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An OMIC approach to elaborate the antibacterial mechanisms of different alkaloids

Fatma Gizem Avci [a, b], Nihat Alpagu Sayar [a], Berna Sariyar Akbulut [a, *]

[a] Department of Bioengineering, Marmara University, 34722, Kadikoy, Istanbul, Turkey
[b] Department of Bioengineering, Adana Science and Technology University, 01250, Adana, Turkey

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ABSTRACT

Plant-derived substances have regained interest in the fight against antibiotic resistance owing to their distinct antimicrobial mechanisms and multi-target properties. With the recent advances in instrumentation and analysis techniques, OMIC approaches are extensively used for target identification and elucidation of the mechanism of phytochemicals in drug discovery. In the current study, RNA sequencing based transcriptional profiling together with global differential protein expression analysis was used to comparatively elaborate the activities and the effects of the plant alkaloids boldine, bulbocapnine, and roemerine along with the well-known antimicrobial alkaloid berberine in *Bacillus subtilis* cells. The transcriptomic findings were validated by qPCR. Images from scanning electron microscope were obtained to visualize the effects on the whole-cells. The results showed that among the three selected alkaloids, only roemerine possessed antibacterial activity. Unlike berberine, which is susceptible to efflux through multidrug resistance pumps, roemerine accumulated in the cells. This in turn resulted in oxidative stress and building up of reactive oxygen species, which eventually deregulated various pathways such as iron uptake. Treatment with boldine or bulbocapnine slightly affected various metabolic pathways but has not changed the growth patterns at all.

1. Introduction

Expeditious increase in bacterial resistance against existent antimicrobials is a challenging worldwide health problem (Arias and Murray, 2015). Therefore, discovery of new antimicrobials gains increasing importance. Traditional use of plants for their diverse bioactivities has attracted attention in the search of new drug leads. Plant-derived molecules may act through mechanisms different from those used by current antibiotics. Furthermore, their structural diversity may enable them to target multiple biochemical pathways (Harvey et al., 2015; Radulović et al., 2013).

Alkaloids are a group of heterocyclic nitrogenous compounds obtained from plants. With their diverse structures, they display different mechanisms in antibacterial and antibiotic enhancing activities. Comprehensive reviews are available that summarize the structure-activity relation (SAR) of alkaloids and especially of aporphinoids. Common functional groups that contribute to antibacterial activity have been reported to be hydroxyl, methoxy, and methylenedioxy in specified locations in the backbone ring structures (Cushnie et al., 2014a, b; Udvardy et al., 2014). Despite the extensive information on SAR of aporphine alkaloids, information on their mechanism of action (MOA) is very limited.

Achievement in the development of analytical tools for identification and isolation of antimicrobial phytochemicals together with the advent of high-throughput data analysis techniques has enabled us to acquire precise information on the MOA of the plant molecules. In this context, OMIC approaches are capable of providing essential data on the MOA of phytochemicals while requiring only small quantities of the active compounds. Thus, they hold promise in the timely discovery of new drugs by capturing instantaneous changes with great precision (dos Santos et al., 2016).

The current work investigates the antimicrobial MOA of three aporphine type alkaloids of plant origin, roemerine, boldine, and bulbocapnine, with different substituents to seek their potential for the development of new pharmaceuticals. Both roemerine and bulbocapnine possess a methylenedioxy group bonded to C1 and

* Corresponding author. Bioengineering Department, Marmara University, 34722, Kadikoy, Istanbul, Turkey.
E-mail addresses: gizemavci@gmail.com (F.G. Avci), alpagu.sayar@marmara.edu.tr (N.A. Sayar), berna.akbulut@marmara.edu.tr (B. Sariyar Akbulut).

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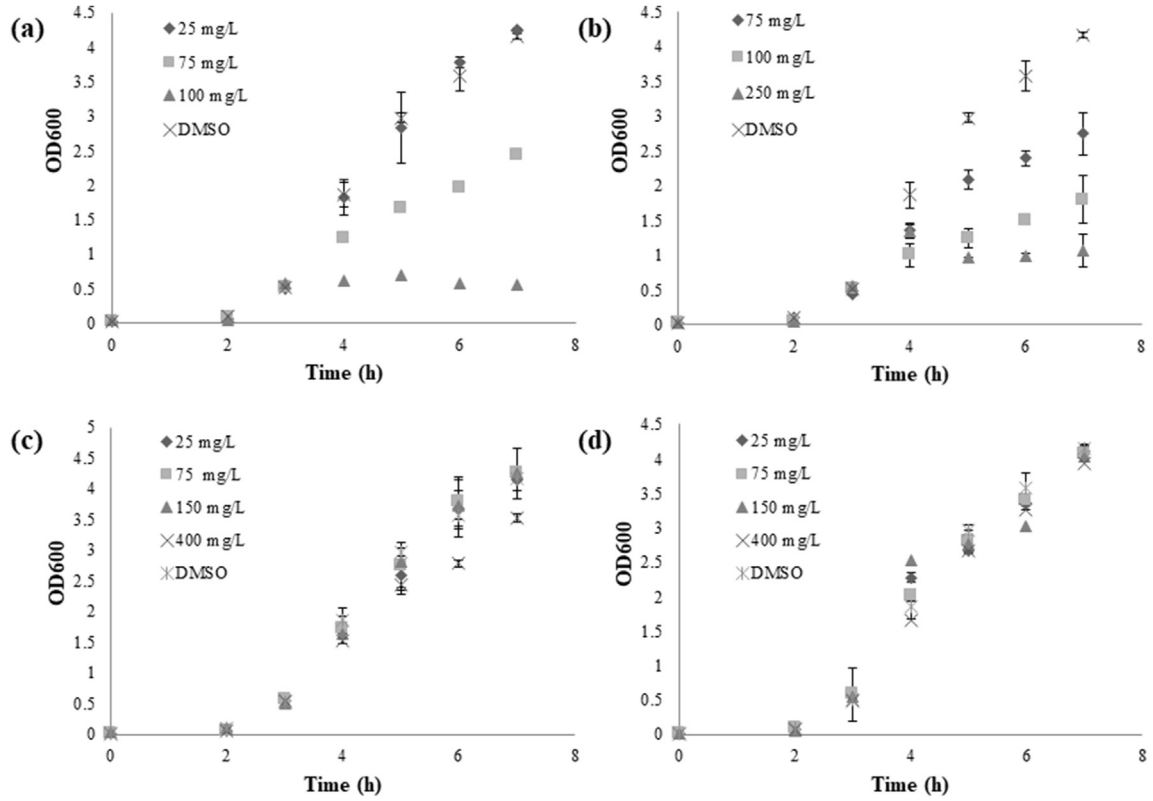

Fig. 1. Growth of *B. subtilis* cells in the presence of alkaloids: a) Roemerine, b) Berberine, c) Boldine, d) Bulbocapnine.

C2, whereas boldine has a tetra-substituted structure with methoxy and hydroxyl groups on the preferred carbon atoms for antibacterial activity. Motivated by the evidence that both roemerine and bulbocapnine display antibacterial activity and the lack of any information on the antibacterial activity of boldine (Gokgoz and Akbulut, 2015; Orhan et al., 2007), we explored the importance of the methylenedioxy group and used a multi OMIC approach to study the mechanistic details of the antibacterial effects of these aporphine alkaloids in *Bacillus subtilis*. The selection of *B. subtilis* is based on its being the model for gram positive microorganisms for which the complete annotated whole genome sequence is available (Harwood, 2007; Michna et al., 2016). The isoquinoline type alkaloid berberine, which is well-known for its MOA in its antibacterial activity (Jin et al., 2010), was selected to comparatively investigate the MOA of different alkaloid classes.

2. Results

2.1. Selection of alkaloid working concentration

The effects of the alkaloids were first examined by monitoring growth in their presence. Based on their effects, different concentrations were tested. Obtained values were plotted as a function of time (Fig. 1). Roemerine was found as the most active with only 100 μg mL^{-1} impeding growth. However, due to endospore formation, its working concentration was set at 75 μg mL^{-1}. With the highest possible concentrations tested (400 μg mL^{-1}), there was no effect of neither boldine nor bulbocapnine. For a comparative analysis with berberine, its lower concentration was set at 75 μg mL^{-1} and upper concentration was set at 250 μg mL^{-1}, a value slightly above its reported minimum inhibitory concentration (MIC) value (Jin et al., 2010). As expected, growth almost ceased with 250 μg mL^{-1} berberine while 75 μg mL^{-1} of the same alkaloid only slightly affected the cells. With these findings, 75 μg mL^{-1} was set as the working concentration.

Table 2
Number of differentially expressed genes following 1-h alkaloid treatment.

Treatment	Number of genes	
	Up-regulated	Down-regulated
Berberine	735	570
Boldine	23	27
Bulbocapnine	14	13
Roemerine	344	221

Table 1
Summary of control and alkaloid treated *B. subtilis* cDNA samples.

Treatment	Read length (bp)	Clean Reads	Clean bases	GC (%)	Mapped Reads (%)
DMSO	100	11128658	1112865800	44.60	94.4
Berberine	100	11083418	1108341800	43.91	93.2
Boldine	100	11136066	1113606600	43.95	94.7
Bulbocapnine	100	10976112	1097611200	44.28	94.2
Roemerine	100	11146966	1114696600	44.87	94.5