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Prediction of the allergic response of extracellular amylase producing bacteria through *in-silico* method

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ABSTRACT

Allergies intolerance is a common problem worldwide. The major difficulties are related to the correct diagnosis of causes which is associated with amino acid sequences present in the epitope region of allergen. So there is a need to find out the factors causing allergies and allergens themselves. In the present study a bioinformatics tool is used to predict amino acid sequence and mast cell association with different integrated approaches. Internet databases for amylase producing bacteria were used in *In-silico* method to check the allergy for microorganism producing the extracellular enzyme. Amylase is an extracellular enzyme isolated from soil bacteria *Salmonella* species and *Proteus vulgaris*. It is very important in the pharmaceutical industry to check the allergenicity of any drug, protein or enzyme that be used in the treatment of diseases or food industries for various purpose. The aim of the present study is isolation and characterisation of extracellular enzyme produced from soil bacteria and to analyzed allergic response through AlgPred tool of bioinformatics. From results, it was concluded that the protein sequence of amylase did not contain any epitope, no hits for mast and blast which proved that it was not an allergen. So, bacterial isolates from the industrial soil are a good alternative source of enzyme production and may be used as an industrial level. Thus, from the results, it may be concluded that microbes from soil sample can be a good source of industrially important enzymes without any allergy.

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INTRODUCTION

The development of allergy or hypersensitivity and food intolerance is increasing day by day. Hypersensitivity is induced by some environmental contamination and also by the microorganism. Allergens are the problem of modern civilization related to body metabolism. (Borchers *et al.* 2010; Sicherer 2011; Ameratunga *et al*. 2016; Di Costanzo *et al.* 2016; Jiménez-Saiz *et al.,* 2015).

Information technologies such as bioinformatics tools can be used to gain prior knowledge about compounds which are obtained from microorganism, plants and animals. For this, internet databases played a special role, which are repositories of information related to classification, nomenclature, physical property, chemical property, structure and function of compound (Scalbert *et al.*, 2011).

Computer-based bioinformatics investigation associated with hypersensitivity or allergic response of food components is known as allergens. It is required to find out authentic information based on the amino acid sequences and structure of allergens, used for *in-silico* studies (Brusic *et al.*, 2003). The major task is related to research on allergen is

finding the responsible sequence for inducing hypersensitivity. Part of antigen or allergen which is responsible for allergy is referred to as epitopes (Wróblewska *et al.*, 2007). Haptens are small molecules which increase the property of allergens. So it is necessary to get information about an allergic reaction, involved allergens and concerned epitopes as well as to find out the data related to haptens. Therefore, haptens might play a crucial role in the immune reaction against a variety of molecules (Goodrow *et al.*, 1990; Singh *et al*., 2004). Selected database and tools of bioinformatics are useful in research and analysis on enzyme as epitopes, allergens, and haptens. Their studies showed additionally examples of the applying of bioinformatics ways in analysis associated with allergies. Bioinformatics tools, as well as databases, have recently been more and more employed in education, particularly within the area of medical and biological research. (Atwood *et al.*, 2015; Brazas and Ouelette, 2016; Minkiewicz *et al.*, 2016).

The aim of the present study was isolation and characterization of extracellular enzyme produced from soil bacteria and to analyzed allergic response through AlgPred tool of bioinformatics.

MATERIAL AND METHODOLOGY

Soil sample collection

Samples of soils were collected from divergent sites of Gwalior region, Madhya Pradesh.

Isolation and Identification of Bacteria

First, the bacteria present in Soil samples were cultured on nutrient agar medium and primary characterization based on the morphology, colony characteristics of organisms, shape, size, odour, margin and surface characteristics. Gram-positive and gram-negative bacteria were differentiated on the basis of gram staining. After phenotypical identification of bacteria, further confirmation was done on the basis of their biochemical characterization viz Mannitol salt agar test, amylase production, hydrolysis of gelatin, IMViC test, catalase test, Widal test, skim milk test, carbohydrate fermentation test, Simmon citrate test and urease test by standard methods.

Evaluation of protein

Protein concentration in the sample is evaluated by Lowry's method. Lowry's method is a very useful method for determining the concentration of protein in the sample. (Dunn, 2013).

AlgPred method

It is a bioinformatics tool for a test of allergy done by Saha *et al*., 2006

RESULT AND DISCUSSION

Bacteria are used in various industrial processes like gelling agents, coagulants, emulsifiers etc. in many food and pharma industries. It is also reported by many researchers that bacteria have the potential to synthesize and release exopolysaccharides which are used for the various purpose in different industries. (Indira *et al.,* 2016)

Bacteria are able to produce antibodies amino acid and drugs which are natural therapeutic products. It can be used to cure various diseases like anaemia, diarrhoea, cancer, obesity etc. These products also play a role as antioxidants, enzymes, enzyme-inhibitors, immuno-suppressants, vitamin, antibiotics etc. (Gupta *et al.,* 2014).

Many researchers are showing interest in the isolation and characterization of soil bacteria especially amylase producing *Bacillus* species. (Singh and Kumari; 2016). In the present study bacterial isolates from soil samples are observed as species of *salmonella*, *Proteus vulgaris* and *Bacillus subtilis.* They are disease producing in nature and also have the capability of producing enzymes like urease, amylase, asparaginase and glutaminase. These all have industrial importance, so it is necessary to check their allergenicity nature before use in food and other pharmaceutical industries. It can be identified with the help of bioinformatics tool AlgPred.

AlgPred plays an important role in the pharmaceutical industry to check the allergenicity of any drug that the particular drug can be used in the treatment of diseases. The presently available amylase is not sufficient to meet industrial demands. Hence, there is a continuous search for new amylase with novel characteristics for industrial application obtained from diverse bacteria isolates, and their allergenicity nature can be identified with the help of bioinformatics tool AlgPred. In AlgPred bioinformatics tools allergenicity is checked on the basis of the following criteria:

- Reactivity with the mast cell
- Mapping of IgE epitopes
- Composition of amino acid,
- BLAST
- Presence of dipeptides.

The sequences from different organisms belonging to the same group as studied in the present work were analyzed for their antigenicity. These results are supported by the work done by Bucholska *et al.* 2018. He used bioinformatics tools to check allergens, haptens, epitopes on the enzyme and the reactivity of mast. This kind of findings has advantages in the field of medicine.

Table 1: Algpred table for Amylase producing *Salmonella enterica subsp*

S. No	Sequence	Source	Alegpred				
			Mast	Mapping of IgE Epitopes	SVM	Blast	DP
1	Accession: AH09 GI: 255170879	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
2	Accession: AD0751 GI: 2528935	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
3	Accession: AGK69992.1 GI: 485084613	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
4	Accession:AGK69495.1 GI: 485084116	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
5	Accession:AGK69366.1 GI: 485083987	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
6	Accession:AGK66530.1 GI: 485081151	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
7	Accession:CGA49954.1 GI: 813564309	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
8	Accession:CHQ61771.1 GI: 877339601	Salmonella enterica subsp. entericaserov	NR	NR	R	NR	R
9	Accession:CGA67540.1 GI: 812993421	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
10	Accession: CHM51659.1 GI: 812885997	Salmonella enterica subsp. Enterica Typhi	NR	NR	R	NR	R
11	Accession:CFZ92988.1 GI: 812495650	Salmonellaenterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
12	Accession: CHM74263.1 GI: 812318785	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
13	Accession:KYN49516.1 GI: 1009432567	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
14	Accession: KYN47775.1 GI: 1009430801	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
15	Accession: CQX05627.1 GI: 804580984	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
16	Accession: CQU84268.1 GI: 804268000	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
17	Accession: CHM96195.1 GI: 813548768	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
18	Accession: CQS40527.1 GI: 900013051	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
19	Accession: CGN57236.1 GI: 877692080	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R
20	Accession: CHQ23660.1 GI: 876884638	Salmonella enterica subsp. Entericaserovar Typhi	NR	NR	R	NR	R

Mahboobi *et al.,* in 2017 showed interest in the application of bioinformatics tools to study bacteria such as *E. coli* model. He reported that *E. coli* is a good source for the production of amylase enzyme. It is also reported that amylase has an anticancer ability.

After Databases analysis from NCBI and Associated bioinformatics tools, it is concluded that AlgPred is very useful to study Allergens, Epitopes and Haptens present in the test organisms. Although the use of such *in-silico* tools provide us rapid and reliable results and minimize the experimental work, cost and time but these results further need to be validated by *in vitro* and *in vivo* studies.

CONCLUSION

The presently available amylase is not sufficient to meet industrial demands. Hence, there is a continuous search for new amylase with novel characteristics for the industrial application. The computer-based methods and information provided in the form of amino acid databases are an important part of allergens findings. The main representative part of allergen is epitopes. Online databases from

Table 2: Algpred table for Amylase producing *Proteus vulgaris*

S.No.	Sequence	Source	Alegpred				
			Mast	Mapping of IgE epitopes	SVM	Blast	DP
1	Accession: KGA57821.1 GI: 685170148	Proteus vulgaris	NR	NR	R	NR	R
2	Accession: ATM99194.1 GI: 1267036235	Proteus vulgaris	NR	NR	R	NR	R
3	Accession: CRL59508.1 GI: 857778412	Proteus vulgaris	NR	NR	R	NR	R
4	Accession: CRL59511.1 GI: 857778413	Proteus vulgaris	NR	NR	R	NR	R
5	Accession: CRL61082.1 GI: 857777606	Proteus vulgaris	NR	NR	R	NR	R
6	Accession: CRL62584.1 GI: 857585732	Proteus vulgaris	NR	NR	R	NR	R
7	Accession: CRL64797.1 GI: 857584720	Proteus vulgaris	NR	NR	R	NR	R

NCBI provide a simple basis to access a variety of information related to the structure and properties of enzyme and proteins. Their fragments that may be responsible for the occurrence of hypersensitivity reactions. Today, Bioinformatics tools are commonly used in immunological research. Amino acid sequences were searched from NCBI and used for analysis of the function of particular molecules. Bioinformatics methods are used in the field of immunology known as allergology. It is concluded that it may be suitable for developing new vaccines. This comes after sequence analysis from NCBI for potential allergenicity of the molecule. It helps in the prediction of epitope structure, location and peptide sequence which is an important factor for analysis of enzyme allergenicity. The analysis and identification of such kind of fragments can improve the knowledge related to hypersensitivity reactions in immunology. Researchers have a great interest in bioinformatics analysis related to enzyme and allergies which is the novel characters for industrially important enzymes.

The present study paves the path for standardisation of different experiments for increasing the enzymatic yield and minimizing the time and cost of the downstream process for the extraction of desired enzymes at the industrial level. The results obtained from the in-silico study are also very promising. Although the use of such *in-silico* tools provide us rapid and reliable results and minimize the experimental work, cost and time but these results further need to be validated by in vitro and in vivo studies.

Conflict of interest

The authors declare no competing or conflicts of interest.

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