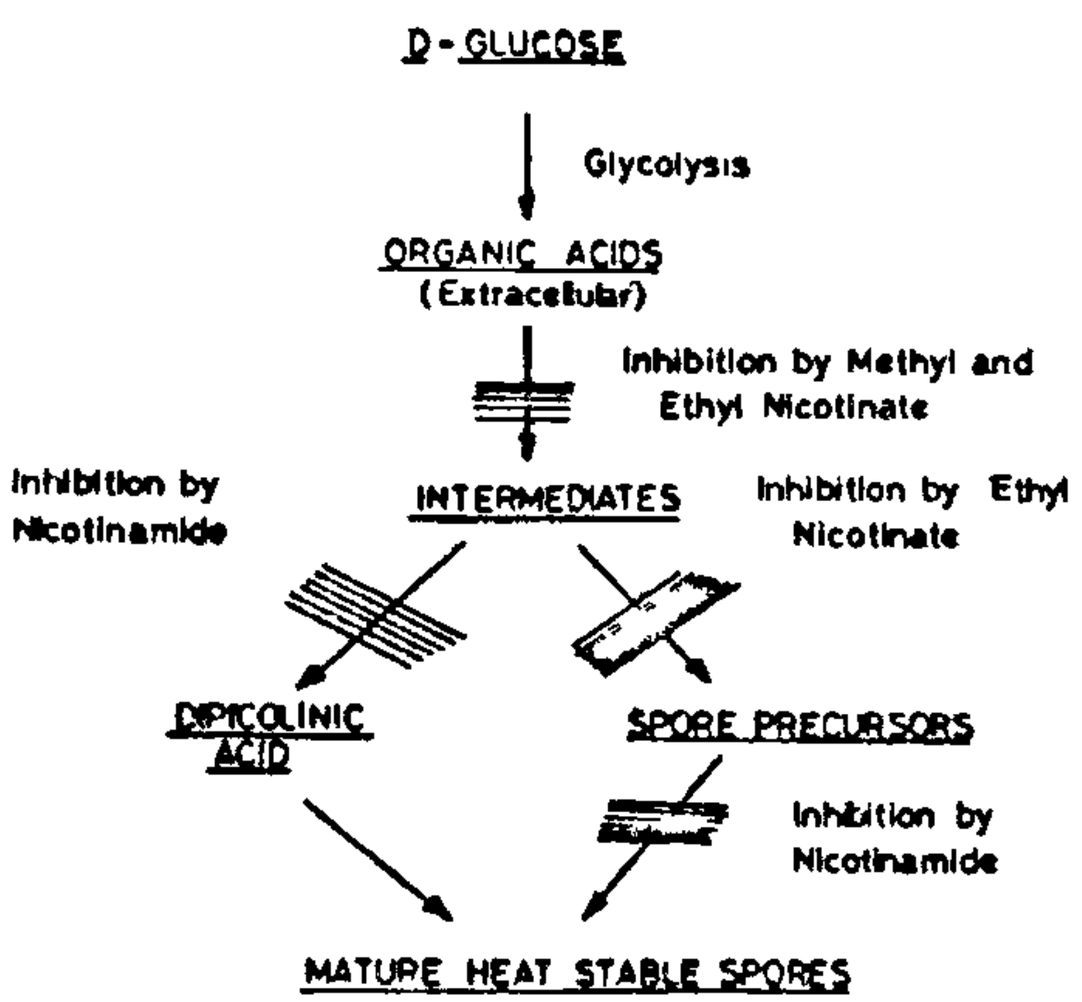


**Figure 1.** Model showing possible sites of action of nicotinic acid derivatives during growth and sporulation of bacilli.

various nicotinic acid derivatives during growth and sporulation of bacilli.

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