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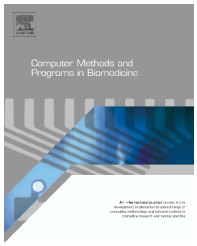

Clavulanic acid production estimation based on color and structural features of streptomyces clavuligerus bacteria using self-organizing map and genetic algorithm

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ABSTRACT

The utilization of antibiotics produced by Clavulanic acid (CA) is an increasing need in medicine and industry. Usually, the CA is created from the fermentation of Streptomycen Clavuligerus (SC) bacteria. Analysis of visual and morphological features of SC bacteria is an appropriate measure to estimate the growth of CA. In this paper, an automatic and fast CA production level estimation algorithm based on visual and structural features of SC bacteria instead of statistical methods and experimental evaluation by microbiologist is proposed. In this algorithm, structural features such as the number of newborn branches, thickness of hyphal and bacterial density and also color features such as acceptance color levels are extracted from the SC bacteria. Moreover, PH and biomass of the medium provided by microbiologists are considered as specified features. The level of CA production is estimated by using a new application of Self-Organizing Map (SOM), and a hybrid model of genetic algorithm with back propagation network (GA-BPN). The proposed algorithm is evaluated on four carbonic resources including malt, starch, wheat flour and glycerol that had used as different mediums of bacterial growth. Then, the obtained results are compared and evaluated with observation of specialist. Finally, the Relative Error (RE) for the SOM and GA-BPN are achieved 14.97% and 16.63%, respectively.

1. Introduction

In general, the fermentation of fungi and bacteria is a common way to product the most important industrial antibiotics. The most important group of bacteria for production belongs to actinomycete group and particularly streptomyces which one of the various kinds of strptomyces is streptomyces clavuligerus (SC) bacteria [1]. The SC bacteria can be produced by 21 secondary metabolites such as Penicillin, Sefamaysyn and an important commercial production that is called Clavulanic acid (CA). The production of these metabolites is closely dependent on the process conditions, medium composition, especially carbon and nitrogen sources. In other words,

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medium composition has a major role in determining output of the fermentation. Since 1981, the produced CA by SC bacteria has been used in clinical application. Also, it is a strong inhibitor of β-lactamase, which can cause irreversible binding to the active site serine of the enzyme, prevents the enzyme operation and protects the β-lactam antibiotic against enzymatic hydrolysis.

In addition to prevent activity of β-lactamase, CA causes pathogen removal by connecting to some of PBP's (Penicillin-Binding Proteins) such as PBP2 and PBP3 in Streptococcus Pneumoniae, effect on PMN cells (Polymorph nuclear) and also increase the power of immune [2,3]. The CA consists of a Clavum nucleus and resembles penicillin, except for the little difference in the five-member loop, Sulphur is replaced by Oxygen and it lacks the Acylamino Penicillin side-chain. Fig. 1 shows some types of lactamase inhibitors that have been used in the clinical usage [4,5].

The CA has just little antibacterial activity and is used in combination with other β-lactams. Also, it has synergistic activity with many Penicillines and Cephalosporins such as Penicillin, Amoxicillin, Ampicillin, Mezlocillin, Cephaloridine, Cefamandole and Ticarcillin. It must be mentioned that the use of β-lactam and lactamase inhibitor depends on many factors and it is not simple to combine every β-lactam with every lactamase inhibitor. Thus, although the CA has synergistic effect on many of β-lactamase but in clinical applications, it is used in combination with Ticarcillin and especially Amoxicillin. The synergistic effect of combination Amoxicillin and CA over a wide range of β-lactamases has been observed and indicated that significantly Amoxicillin is protected against many bacteria resistant to the antibiotic. The CA acts on many pathogenic bacteria and it can be used to stop the action of these bacteria by its mechanism [2]. The combination of CA and Amoxicillin (that is known as the brand names of Augmentin or Co-Amoxiclav) is used in the treatment of upper and lower respiratory tract infections, skin infections, dental or head or neck infections and urinary tract infections [4–6]. For the first time, Co-Amoxiclav was used in England in 2002 and then has been used in more than 150 countries [7].

Producing CA by using of SC bacteria depends on the production process conditions and also the composition of medium, specially carbon and nitrogen resources. It also depends on the compactness level (in other words, bacterial density) of the produced bacteria. Therefore, nutritional conditions should be adjusted separately by a suitable way for both cellular growth and antibiotic production. For the most of instances, the medium is designed for a two-phase fermentation. In the first phase, rapid cellular reproduction takes place without production and in the next phase optimal conditions for antibiotic biosynthesis are provided [8]. The production of antibiotic takes place via special biosynthetic pathways, in a particular time during the bacterial growth cycle. The mentioned stage is severely influenced by the limiting factors of growth such as nitrogen, carbon or phosphate resources. The effect of changes in carbon resources such as glycerol, malt, and starch and wheat flour in different densities on the production level has been investigated as a major determinate factor of the medium. Although, it is recognized that the CA production rate is strongly affected by the nitrogen source, but little information regarding the effect of different sources of nitrogen in the production of CA by SC bacteria has not been published. Other important conditions that SC bacteria need to grow are PH, temperature of medium and the proper place to live. SC bacteria live in soil and the optimum temperature range to grow is 26–30 °C and the ideal temperature is 28 °C. Also, there is no growth upper than 37 °C. Moreover, the bacteria grow in mediums with a PH value 5–8.5 and PH range of 5–6 is optimum for growing.

In typical experimental procedure, three days after the injection of bacterial spore into different mediums and the coloration of the resulting hyphal mass, microscopic photography of the productive mass is conducted. Finally, the CA production level for every medium is calculated. In this experimental methods, the usage of complex statistical equations and also spending a relatively long time for reporting the CA production level creates the need for a fast and automatic algorithm for achieving the CA production level in each medium. However, by reviewing other works in the literature, this fact is demonstrated that there is not any special algorithm for this purpose.

Studying the results of microbiologists' discoveries and observations reveals that the morphology of hyphae growing, their manifested level of warm colors or a blue-violet appearance in the medium, the number of new branches ramified from the growing hyphae, the proper density of bacterial, and also the PH and biomass of medium have a direct relationship with the production level of CA [9]. Actually, these factors represent the physiological status of the bacteria as the building blocks of antibiotics.

Considering these findings and the performed study in [10], in this paper, we propose a fast and automatic production level estimation of CA using visual features of SC bacteria. In this algorithm, structural features such as the number of newborn branches, thickness of hyphal and bacterial density and also color features as acceptance color levels are extracted from the SC bacteria. Moreover, PH and biomass of the medium provided by microbiologists are considered as specified features. In next stage, the extracted features are used in different networks such as Back Propagation Network (BPN), the hybrid model of Genetic algorithm with back propagation network (GA-BPN), and the self-organizing map (SOM) as inputs and finally the produced level of CA in according to our extracted visual features from SC bacteria is estimated.

The rest of this paper is organized as follows. Section 2 explains the details of pre-processing stage and visual features of SC bacteria. In Section 3, our proposed CA production estimation algorithm is discussed. The obtained results on three networks and statistical analysis by ANOVA are provided in Section 4 and finally a conclusion is presented in Section 5.

2. The proposed pre-processing and feature extraction

In this section, our proposed pre-processing and feature extraction algorithms for the production level estimation of CA are described. First, there are many inappropriate factors on the obtained images, which need to be process by a set of proper pre-processing procedures. For this purpose, using different methods such as Laplacian filter, a thresholding method