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Effects of cultural conditions on dextran production by *Leuconostoc* spp.

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Abstract

In this study, attempts were made to investigate the influence of various cultural conditions such as sources of carbon and nitrogen, vitamins, amino acids, initial pH of the medium, temperature of incubation and agitation on dextran production by a recently isolated species of *Leuconostoc*. Among the various carbon source studies, potato peels at a concentration of 20% (w/v) gave the highest amount of dextran (7.9 g/100 ml) while corn steep liquor-containing medium gave the lowest amount of dextran. The effect of various nitrogen sources revealed that casein at 0.5% (w/v) enhanced the highest (8.5 g/100 ml) production of dextran while the lowest amount (3.3 g/100 ml) was recorded in a medium containing urea. The effects of vitamins and amino acids revealed that ascorbic acid and glutamic acid at a concentration of 0.05% gave the highest dextran production of 8.3 g/100 ml and 8.2 g/100 ml respectively. Inoculum size of 6% was found to be optimum for dextran production (9.2 g/100 ml) by this *Leuconostoc* spp while an incubation time of 20 h produced the highest dextran weight of 8.0 g/100 ml. The effect of initial pH of the medium and temperature of incubation of the culture revealed that pH 6.5 and incubation at 25ºC gave the optimum dextran production of 8.2 g /100 ml each.

Introduction

Microbial polymers are important sources of polymeric materials having great potentials for commercialization due to their structural diversity and peculiar characteristics, and they have been used extensively in food, pharmaceutical, agricultural industries and in medicine. An example of these biopolymer is dextran which is mostly produced by species of *Leuconostoc* especially *Leuconostoc mesenteroides* (Raymond and Scott, 1996; El-Tayeb and Khodair, 2006; Shah et al., 2006). Dextran is an extracellular bacterial polymer of D-glucopyranose with predominantly α-(1→6) linkage in the main chain and a variable amount of α-(1→2), α-(1→3), α-(1→4) branched linkages (Mosan et al., 2001). It is a commercially important biopolymer owing to its wide applications in various industries such in medicine (used as blood plasma volume expander), food industry (used as adjuvant, emulsifier, carrier and stabilizer and thickener for jam and ice cream) (Goulas *et al*., 2004; Naessens *et al*., 2005). It is also reported to prevent the crystallization of sugar, improves moisture retention, and maintains flavour and appearance of various food items (UL Qader e*t al*., 2005).

The production of dextran by various microorganisms have been reported in literatures and the various process parameters affecting production optimized (Kim *et al.*, 2000; Santos *et al*., 2005; Son *et al*., 2008; Sarwat *et al.*, 2008). The amount of dextran produced however is practically insufficient to meet the dextran-requirements of the various industries, hence the need for the isolation and characterization of more dextran-producing organisms with potentials for industrial application. The isolation of a new dextran-producing organism as well as the investigation of the various parameters affecting dextran production by this new organism will go a long way in providing information necessary to develop an industrial process for dextran production so as to meet the large dextran demands of the various industries. This paper therefore reports the effect of various environmental factors on dextran production by a newly isolated *Leuconostoc* spp. isolated from field-spoilt sugar cane within a local farm at the University of Ibadan, Nigeria.

Materials and Methods

The *Leuconostoc* spp used in this study was obtained from the culture collection of The Department of Microbiology, University of Ibadan, Nigeria. It was isolated from a field-spoilt sugarcane

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and maintained on an MRS slant at 5ºC.

Inoculum preparation

Inoculum used in this study was prepared by inoculating a 10 ml aliquot of sterile sucrose broth with a loop full of 24 h old culture of Leconostoc spp. Incubation was done for 24 h at 30ºC. 1 ml of this (containing 6.7 x 107 CFU/ml) was used as the inoculum unless otherwise stated.

Dextran production

For dextran production, 100 ml of the medium described by Sarwat *et al.* (2008) which contained (g/l): sucrose, 150; bacto-peptone, 5.0; yeast extract, 5.0; K_2HPO_4, 15.0; $MnCl_2.H_2O$, 0.01; NaCl, 0.01; $CaCl_2$, 0.05, was used. Incubation was done at 30ºC for 20 hours after which the amount of dextran produced was determined by precipitation followed by weighing as described by Sarwat *et al.* (2008).

Purification and partial characterization of dextran

The purification of the produced dextran was done as described by UL Qader *et al*. (2001), while the partial characterization was done by taking into consideration the color, texture and pH of the produced dextran. The total protein content was determined as described by Lowry *et al.* (1951) using bovine serum albumin as standard, the total Carbohydrate as described by Anthrone method (Hedge and Hofreiter, 1962) and total reducing sugar was determined using the method of Miller (1955).

The effect of various agro-wastes as carbon sources on dextran production

100 ml of the medium described above was also employed. However, sucrose was replaced with each of sugarcane bagasse, corn steep liquor, wheat bran, palm kernel cake, potato peel and rice bran and inoculated with 1 ml of the inoculum. Incubation was carried out at 30ºC for 20 hours after which the amount of dextran produced was estimated as described above.

Effect of Nitrogen sources on dextran production

This was done using the medium as described for carbon sources. However, Yeast extract was substituted with peptone, casein, urea, soy meal preparation, ammonium di-hydrogen orthophosphate, ammonium sulfate, ammonium nitrate, ammonium chloride potassium nitrate and sodium nitrate at 0.5% (w/v). The pH of the broth medium was adjusted to 7.0 before sterilization at 121ºC for 15 min. Inoculation was done as described above and incubation carried out for 20 h at 30ºC.

Effect of vitamins on dextran production

Influence of vitamins on dextran production was investigated using the medium containing the best carbon and nitrogen sources above and supplemented differently with each of folic acid, thiamine, ascorbic acid, nicotinic acid and riboflavin at 0.05% (w/v). This was then inoculated and incubated at 30ºC for 20 hours. Dextran produced was precipitated as described earlier.

Effect of amino acids on dextran production

For the investigation of the effect of amino acids on dextran production, different amino acids namely aspartic acid, glutamic acid, alanine, tryptophan and methionine were supplemented into the medium at 0.05% concentration. The medium was inoculated and incubation carried out at 30ºC for 20 hours. Dextran produced was precipitated as described earlier.

Effect of inoculum size on dextran production

The influence of inoculum size on dextran production was studied by introducing 2, 4, 6, 8, and 10% of the standard inoculum (6.7 x 10^7 CFU/ml) in the broth medium, pH of the broth medium was adjusted to 7 before sterilization and the cultivation media were incubated at 30ºC for 20 hours.

Effect of incubation time on dextran production

The effect of incubation time on dextran production by the *Leuconostoc* spp was done as described by Sarwat *et al.* (2008) using the medium containing the best carbon, nitrogen, vitamin and amino acid source. Incubation was done at 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48 h after which dextran production was estimated.

Effect of temperature of incubation

For studying the influence of temperature on dextran production, cultures were incubated at temperatures of 20, 25, 30, 35, 40 and 45°C. Thereafter the amount of dextran produced was estimated.

Effect of initial pH on dextran production

To investigate the effect the initial pH of the culture medium on dextran production, the initial pH of the medium was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8 separately, before sterilization. Incubation was done for 20 h at 30ºC.

Effect of agitation on dextran production

The different agitation speeds considered in this study were 50, 100, 150, 200 and 250 rev min^{-1}.

Cultures were incubated at 30ºC for 20 h.

Statistical analysis

Results obtained in this study were subjected to analysis of variance (ANOVA) and separation of means was carried out by Duncan's Multiple Range Test (Duncan, 1955).

Results

The result of the amount of dextran produced revealed that this species of *Leuconostoc* is capable of producing 6.5±0.06 g/100 ml at 30ºC and 20 h. Table 1 shows the results of the partial characterization of the dextran. It was observed to be granular, whitish and highly soluble in water giving a homogenous mixture with a moisture content of 8.8 ± 0.02%, total carbohydrate of 48 ± 1.00% (Table 1). The results of the effect of various agro-residues (as carbon sources) on dextran production by *Leuconostoc* spp is presented in Figure 1. All the carbon sources investigated supported dextran production. However, potato peels at a concentration of 20% (w/v) appears to stimulate the highest production of dextran giving a dextran production of 7.9 g/100 ml. This is closely followed by wheat bran also at 20% (w/v) producing 7.7 g per 100 ml of culture medium. The lowest dextran production was observed in a medium containing corn steep liquor as the carbon source (Figure 1).

Table 1. Characteristics of dextran produced by *Leuconostoc* spp.

Characteristics	Obtained Character
Color and Texture	White, granular
pH	5.9±0.020
Ash Content	10.2 ±0.028
Moisture Content	8.8 ±0.30
Total Carbohydrate (%)	48 ±1.00
Total Protein (%)	2.3 ±0.06
Total reducing sugar (%)	12.01±0.06

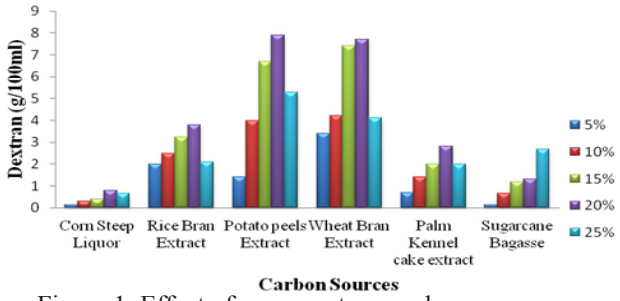

Figure 1. Effect of agro-waste as carbon sources on dextran production by *Leuconostoc* spp.

All the nitrogen sources investigated in this study supported the growth and dextran production as revealed in Table 2. Casein was however found to support the highest production of dextran (8.5 g/100 ml) closely followed by Yeast extract (5.3 g/100 ml) and Sodium Nitrate (5.0 g/100 ml) while the lowest production (3.3 g/100 ml) was observed in a urea-containing medium as the sole nitrogen source (Table 2).

Table 2. Effect of different nitrogen sources on dextran production by *Leuconostoc* spp.

Nitrogen sources (0.5% w/v)	Dextran production (g/100 ml)
Peptone	4.0±0.003^{c}
Yeast extract	5.3±0.066^{b}
Casein	8.5±0.331^{c}
Urea	3.3±0.021^{e}
Soy meal preparation	4.8±0.001^{a}
Potassium nitrate	4.7±0.000^{d}
ammonium sulpahte	3.4±0.010^{a}
Ammonium nitrate	3.2±0.023^{c}
Sodium nitrate	5.0±0.021^{b}
Ammonium dihydrogenphospahte	3.0±0.000^{c}

Data are means of three replicates ± Standard error of mean.

Investigation of the influence of vitamins on dextran production as presented in Figure 2 revealed that that ascorbic acid supported the highest production of dextran (8.3 g/100 ml), closely followed by thiamine, and folic acid with 8.2 g/100 ml and 8.1 g/100 ml respectively while the lowest dextran production was recorded in a medium containing Riboflavin 6.6 g/100 ml (Figure 2).

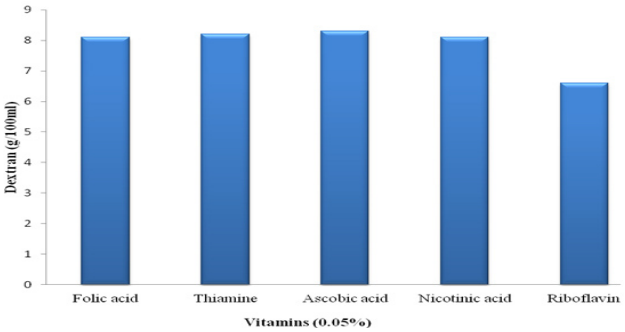

Figure 2. Effects of vitamins on dextran production by *Leuconostoc* spp.

The results of the influence of amino acids on dextran production are shown in figure 3. Glutamic acid at 0.05% (w/v) was found to support the highest amount of dextran (8.2 g/100 ml) closely followed Aspatic acid 8.1 g/100 ml and Alanine 8.06 g/100 ml. The lowest production (6.72 g/100 ml) was however recorded in a medium containing Tryptophan as amino acid.

Investigation into the influence of physical parameters on dextran production by the newly isolated *Leuconostoc* spp. took into consideration the

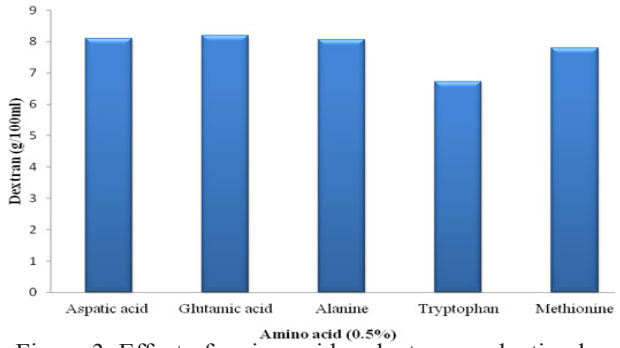

Figure 3. Effect of amino acid ondextran production by *Leuconostoc* spp.

effect of inoculum size, agitation, temperature and pH. The effect of inoculum size on dextran production indicated that there was a gradual increase in dextran production as the inoculum size increase from 6.7g/100 ml at 2% inoculum to 8.4 at 4% reaching a peak of 9.2 g/100 ml at 6% before a gradual decrease to 8.0 g/100 ml at 8% inoculm size and 7.5 g/100 ml at 10% inoculum size (Figure 4).

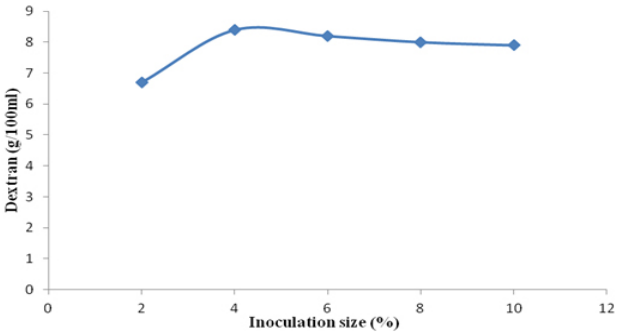

Figure 4. Effect of Inoculums size on dextran production by *Leuconostoc* spp.

Figure 5 shows the effect of the time of incubation on dextran production by *Leuconostoc* spp. Dextran production was found to gradually increase with an increase in the incubation time from 0.8 g/100 ml at 4 h to 1.2 g/100 ml at 6 h and 5.3 g/100 ml at 12 h and reaching a peak of 8.0 g/100 ml at 20 h of incubation after which a gradual decrease was observed from 7.8 g/100 ml at 24 h to 6.9 g/100 ml at 36 h and 6.0 g/100 ml at 48 h (Figure 5).

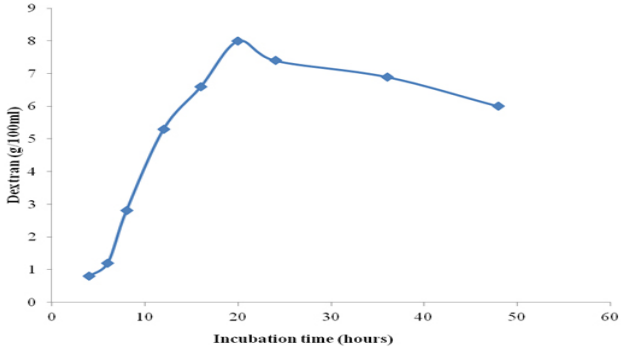

Figure 5. Effect of incubation time on dextran production by *Leuconostoc* spp.

The optimum temperature recorded for dextran production in this study as presented in figure 6 was 25ºC with 8.2 g/100 ml of dextran produced. After this temperature a gradual decrease in dextran production was recorded with an increase in temperature with 7.8 g/100 ml of dextran produced at 30ºC. However, a sharp decrease in dextran production was observed after 30ºC (Figure 6).

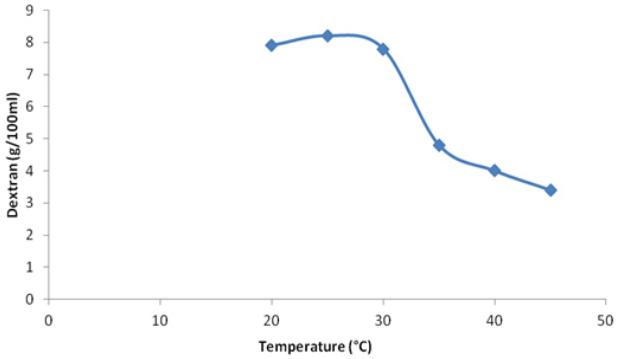

Figure 6. Effect of Temperature on dextran production by *Leuconostoc* spp.

Figure 7 represents the effect of pH on dextran production. A gradual increase in dextran production was observed with an increase in the initial pH of the culture medium with 4.8 g/100 ml recorded at pH 5.0, 5.0 g/100 ml at pH 5.5 and 6.0 g/100 ml at pH 5.3. An optimum production of 8.2 g/100 ml was recorded at pH 6.5. After the optimum pH of 6.5, an increase in pH led to a decrease in dextran production (Figure 7).

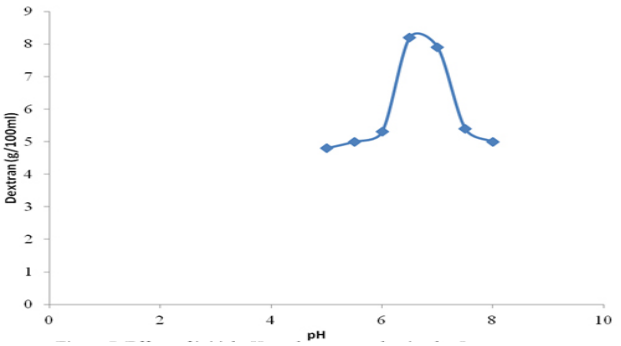

Figure 7. Effect of initial pH on dextran production by *Leuconostoc* spp.

The result of the effect of agitation on dextran production is presented Figure 8. There was a gradual increase in the dextran production as the speed of revolution increased from 8.1 g/100 ml at 50 rpm to 8.3 g/100 ml at 150 rpm reaching an optimum production of 8.4 g/100 ml at 200. Thereafter an increase in agitation led to a decrease in dextran production (Figure 8).

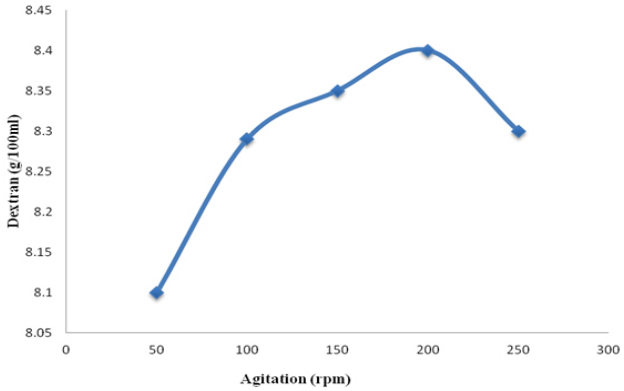

Figure 8. Effect of agitation speed on dextran production by *Leuconostoc* spp.

Discussion

Dextran production by various *Leuconostoc* species especially *Leuconostoc mesenteroides* has been reported in Literatures (Raymond and Scott, 1996; Shah *et al*., 2006; Sarwat *et al*., 2008; Majumder *et al.*, 2009). This study also investigated the effect of cultural condition on dextran production by *Leuconostoc* spp. isolated from field-spoilt sugarcane.

Carbon is an important constituent of the cellular materials and it plays a central role in energy generation in living cells. All the agro-wastes investigated as carbon sources in this study supported dextran production at varying degree. This is of particular importance when considering the cost of dextran production which is mostly based on sucrose-containing medium. Potato peels was found to support the highest production of dextran relative to all the carbon sources investigated in this study. Similar results have also been reported in earlier literatures (Behravan *et al*., 2003; Abdel-Azeem *et al.*, 2009). Potato peels supporting the highest dextran production could be a function of its various compositions since it is reported that various agro-wastes besides acting as carbon source contain other constituents such as peptone, which also affect microbial growth and product formation (Kali *et al*., 1999).

Similarly, the various organic and inorganic nitrogen sources were also found to affect dextran production at varying amounts. Casein, an organic nitrogen source was however found to support the best dextran production. This trend has also been earlier observed by Abdel-Azeem *et al.* (2009) who reported that organic nitrogen sources have a significant effect on dextran production by *Leuconostoc mesenteroides*. Nitrogen is reported to be essential for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors and other substances by the cells (Zang *et al.*, 2007). The cheap and readily available nitrogen sources are of particular interest since these could prove as alternative sources of nitrogen for dextran production thereby reducing the cost of production.

The effect of vitamins and amino acid showed that added vitamins and amino acid have a significant effect on dextran production. Vitamins are reported to be necessary for the activity of certain enzymes while the amino acids are the building blocks of proteins (Moat *et al.*, 2002). The preferential utilization of glutamic acid could be the ease with which it is transported across the cell membrane.

The inoculum size of 4% producing the highest amount of dextran in this study is in the range of 3-5% inoculum reported by Abdel-Azeem *et al.* (2009) as being optimum for dextran production by the species of *Leuconostoc*. Low dextran production at below the 4% inoculum could result from a low cell mass to utilizing the available nutrients necessary for optimal dextran production. Reduced dextran production beyond 4% inoculum level could be due to high concentrations of inoculum depleting the substrate nutrient concentrations necessary for optimum product formation (Onilude *et al*., 2011).

An incubation time of 20 h was found to be the optimum incubation time for this *Leuconostoc* sp. in this study. This is in agreement with the work of Sarwat *et al.* (2008), who studied the effect of incubation time on dextran production by *Leuconostoc* mesenteroides CMG713. Low dextran production below the optimum incubation time could be as a result of the fact that the bacterial cells are still in the lag phase and still adapting to the environment while a decline in after 20 h of incubation might be due to exhaustion of the carbon source (Onilude *et al*., 2011). However, the result above is a shift from that of Abdel-Azeem *et al.* (2009) who reported maximum dextran production at 16 h of incubation. This shift may be due to environmental influences on the growth and metabolite produced by the respective organisms.

Earlier reports found that dextran production is greatly influenced by temperature (Kim *et al*., 2003; Sarwat *et al*., 2008; Abdel-Azeem *et al*., 2009). The result of the effect of temperature on dextran production in this study is also a pointer to this fact. A decline in dextran yield after the 25ºC optimum temperature has been attributed to low multiplication rate of the bacterial cell leading towards less dextran production as compared to optimum temperature (Sarwat *et al*., 2008).

The pH which is a measure of the hydrogen ion concentration of a particular solution has also been

observed to affect the amount of enzyme production in this study. The optimum pH for dextran production by this *Leuconostoc* sp. was found to be 6.5. This is in the range of pHs of 5.5-8.0 that have been reported to be optimum for various sp of *Leuconostoc* sp (Santos *et al*., 2000; Kim *et al.*, 2003; Sarwat *et al.*, 2008). Decrease in dextran production outside the optimum pH range could be due to reduced metabolic activities of the bacterial cells resulting in low dextran yield.

Effects of agitation on dextran yield revealed that for optimum dextran production agitation at 200 rpm is necessary. This result is similar to the report of Vedyashkin *et al*. (2004), who studied the effect of agitation speed on dextran production by *Leuconostoc mesenteroides* and obtained the optimum agitation speed at 200 rpm.

In conclusion, the use of pure materials as components of fermentation medium in dextran production imposes high cost on the industry; this study however showed that dextran production can be economized by using local and cheap sources of carbohydrate and nitrogen. In addition to the use of cheap and readily available sources of carbon and nitrogen, dextran production by this *Leuconostoc* sp. can also be improved by regulating the environmental conditions such as initial pH of the medium, temperature and composition of the medium. A significant increase in dextran production was achieved when the organism was grown at 25˚C, pH 6.5, for 20 hours in the broth medium. These optimized conditions can be used as a standard in commercial production of dextran.

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