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Analysis of the antagonistic properties of yeasts isolated from North East India.

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Abstract

The microflora of North Eastern India is considered as the most unexplored and untouched . A total of 10 probiotic yeasts were isolated and and evaluated and examined on the basis of biochemical and morphological characteristics and theirantagonistic activity were examinedfor their againstvarious food borne pathogensi.e*Staphylococcus aureus, Leuconostocmesenteroides,Bacillus cereus, Pectobacteriumcarotovorum, Escherichia coli,Pseudomonas syringae , Enterococcus faecalis , Listeria monocytogenes* , and *Clostridium perfringens*.

Keywords

sidra, morphological, biochemical and antagonistic activity.

Introduction

The geographical location of North East (NE) India is located within the Eastern Himalayas and Purvanchal Himalayas. The Eastern Himalayan region lies between the latitudes 26° 40'-29° 30' North and longitudes 88° 5' - 97° 5' East and covers a total area of 93,988 km2 comprising two North East states, viz. Sikkim and Arunachal Pradesh, besides eastern Nepal, Darjeeling hills in India, Bhutan and Tibetan Autonomous Region in China (Tamang JP 2010). Ethnic fermented foods and alcoholic beverages and drinks have been consumed by the ethnic people of North East India for more than 2500 years old as per the historical records (Tamang JP 2010).

Seafood is atypical to North East India, people catch the available fishes from the various sources and preserve them traditionally (Tamang, 2010). The ethnic people use their indigenous knowledge to preserve the fish, these methods have still being used to preserve fishes which are located near water bodies with plenty of freshwater fishes. These fish products are generally consumed in the daily diet of the people. Fermented foods have heterogeneousness of traditions and cultural preferences found in the different geographical areas, where they are produced.

They have been consumed since ancient times due to their prolonged shelf life, reduced volume, shorter cooking times and superior nutritive value as compared to the non-fermented ingredients. Fermentation processes are considered as developed in order to preserve food (Rolle and Satin, 2002).

These traditionally preserved fish are smoked and sun dried by the traditional people of North East India. *Sidra* is an ethnic fermented sun-dried fish product commonly consumed in the North East India, its pickle is a popular dish, the microbial composition of *sidra* includes bacteria (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. plantarum*, *L. mesenteroides*, *E. faecium*, *E. f aecalis*, *P. pentosaceus*, and *Weissellaconfusa*) and yeasts (*Candida chiropterorum*, *C. bombicola*, and *Saccharomycopsis* spp.). Preservation of perishable fishes by the indigenous people residing near the coastal regions is done through fermentation, sun drying, smoking, and salting without refrigeration, andare consumed as seasoning, condiments, and side dishes (Salampessy*et al.* 2010).

The isolated strains of yeasts strains were further tested against various food borne pathogens

Materials and Methods

The current research was divided in some steps, as follows: a) yeast isolation from fermented fish product sidra; b) phenotypic and genotypic characterization

Yeast isolation

A total of, 10 yeast strains were isolates were isolated from sidra.Fermentation of sidra was conducted by the indigenous people household scale. Sample was taken 1gm and it was diluted with 9 ml of a sterile saline solution (0.9% NaCl) and homogenized for 60 s. The homogenates were serially diluted in saline solution and plated onto the Yeast Universal Medium (YM) (Yeast Extract, 3.0 g/L; Malt Extract, 3.0 g/L; Peptone, 5.0 g/L; Glucose, 10.0 g/L; agar, 15 g/L), incubated at 25 ºC for 2-4 days for the evaluation of yeasts. Plates were incubated for 72 h at 28 °C.Yeasts, showing the typical appearance of *Saccharomyces* and*Candida* (white-to-yellow colonies) andthe typical cell appearance at the microscope, were randomly selected and labeled with code names. The selected yeast strains were further purified by successive streaking on YM media. All isolates were maintained at −80 °C in 20% (v/v) glycerol (Hi-Media). Isolates were propagated in YM broth

Identification of yeast isolates to species level by genotypic andphenotypic tests

Biochemical and Phenotypic characterization

The selected yeast isolates were phenotypically characterized according to Kurtzman*et al.* (2011b), and further biochemical tests were carried out i.e catalase reaction, carbohydrate fermentation, hydrogen sulphide formation, urease activity and growth at different temperature

Biochemical characterization

Catalase activity

yeast strains were evaluated by adding 3% (v/v) of hydrogen peroxide onto the cultured colonies, according to the Whittenbury method (1964). The results were expressed as positive (+) or negative (-).

Carbohydrate fermentation

Carbohydrate fermentation was performedfor the yeasts isolates following the method of Aneja, 2003.

Hydrogen sulphide production

Hydrogen sulphide production was evaluated following the method of Aneja, 2003.

Antimicrobial

activity

The antimicrobial activity was assessed by bit/disk method. Spoilage causing microorganisms i.eviz*Staphylococcus aureus* IGMC, *Leuconostocmesenteroides* MTCC 107, *Bacillus cereus* CRI, *Pectobacteriumcarotovorum* MTCC 1428, *Escherichia coli* IGMC, *Pseudomonas syringae* IGMC *Enterococcus faecalis* MTCC 2729, *Listeria monocytogens* MTCC 839 and *Clostridium perfringens* MTCC 1739, were used to check antagonistic activity of the yeast isolates.

Results and Discussion

Phenotypic and biochemical characterization

Physiological and Biochemical characterization of the yeasts isolates had been done and their characteristics were noted down in Table 1-3.

Pedersen *et al.* (2012) , reported yeast isolates from Fura, a spontaneously fermented pearl millet product consumed in West Africa, and these were identified by pheno- and genotypic methods. Perricone (2014) , isolated probiotic yeasts from cereal based food and beverages following technological and biochemical characterization.

Table 1 Morphological characteristics of probiotic yeasts isolates

S.No	Isolate	Source	Color	Surface	Margin	Elevation	Cell-shape
1	YS	Sidra	Cream	Round	Undulate	Convex	Round
2	YS1	Sidra	Cream	Round	Undulate	Convex	Ellipsoid
3	Y5	Sidra	Cream	Round	Entire	Convex	Oval
4	Y5(A)	Sidra	Cream	Round	Entire	Raised	Ellipsoid
5	Y4	Sidra	Cream	Smooth	Entire	Raised	Ellipsoid
6	F1	Sidra	Cream	Wrinkled	Entire	Convex	Rough
7	F2	Sidra	White	Smooth	Undulate	Convex	Rough
8	F3	Sidra	White	Rough	Undulate	Raised	Ellipsoid
9	F4	Sidra	Yellow	Rough	Etire	Raised	Ellipsoid
10	F5	Sidra	Yellow	Smooth	Entire	Convex	Rough

Table 2 Biochemical characteristics of probiotic yeasts isolates

Sr. No.	Isolate	Catalase test	Carbohydrate utilization	H_2S production	Urease test
1.	YS	-ve	AG^-	-ve	-ve
2.	YS1	+ve	AG	-ve	-ve
3.	Y5	-ve	AG^-	-ve	-ve
4.	Y5(A)	-ve	AG	-ve	-ve

5.	Y4	-ve	AG^-	-ve	-ve
6.	F1	+ve	AG^-	-ve	-ve
7.	F2	+ve	AG	-ve	-ve
8.	F3	-ve	AG^-	-ve	-ve
9.	F4	-ve	AG	-ve	-ve
10.	F5	+ve	AG^-	-ve	-ve

Antimicrobial activity

Probiotic yeast strains isolated from sidra were tested for their antagonistic activity against selected food borne/spoilage causing microorganisms viz. *Staphylococcus aureus* IGMC; *Leuconostocmesenteroides* MTCC 107; *Bacillus cereus* CRI; *Pectobacteriumcarotovorum* MTCC 1428 ; *Escherichia coli* IGMC ; *Pseudomonas syringae* IGMC ; *Enterococcus faecalis* MTCC 2729 ; *Listeria monocytogens* MTCC 839 ; *Clostridium perfringens* MTCC 1739. The data on inhibitory spectrum of probiotic yeast by bit/disc method had been shown in Table 4.

Table 4: Preliminary screening of probiotic yeasts isolated traditional fermented food matrices of North-East India on the basis of their antagonistic pattern against test indicators by bit/disc method

Sr. No.	Name of isolate	Source	*S. aureus* (mm)	*L. mesenteroides* (mm)	*B. cereus*(mm)	*P. carotovarum* (mm)	*E. coli* (mm)	*P. syringia* (mm)	*E. feacalis* (mm)	*L. monocytogens* (mm)	*C. perfringenes* (mm)	Mean	Percent Inhibition (%)
1.	YS	Sidra	-	-	-	-	-	-	-	-	-	-	-
2.	YS1	Sidra	-	-	-	-	-	-	-	-	-	-	-
3.	Y5	Sidra	-	-	9	-	-	-	-	-	-	1	11.11%
4.	Y5(A)	Sidra	-	-	-	-	-	-	-	-	-	-	-
5.	Y4	Sidra	14	-	-	-	-	9	-	-	-	2.55	22.22%
6.	F1	Sidra	12	-	-	-	-	10	-	-	-	2.44	22.22%
7.	F2	Sidra	-	-	-	-	-	-	-	-	-	-	-
8.	F3	Sidra	15	10	-	-	-	20	17	15	16	10.33	66.66
9.	F4	Sidra	9	-	-	9	18	9	-	-	-	5	44.44%
10.	F5	Sidra	9	-	-	-	17	-	-	-	-	2.88	22.22%

Conclusion

In the present study, 10 yeast strains were isolated, and characterized from sidra based on their morphological and biochemical characteristics and among them, two i.e F6 and F3 were potentialproducers of antimicrobial substances against various food borne pathogens.

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